Review

Protective effects of sulforaphane against toxic substances and contaminants: A systematic review☆

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ABSTRACT

Background: Sulforaphane (SFN) is a dietary isothiocyanate, derived from glucoraphanin, present in cruciferous vegetables belonging to the *Brassica* genus. It is a biologically active phytochemical that acts as a nuclear factor erythroid 2-related factor 2 (Nrf2) inducer. Thus, it has been reported to have multiple protective functions including anticancer responses and protection against a toxic agent's action.

Purpose: The present work systematically reviewed and synthesised the protective properties of sulforaphane against a toxic agent. This review reveals the mechanism of the action of SFN in each organ or system.

Methods: The PRISMA guideline was followed in this sequence: researched literature, organised retrieved documents, abstracted relevant information, assessed study quality and bias, synthesised data, and prepared a

comprehensive report. Searches were conducted on Science Direct and PubMed using the keywords "Sulforaphane" AND ("protective effects" OR "protection against").

Results: Reports showed that liver and the nervous system are the target organs on which attention was focused, and this might be due to the key role of oxidative stress in liver and neurodegenerative diseases. However, protective activities have also been demonstrated in the lungs, heart, immune system, kidneys, and endocrine system. SFN exerts its protective effects by activating the Nrf2 pathway, which enhances antioxidant defenses and reduces oxidative stress. It also suppresses inflammation by decreasing interleukin production. Moreover, SFN inhibits apoptosis by preventing caspase 3 cleavage and increasing Bcl2 levels. Overall, SFN demonstrates multifaceted mechanisms to counteract the adverse effects of toxic agents.

Conclusion: SFN has potential clinical applications as a chemoprotective agent. Nevertheless, more studies are necessary to set the safe doses of SFN in humans.

Introduction

Sulforaphane (SFN) is a natural compound within the isothiocyanate group of organosulfur compounds. The chemical formula correspond to 1-isothiocyanate-(4R)-(methylsulfinyl)butane (Fig. 1). SFN has undergone extensive investigation in recent years for its protective efficacy across various *in vivo* pathologies, alongside *in vitro* studies conducted on experimental models. SFN exerts influence on oxidative stress and antioxidant capacity, neuroinflammation, and numerous other biochemical irregularities (Baralic et al., 2024).

SFN is a dietary isothiocyanate derived from glucosinolates which is present in several cruciferous vegetables belonging to the *Brassica* genus. These vegetables include cauliflower, broccoli, kale, cole crops, cabbage, collards, Brussels sprouts, as well as other genera such as radish, mustard, and cress (Fahey et al., 2001). SFN is produced by the action of the enzyme β-thioglucoside glucohydrolase or myrosinase on glucosinolates. However, this enzyme is physically separated from the substrate, so it is necessary for the plant to suffer previous aggression processes to generate the enzymatic hydrolysis and the production of SFN, which is the primary product of the reaction (Shapiro et al., 2001). Consuming a diet rich in fruits and vegetables has been associated with a reduced risk of developing metabolic diseases. However, not all fruits and vegetables exhibit uniform effectiveness in this regard (Padayachee et al., 2017). In particular, the consumption of cruciferous vegetables, such as broccoli, has shown greater potential to mitigate the risk of metabolic disorders, including cancer and diabetes, compared to other vegetables (Latté et al., 2011; Marshall et al., 2023). This positive impact is attributed to the content of glucosinolates, with glucoraphanin (4-methylsulfinylbutyl glucosinolate) being a major component. Young broccoli has been found to contain significantly higher levels of glucosinolates, particularly glucoraphanin, with concentrations 20–50 times higher than those found in mature broccoli (Vanegas et al., 2022).

Under specific reaction conditions, such as pH, temperature, and presence of iron, other reaction products different from glucosinolates, such as thiocyanates and nitriles, can also be generated. These nonenzymatic, intramolecular rearrangements contribute to the formation of the aforementioned additional products (Hayes et al., 2008; Guerrero-Beltrán et al., 2012). Usually, the precursor of SFN is found in broccoli in high concentration: 0.8–21.7 μmol/g of dry weight (Guerrero-Beltrán et al., 2012). The consumption of 200 mg of broccoli can result in approximately 2 μM SFN in the plasma after about 2 h (Gasper et al., 2005). Furthermore, the highest level reported in plasma was 7.3 μM after consumption of 100 g of high glucosinolate broccoli containing 345 μmol SFN and its metabolites (Ye et al., 2002). Although SFN and other isothiocyanates (ITCs) from cruciferous vegetables are recognised for their beneficial effects, it is important to note that moderate intakes, typically within the range of 100 to 200 gs of fresh cruciferous vegetables per day, are considered chemoprotective agents. However, elevated levels of ITCs can potentially induce stress-related cytotoxicity. A study proposed that SFN, when administered at concentrations ranging from 10 to 30 μM, led to the induction of DNA single-strand breaks in cultured human HUVEC cells (Sestili et al., 2010).

SFN is an inducer of nuclear factor erythroid 2-related factor 2 (Nrf2) and produce powerful cytoprotective effects (Dinkova-kostova et al., 2017; Kubo et al., 2017). Moreover, this transcription factor plays a key role in redox homeostasis (Hashimoto, 2018; Yamamoto et al., 2018, Yu and Xiao, 2021). SFN has been shown to induce many health benefits (Juge et al., 2007), including anti-inflammatory properties. It inhibits the production of pro-inflammatory cytokines, such as IL-6, in various human cell lines and *in vivo* models (Kawarazaki et al., 2017; Burnett et al., 2017; Folkard et al., 2014).

The first known chemotherapeutic properties of SFN were anti-proliferative and anticancer. However, nowadays, research on the new protective effects of SFN has been increased. Current research has also focused on the positive impact of SFN on pathologies such as brain, liver, kidney, cardiovascular system, lungs, and muscle among others (Klomparens and Ding, 2019; Aranda-Rivera et al., 2022; Chang R., 2022; Brasil et al., 2023). Due to these reported beneficial effects, SFN has also been used to protect against toxic agents (Guerrero-Beltrán et al., 2012). Humans are exposed to an increasing number and diversity of chemicals from the environment (Sturla and Wang, 2023); therefore, to reveal chemoprotectants such as SFN that could prevent or reverse the potential toxicity induced by toxic agents is of interest. The purpose of the present systematic review is: 1) to comprehensively and rigorously evaluate existing literature on sulforaphane's protective effects and the mechanisms of actions in different organs following the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines; 2) to analyze the risk of bias present in the selected literature; 3) to know the future perspectives and limitations of SFN with regard to its potential therapeutic applications against different toxic agents.
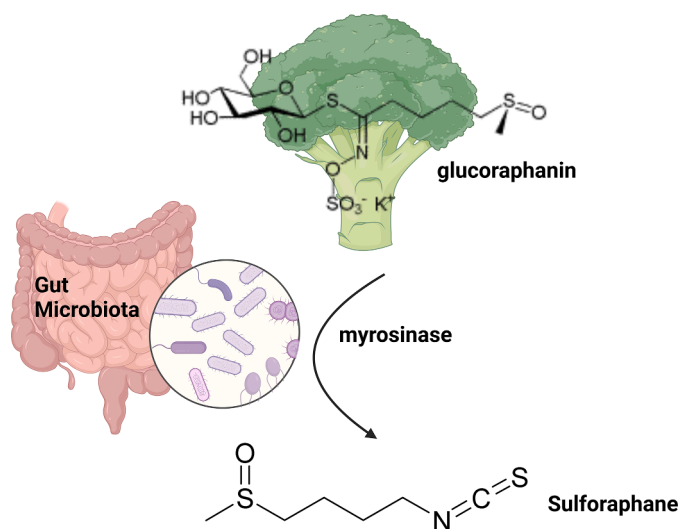
Fig. 1. Metabolism and chemical structure of glucoraphanin and sulforaphane. Created with BioRender.com.

Materials and methods

The present investigation constituted a systematic review conducted in accordance with the Preferred Reporting Items for Systematic Reviews (PRISMA) guidelines, as outlined by Cascajosa-Lira et al. (2022). These guidelines serve as a structured framework for the planning and execution of systematic review studies. Our methodology started with a comprehensive literature search, proceeded with the organization of retrieved documents, abstracting pertinent information, and assessing the quality and bias of each individual study. Subsequently, data synthesis was conducted, culminating in the preparation of a comprehensive report.

Information sources and search strategy

Two electronic research databases, Science Direct (https://www.sciencedirect.com/) and PubMed (https://pubmed.ncbi.nlm.nih.gov/), were searched on April 1, 2023. The keywords strings chosen were: ("Sulforaphane") AND ("protective effects" OR "protection against"). The searches included works published in all languages. The Science Direct option search was 'all fields except full text (NOFT)' and the PubMed option search was 'all fields'.

Study selection. Eligibility and exclusion criteria

Once the exploration had been performed, a three-step process was carried out to review all records according to the eligibility criteria: first,
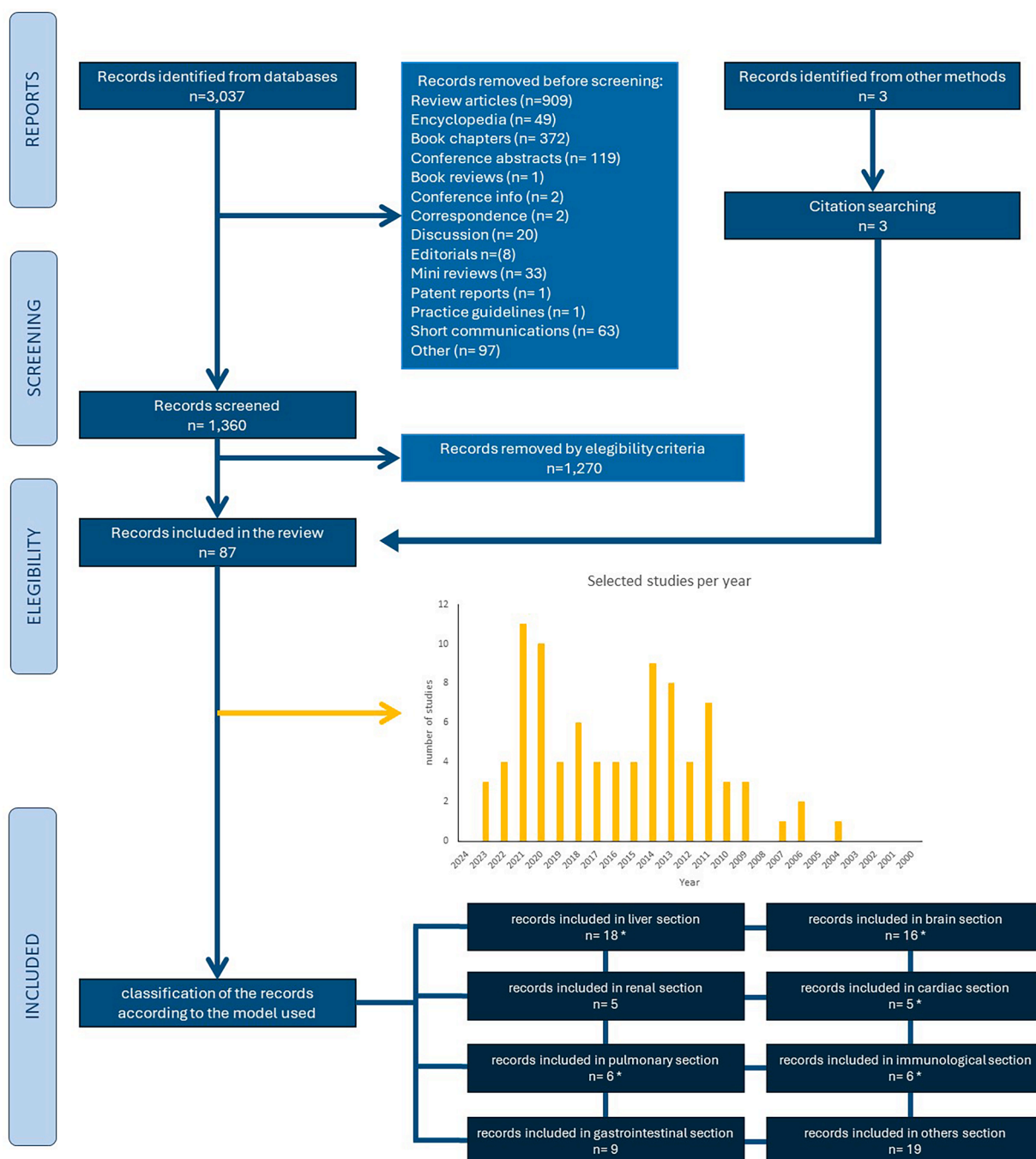
Fig. 2. PRISMA flowchart of studies selection. * These sections share one or more records.

the title was read, second the abstract, and third the entire text of the publication. The works obtained by the two databases were crossed with EndNote X9 software to identify possible duplicates and to classify the works according to the exclusion and inclusion criteria. Conflicts about whether a given reference should be incorporated were determined by agreement of the authors. Also, some of the records include information from two models, so they were classified in both sections. The details of the search method and the classification of records are presented in Fig. 2.

All international studies were considered. The eligibility criteria for inclusion in this work were the following: 1) articles available in English; 2) articles published prior to April 1, 2023 and after January 1, 2000; 3) studies conducted in animal or *in vitro* models; 4) research investigating the effects of SFN as a protective agent against toxicity induced by chemical substances, environmental pollutants, or naturally occurring toxic agents; and 5) studies providing relevant information on the mechanisms of action of SFN in protection against toxicity.

The following exclusion criteria were applied: 1) non-systematic and narrative reviews; 2) articles published in languages other than English; 3) proceedings and dissertations 4) books or book chapters; 5) editorial material; 6) studies not focusing on SFN as a protective agent against toxic agents; 7) studies in which SFN is combined with other compounds without specifically evaluating its protective effect and 8) studies that do not provide relevant information on the protective activity of SFN in relation to toxicity. Criteria and exclusions are visually represented in the PRISMA diagram of the study selection process (Fig. 2).

Data extraction and data items

After a comprehensive reading of each of the articles selected for the review, the following items were established: Toxic substance; Sulforaphane concentration or dose; Experimental model; Exposure condition, and Main results.

Risk of bias

The authors meticulously assessed the risk of bias in selected studies by rigorously evaluating several key criteria. First, they scrutinized whether the studies had a clear objective, ensuring that the research goals were well-defined and articulated. Second, the authors examined whether the product under investigation was adequately characterised, ensuring clarity regarding its composition and properties. Third, they assessed the reproducibility of the assay employed in the studies, verifying whether the experimental procedures could be reliably replicated to yield consistent results. Additionally, the authors evaluated the comparability of the experimental groups, ensuring that any differences observed were attributable to the interventions being studied rather than extraneous factors. Finally, they scrutinised the statistical analyses performed in the studies, ensuring that appropriate statistical methods were employed to accurately interpret the data and draw valid conclusions. This comprehensive approach allowed the authors to thoroughly assess the risk of bias across multiple dimensions of the selected studies. The results of this preliminary assessment are represented in table S1, in which the risks of bias of the selected studies are included. Most of the studies selected presented low risk of bias.

Kinetic and mechanism of action of sulforaphane

Kinetic and distribution of sulforaphane

Sulforaphane kinetic and distribution studies show a wide organ distribution (Veeranki et al., 2013), even crossing the blood-brain barrier (Jazwa et al., 2011). Although the cross-through of the placental barrier has not been studied as far as we know, there is a study that reported the presence of SFN metabolites in the plasma of newborns (Shorey et al., 2013). Therefore, SFN can exert its beneficial effects in multiple organs as represented in the results of the present review.

Mechanism of action of sulforaphane

The main mechanism of action of SFN is as an indirect antioxidant (Fig. 2), and this fact has been outlined in numerous works included in this review (Tables 1-8). SFN initiates the expression of detoxification enzymes through the Nrf2/ Keap1/ARE signaling pathway when exposed to oxidative and/or electrophilic conditions. Moreover, it indirectly influences Nrf2 by promoting its translocation and accumulation in the nucleus, potentially phosphorylating Nrf2 through activation of various kinases, including MAP (mitogen-activated protein kinase), PKB/Akt (protein kinase B) and PKC (protein kinase C). Studies have reported the activation of more than 500 genes by SFN via the Nrf2/ARE signaling pathway (Ruhee et al., 2020). ARE, which acts as a cis-acting enhancer sequence, regulates the basal expression of phase 2 detoxification and antioxidant genes. In addition to its antioxidant activity primarily through Nrf2 activation, SFN demonstrates the ability to mitigate inflammation reducing phase 1 cytochrome P450 enzymes, improves phase II enzymes, and decreases HIF alpha and COX 2, among other enzymes (Zhou et al., 2014). Furthermore, Nrf2 significantly contributes to the inhibition of the nuclear factor-kappa beta (NF-κB) signaling pathway, which is pivotal in the regulation of inflammation (Russo et al., 2018). In relation to carcinogenesis, SFN-induced activation of Nrf2 leads to the upregulation of various cytoprotective genes recognised for their anticarcinogenic effects (Russo et al., 2018). In terms of its antidiabetic properties, SFN has been observed to mitigate insulin resistance through modulation of the PI3K/Akt and JNK/IKK, AMPK/mTOR pathways. Furthermore, it enhances glucose transport via the IRS-1/Akt/GLUT4 and PPAR/GLUT4 pathways, while also improving blood glucose levels through the PPAR/GSK/GS pathway (Wang et al., 2022a).

Hepatoprotective effects of sulforaphane

The hepatoprotective effects of SFN reported in the selected studies are summarised in Table 1. Its efficiency has been demonstrated using both *in vitro* and *in vivo* models. The effects of SFN are mainly mediated by its antioxidant, anticarcinogenic, anti-endoplasmic reticulum (ER) stress and lipid and alcoholic metabolism-regulating activity. According to the findings obtained after the revision, the following whole mechanism of action is proposed (Fig. 3).

The protective action of SFN has been demonstrated *in vitro* in the following cell lines: HepaRG, LX-2, LO2, HepG2, and HHL5, in addition to primary cultures. The concentrations tested ranged from 2 to 100 μM, but the concentration most commonly used to demonstrate a protective effect was 5 μM. These studies have tested SFN against the toxic mechanism of lipopolysaccharides (LPS) (Al-Bakheit et al., 2020; Ishida et al., 2021), medications such as acetaminophen (Noh et al., 2015), plasticisers such as bisphenol-A (Hong et al., 2023), metals such as cadmium (He et al., 2021) or vanadium (Visalli et al., 2017), H_2O_2 (Li et al., 2012; Liu et al., 2019), ethanol (Zhou et al., 2014) and cyanotoxins such as MC-LR (Gan et al., 2010). The mechanism of action of these toxins is mainly to create an inflammatory reaction (LPS and Bisphenol-A) and to promote oxidative stress (H_2O_2, metals, Ethanol, and Acetaminophen). In addition, MC-LR is able to inhibit hepatic phosphatases (PP1 and PP2A), resulting in necrosis and apoptosis.

According to the findings obtained after the revision, the following whole mechanism of action is proposed (Fig. 4). The mechanism of action of SFN is mainly due to its antioxidant activity as an inducer of the Nrf2 factor, which increases the amount of GSH available and thus reduces the oxidative stress that can be caused by toxicants (Gan et al., 2010). Furthermore, some antioxidant genes seem to be up-regulated in the presence of SFN such as HMOX1, NQO1, GSTM3, TrxR-1, and OH-1 (Li et al., 2012; Noh et al., 2015; Ishida et al., 2021). On the other hand, *in vitro* anti-inflammatory activity has been demonstrated by reducing

Table 1
Overview of studies reporting the protective effects of SFN in liver.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Acetaldehyde Lipopolysaccharide (LPS) Ethanol/ CCl 4	0–40 μM 5 μmoL/d/b.w.	Cells were treated with different concentrations of acetaldehyde (0–200 μM) and SFN (0–40 μM) for 12 to 48 h. LX-2 cells were exposed to 5 or 20 μM of SFN and 100 ng / ml of LPS for 12 h. SFN was administered orally as a mixture of diet (5 μmoL/d/b.w.) once a day for 14 days. On the 13th day, mice were fed a 2.5 % liquid diet and received i.p. injections of CCl4 twice weekly (1 ml/kg b.w.)	HepaRG and LX-2 cells, human hepatoma, and liver stellate cell lines. Mouse model of alcoholic liver fibrosis induced by EtOH/CCl 4.	SFN induced ADH activity of ADH in HepaRG cells and suppressed ADH-induced proliferation and profibrogenic activity in LX-2 cells with up-regulation of Nrf2-regulated antioxidant genes (HMOX1, NQO1 and GSTM3). SFN attenuated the LPS/toll-like receptor 4-mediated sensitisation to transforming growth factor- β with down-regulation of NOX1 and NOX4. In EtOH/CCl$_4$ treated mice, SFN significantly inhibited Kupffer cell infiltration and fibrosis, decreased fat accumulation and LPO, and induced Nrf2-regulated antioxidant response genes.	Ishida et al., 2021
Acetaminophen (APAP)	10 μM for hepatocytes. 5 mg/kg pretreatmen in mice	Primary hepatocytes were pretreated with SFN for 6 h, then with APAP (15 mM) and incubated for 14 h. Hepatic injury was induced in mice by injection of 300 mg / kg of APAP after SFN treatment (5 mg/kg) and sacrificed after 6 h.	Primary hepatocytes and also mice	In primary hepatocytes: SFN pretreatment reduced cell death and MDA production after APAP exposure; attenuated intracellular GSH and preserved genes associated with GSH synthesis; increased Nrf2 target genes expression, especially HMOX-1. In the mouse model, SFN pretreatment: lowered AST and ALT levels and inhibited APAP-induced liver histological damage induced by APAP; blocked LPO and ROS production in the liver; regulated Nrf2 target gene expression and inhibited GSH depletion in the liver.	Noh et al., 2015
Arsenic (As)	20, 40, 80 mg/ kg b.w. of SFN	Six groups were established: control, As (5 mg / kg b.w.), SFN (20, 40, 80 mg/ kg b.w.) + As (5 mg/kg b.w.); and Vit. C (100 mg/kg b.w.); + 5 mg / kg b.w.).	Male Wistar rats	The As-induced oxidative damage was confirmed by a significant increase in the levels of δ-aminolevulinic acid dehydratase, and depletion in antioxidant content. As also increased the pro-apoptotic marker (Bax) and DNA damage, with decreased Nrf2 protein responsible for liver protection. SFN provided therapeutic and prophylactic efficacy against As-induced oxidative hepatic damage through its strong antioxidant property. SFN ameliorated As-induced alternations in liver through the activation of Nrf2 by the PI3K / Akt-mediated pathway.	Thangapandiyan et al., 2019
Bisphenol-A (BPA)	100 μM 10 mg/kg b.w.	LO2 cells were treated with 100 nM BPA and 0, 0.5, 1, 10 and 100 μM of SFN for 24 h. Mice were exposed to 10 mg / kg b. w. of SFN, along with 100 μg/kg b. w. of BPA ip for 6 weeks.	LO2 cell line (Human hepatocyte cells) C57/BL6J mice	SFN improves BPA-induced lipid metabolic abnormalities (number of lipid droplets, levels of triglycerides and mRNA expression of lipogenesis-related genes) and stress of the reticulum endoplasmic (ER) in both LO2 cells and in mice.	Hong et al., 2023
Cadmium (Cd)	0–80 μM for 24 h. 0.5, 1 and 2 mg/ kg/b.w. daily for 6 weeks	Mice were orally exposed to 0.5, 1 or 2 mg / kg / b.w. daily of SFN for 6 weeks + 10 mg / kg / b.w. CdCl$_2$ during the last 4 weeks.	HepG2 cells Kunming mice	SFN decreased Cd cytotoxicity in HepG2 cells and induced liver damage in mice (such as haemorrhage in the liver parenchyma, cell degeneration, focal hepatocyte necrosis, hepatocyte disarrangement, and congestion of portal vein congestion) in a dose-dependent manner. SFN (dose of 2 mg / kg / b.w. mainly) restores AST, ALT, TBil, SOD, CAT, GST, GSH, MDA, and T-AOC levels to control values. SFN showed antioxidant and anti-inflammatory effects against Cd-induced liver damage, associated	He et al., 2021

Table 1 (*continued*)

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Cisplatin	500 µg/kg/d for 3 days	Rats were pretreated i.p. with SFN (500 µg for 3 days) and then exposed to cisplatin (single i.p. injection, 10 mg/kg) and sacrificed 3 days later.	Wistar rats	with the modulation of the intrinsic Nrf2/ARE and NF-κB pathway. SFN prevented cisplatin-induced liver damage and attenuates histopathological alterations of the ALT and AST enzymes observed in exposed rats. SFN protected against cisplatin-induced oxidative stress in the rat liver by reversing the increase in LPO and protein carbonyl content and the decrease in GSH and antioxidant enzymes (CAT, SOD, GPx, GR). SFN has a high scavenging capacity for free radicals such as peroxynitrite anion, superoxide anion, singlet oxygen, and peroxyl radicals, and prevented mitochondrial alteration of oxygen uptake and decreased complex I activity.	Gaona-gaona et al., 2011
Cuprizone (CPZ)	2 mg/kg/day for two weeks	Rats were fed a CPZ-contained diet (0.2 %) for four weeks after which they were SFN i.p. administrated (2 mg/kg/day) for two weeks.	Wistar rats	SFN treatment reversed all the biochemical alterations produced by CPZ such as ALT and AST. In addition, SFN produced in decrease a LPO and enhanced T-AOC levels and CAT activities in rats pretreated with CPZ, reducing CPZ-induced oxidative stress in hepatic tissue.	Fouad et al., 2023
D-galactosamine (GalN) and lipopolysaccharide (LPS)	3 mg/kg	The rats were divided into 4 groups: control, SFN treated and 2 groups i.p. injected with GalN (300 mg/kg) and LPS (30 µg/kg), one of them also exposed i.p. to SFN.	Male Wistar rats	SFN provided protection against GalN/LPS-induced fulminant liver failure. This was demonstrated with reduced mortality rates, reduced serum AST and ALT activities, restored SOD, CAT, GPx and Oh-1 activities, and improved pathological liver changes.	Sayed et al., 2014
Ethanol	*In vitro:* 6 µM *In vivo:* 0.05 g/kg	*In vitro*: cells were treated with or without 100 mM ethanol in the absence or presence of SFN. *In vivo*: mice were gavaged twice a day with 30 % ethanol at a dose of 3 g/kg b.w. for 5 days. Some mice were injected i.p. with SFN, at the dose of 0.05 g/kg once a day for 5 days.	*In vitro*: HepG2, E47 cells *In vivo*: SV129 humanised male CYP2E1 knockinknocking mice	*In vitro* SFN elevated Nrf2 levels and decreased lipid accumulation in cells cultured with ethanol. *In vivo*, SFN activated Nrf2, increased levels of Nrf2 target heme oxygenase-1 and decreased oxidant stress as shown by the decrease in LPO and 3-nitrotyrosine protein adducts and increased GSH levels. It decreased the levels of triglycerides and cholesterol, and Oil Red O staining. It proved to be an effective *in vivo* inhibitor of acute ethanol-induced fatty liver in mice. Similarly, *in vitro*, SFN elevated Nrf2 levels and decreased lipid accumulation in cells cultured with ethanol.	Zhou et al., 2014
Ethanol	0.1 ml/g b.w. SFN	Six groups of mice were established: normal control group (A), model control (B), bifendate pill (BDP) intervention group (C,) aerobic exercise intervention group (D), SFN intervention group (E) and group treated with SFN integrated with aerobic exercise (F). Mice in groups A and B were intragastrically administrated with 50 % ethanol for 8 ml/kg b.w. for 12 weeks. From the fifth week, the intervention group C was given 0.1 ml/g b.w. of BDP before ethanol, while the same amount of SFN solution was given to intervention groups E and F; and group B received an equal amount of distilled water. The training time, in groups D and F, was 60 min a day, 5 days a week and 8 weeks in total.	Mice SPF	SFN administration has a stronger treatment effect than aerobic exercise alone, and the combination of SFN and aerobic exercise has the strongest protective effect on acute alcoholic hepatic injury in mice. The SOD activity in liver experienced significant increases and the MDA and triglyceride (TG) experienced significant decreases in the SFN group and the group treated with SFN administration integrated with aerobic exercise. VLDL in the serum of mice also experienced significant decreases in this latter group. Thus, both SFN and aerobic exercise can impose a certain protective effect on acute alcoholic liver injury in mice and help reducing the damage caused by alcohol in liver cells.	Wang and Zhou, 2020

Table 1 (*continued*)

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Hydrogen peroxide (H_2O_2)	2,5–80 μM	Cells were exposed to several concentrations of SFN and / or Selenium (Se) for different times.	HHL-5 cells	SFN and Se synergistically induced TrxR-1 expression in HHL-5 cells. SFN can protect against H_2O_2-induced cell death, and this protection was also synergically enhanced with Se. By using siRNA to kill TrxR-1 or Nrf2, the protection of SFN (5 μM) was reduced. SFN-induced TrxR-1expression was associated with significant levels of Nrf2 translocation into the nucleus.	Li et al., 2012
Hydrogen peroxide (H_2O_2)	5 μM	Cells were exposed to several concentrations of SFN for 24 h and were treated with 700 μM H_2O_2 for another 24 h.	HHL5 and HepG2 cells	Pretreatment with low concentration of SFN ($\leq$ 5 μM) reduced the cytotoxicity induced by H_2O_2; moreover SFN reduced DNA damage caused by H_2O_2 in HepG2 but not in HHL5 cells. The Nrf2 / GSH signaling pathways play a crucial role in the protective effects of SFN against H_2O_2.	Liu et al., 2019
Lipopolysaccharide (LPS)	0.13- 0.39 mg/kg	Mice were treated with LPS (15 mg / kg i.p.) and with SFN at different concentrations (0.13 or 0.26 or 0.39 mg / kg i.v.) 12 h after LPS injection.	Male c57BL/6 mice	SFN decreased the mortality and the lethal liver injury previously increased by LPS at the highest concentration tested. SFN decreased serum levels of ALT and AST. Furthermore, SFN decreased the expression of inflammatory cytokines and the expression of the toll-like receptor 4 (TLR4) protein.	Lee et al., 2020
Lipopolysaccharide (LPS)	2 μM	Cells were treated with SFN for 24 h and subsequently they were exposed to 1 μg/ml LPS for 24 h.	Hep G2 cells	SFN suppressed hepcidin secretion LPS-induced transcription and interleukin-6 (IL-6) reducing the inflammatory responses in these cells without decreasing the cells viability.	Al-Bakheit and Abu-Qatouseh, 2020
Microcystin-LR (MC-LR)	10 μM	HepG2, BRL-3A and NIH 3 T3 cells were treated with 10, 5 or 40 μM MC-LR for 24 h with and without pretreatment with SFN (1–20 μM) for 12 h.	HepG2, BRL-3A and NIH 3 T3 cell lines	SFN protects in a time- and concentration- dependent way against the cytotoxicity caused by MC-LR. SFN produced a significant increase in intracellular GSH levels in HepG2 cells, increased the number of cell colonies, and reduced cell death by apoptosis in all cell lines tested.	Gan et al., 2010
Microcystin-LR (MC-LR)	5 μmol/animal	Mice were injected daily i.p. for 10 days with 40 and 50 MC-LR; with and without SFN (12 h prior to exposure to MC-LR).	Male BALB/c mice	SFN prevents MC-LR-induced hepatotoxicity and animal death in mice. In fact, it can block MC-LR-induced apoptosis. Furthermore, SFN stabilised Nrf2 *in vivo* and inhibited LPO and GSH reduction.	Sun et al., 2011
Olanzapine (OLZ)	90 mg/kg/d	Mice were treated with 8 mg/kg/d of OLZ by osmotic minipump for 4 weeks and SFN (90 mg/kg/d) by gavage starting 1 week prior to administration of OLZ.	C57BL/6 mice	SFN can partially prevent the dysregulated glucose and lipid metabolism produced by OLZ together with a high-fat diet, representing protection against liver injury. SFN increased Nrf2 target gene (NQO1) and decreased OLZ-produced 4-HNE adducts produced by OLZ.	Isaacson et al., 2020
Vanadium (VOSO4)	5 μM	Cells were exposed to VOSO4, SFN, and a mixture of both.	HepG2, Caco-2, and Vero cells	Intracellular ROS, DNA, and lysosomal oxidative damage underlined the indirect antioxidant activity of SFN, confirmed by the increase in GSH. SFN neutralised the metal-induced imbalance of redox homoeostasis	Visalli et al., 2017

4-HNE: 4-Hydroxynonenal; ADH: aldehyde dehydrogenase; Akt: serine/threonine protein kinase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; APAP: Acetaminophen; Bax: bcl-2-like protein 4; BRL-3A: fibroblast-like cell isolated from the liver of a rat; Caco-2: human colon adenocarcinoma; CAT: catalase; galN: D-galactosamine; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GST: glutathione-S-transferase; GSTM3: Glutathione S-transferases mu3; HepaRG: human hepatoma-derived cell line; HepG2: human liver cancer cell line; HMOX1: heme oxygenase-1; IL: interleukin; LPS: bacterial lipopolysaccharide; LPO: lipid peroxidation; LO2 cells: Human hepatocyte cells; LX-2: Hepatic stellate cells; MC-LR: Microcystin-LR; MDA: malondialdehyde; mTOR: mammalian target of rapamycin; NF-κB: Nuclear factor kappa light chain enhancer of activated B cells; NIH 3 T3: embryonic mouse fibroblast cell line; Nrf2: nuclear factor erythroid 2-related factor 2; NOX1: NADPH oxidase 1; NOX4: NADPH oxidase 4; NQO1: NAD(P)H dehydrogenase [quinone] 1; OLZ: olanzapine; ROS: reactive oxygen species; SOD: superoxide dismutase; T-AOC: total antioxidant capacity; Tbil: Total bilirrubine; TLR4: toll-like receptor 4; TNFα: tumour necrosis factor alpha; Vero cells: monkey kidney epithelial cell line.
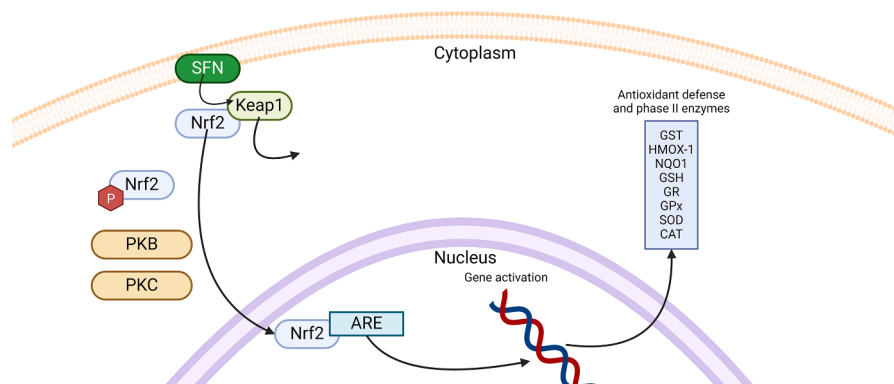
Fig. 3. SFN acts as an indirect antioxidant through the Nrf2/ Keap1/ARE signaling pathway. Created with BioRender.com. CAT – Catalase; GSH – Glutathione; GPx - Glutathione Peroxidase; GR - Glutathione Reductase; GST - Glutathione S-Transferase; HMOX-1 - Heme Oxygenase-1; NQO1 - NAD(P)H Quinone Dehydrogenase 1; SOD - Superoxide Dismutase. Created with BioRender.com.
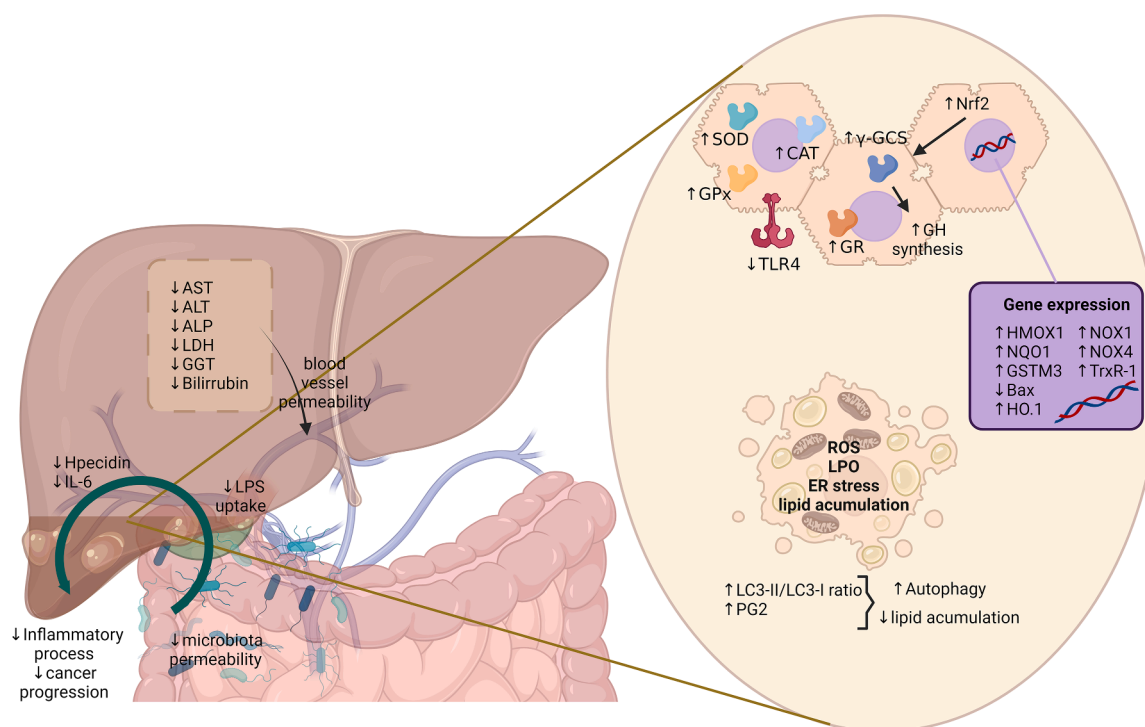
Fig. 4. Mechanism of action of SFN in liver. Created with BioRender.com.

hepcidin and IL-6; consequently, the inflammatory response and cancer progression could be inhibited (Al-Bakheit et al., 2020). Additionally, SFN has been able to repair DNA and lysosomal oxidative damage (Visalli et al., 2017). SFN has been shown to inhibit the catalytic activity in isolated microsomes (oxidation of para nitrophenol) and to block the genotoxicity of nitrosodimethylamine, a substrate for oxidation by CYP2E1. Therefore, Nrf2 activation coupled with possible inhibition of CYP2E1 was believed to make SFN an attractive chemical to blunt the toxic actions associated with CYP2E1 (Zhou et al., 2014) (Fig. 4).

Regarding *in vivo* studies, SFN has been assayed in the following models: Wistar rats (mainly males), SFP mice, C57BL/6 J mice, Kunming mice, BALB/C mice. The doses tested varied widely from 500 μg/kg to 100 mg/kg. These studies have investigated the effects of SFN on countering the toxic actions of d-galactosamine and lipopolysaccharide (LPS) (Sayed et al., 2014; Lee et al., 2019), drugs such as acetaminophen (Noh et al., 2015), cuprizone (Fouad et al., 2023), olanzapine (Isaacson et al., 2020) and cisplatinum (Gaona-Gaona et al., 2011); metalloids

such as arsenic (Thanga-pandiyan et al., 2019), plasticisers such as bisphenol-A (Hong et al., 2023), metals such as cadmiun (he et al., 2021), ethanol (Zhou et al., 2014; Wang and Zhou, 2020), cyanotoxins such as Microcystin-LR (Sun et al., 2011). Similar to the *in vitro* findings, these toxics induced an inflammatory responses accompanied by pro-oxidant conditions *in vivo* models. Furthermore, MC-LR primarily elicits toxic effects in the liver due to the use of specific transporters.

The primary route of administration for SFN was oral, with intraperitoneal (i.p.) injection being the secondary option. *In vivo* research validates the mechanism of action, with its primary attributes being the antioxidant and anti-inflammatory activities, which are responsible for the protective effects. When exposed to certain toxins, cells can become more vulnerable to increased permeability, leading to elevated levels of plasma enzymes from the liver such as alanine aminotransferase (ALT), aspartate aminotransferase (ASP), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), total bilirubin, and gamma-glutamyl transferase (GGT). Furthermore, the intestinal membrane becomes more susceptible

to potential transfer of LPS from the intestinal microbiota to the liver, thereby triggering inflammatory processes. SFN can effectively decrease permeability, restore cell membrane integrity, and decrease the infiltration of enzymes and inflammatory substances. On the other hand, SFN could restore antioxidant enzyme levels when they are affected by toxics, primarily mediated by the increase in Nrf2 factor. Regarding histopathological studies, SFN-pretreated animals exposed to Cd show markedly attenuated abnormalities such as vacuolization, inflammatory cell infiltration, sinusoidal dilatation, and distinct damage of cytoplasmic organelles (He et al., 2021).

Recently, Hong et al. (2023) reported that SFN demonstrated a mitigating effect on lipid metabolism disorder and stress of the ER induced by BPA in both *in vitro* and *in vivo* models. *In vitro* findings suggested that SFN reduced lipid accumulation in hepatocytes and lowered the levels of crucial lipogenic enzymes by inhibiting the ER pathways. However, *in vivo*, SFN did not cause a reduction in the liver/body weight ratio in animals treated with BPA. New research in order to elucidate the role of ER stress *in vivo* is needed.

Neuroprotective effects of sulforaphane

The main neuroprotective effects of SFN reported in the selected literature are reviewed in Table 2. Its efficacy has been demonstrated using both *in vitro* and *in vivo* models, *in vitro* models being the most common. The effects of SFN are mainly related with the anti-inflammatory and antioxidant activity that prevents neuronal degeneration.

Studies carried out *in vitro* have been performed by SFN exposure to several cell lines and primary cultures from different parts of the nervous system: SH-SY5Y (Lee et al., 2014; Zhou et al., 2016; Brasil et al., 2023), neural crest cells (Li et al., 2019), BV-2 cells (Konwinski et al., 2014; Wang et al., 2020; Yang et al., 2023), N2A cells (Kwak et al., 2007), primary microglial and cortical neurons (Yang et al., 2023), or primary astrocytes (Bergstrom et al., 2011). The *in vitro* protective effects of SFN are investigated against proteins such as amyloid beta (Yang et al., 2023) or prion (Lee et al., 2014), insecticides such as chlorpyrifos (Brasil et al., 2023) or rotenone (Zhou et al., 2016), herbicides such as paraquat (Mizumo et al., 2011), chemicals such as H_2O_2 (Konwinski et al., 2004; Kwak et al., 2007; Bergmenton et al., 2011; Mizuno et al., 2011) and Lipopolysaccharide (Holloway et al., 2016; Wang et al., 2020). The majority of neurotoxic agents under investigation act as acetylcholinesterase (AchE) inhibitors, encompassing insecticides and herbicides. Additionally, research has also focused on toxics implicated in cognitive dysfunction, such as amyloid beta and prions.

There is only one study using an ex vivo model, with organotypic cultured rat hippocampal tissue from rats exposed to scopolamine (SCOP) and SFN (Park et al., 2021). In this study, the authors showed that SFN exhibited a concentration-dependent increase in overall fEPSP (field excitatory postsynaptic potential) after high-frequency stimulation and mitigated the interference of SCOP-induced fEPSP in the CA1 area of the hippocampus. Furthermore, SFN prevented the long-term potentiation (LTP) and cognitive abilities induced by cholinergic and muscarinic receptor blockade. These findings indicate that SFN mitigates the decline induced by SCOP in short-term working memory, long-term spatial memory, and avoidance memory in rats. These effects are correlated with the induction of brain-derived neurotrophic factor (BDNF) and cAMP response element binding protein (CREB) expression in the hippocampus, along with the enhancement of synaptic activity. Therefore, SFN merits further investigation as a potential agent for preventing and treating Alzheimer's disease (AD) or disorders related to learning and memory deficits in individuals affected by neurodegenerative disorders (Park et al., 2021) (Fig. 5).

In terms of *in vivo* studies, only three different models have been used: C57BL/6 mice, Wistar and Sprague-Daley rats. The *in vivo* protective effects of SFN were investigated against 6-hydroxydopamine (Morroni et al., 2013), cis-platinum (Fouad et al., 2022), ethanol (Li

et al., 2019; Xu et al., 2020), drugs such as pilcarpine (Folbergrová et al., 2023), and lipopolysaccharide (LPO) (Holloway et al., 2016; Wang et al., 2020). Similar to what has been studied *in vitro*, toxic agents studied *in vivo* also caused mainly cognitive and coordination dysfunctions.

The protective effect of SFN *in vivo* has been shown to have 3 main pathways: 1) by antioxidant effects, 2) by anti-inflammatory effects, and 3) by improving motor coordination and reflexes. SFN exhibited a multifaceted protective role in various contexts. In the context of neurodegenerative oxidative damage to the basal ganglia, SFN restored nigral GSH levels and enhanced GST and GR, thus increasing the antioxidant potential (Morroni et al., 2023). Furthermore, SFN inhibited ROS production and MDA accumulation in mice with rotenone-induced dopaminergic neural loss (Zhou et al., 2016). Consequently, SFN could serve as a prevention of neurodegenerative processes such as Parkinson's disease and improve locomotor activity. Furthermore, SFN exerted anti-inflammatory effects on LPS by suppressing NFκB signaling, as reported by Wang et al. (2020). Additionally, SFN demonstrated its efficacy in mitigating alcohol-induced loss of righting reflex (LORR) duration without affecting latency, as observed in the study by Xu et al. (2020). Taking into account the *in vitro* and *in vivo* information reported, the entire mechanism of action is proposed in Fig. 5.

Lastly, SFN is involved in the prevention of adverse effects caused by cis-platinum. SFN increased in AchE activity and restored redox status by regulating LPO, NO, and GSH levels (Fouad et al., 2022) after exposure to pilocarpine and LiCl. The protective effects of SFN extended to the hippocampal and the dentate gyrus, where it prevented damage induced by oxidative stress (Folbergrová et al., 2023).

Nephroprotective effects of sulforaphane

Table 3 includes the nephroprotective effects of SFN found in the scientific literature, in both *in vitro* and *in vivo* models. The anti-inflammatory and antioxidant activities of SFN may be responsible of its efficient prevention of renal injury. In relation to this protective effect, more *in vivo* studies have been found than *in vitro* ones, in contrast to what has been found in the previous tissues. Only one *in vitro* study has been found that attributes protective effects of SFN using LLC-PK1 cells against the toxic effects of cis-platinum by restoring mitochondrial membrane potential (Guerrero-Beltrán et al., 2010). The rest of the studies have been carried out in Wistar rats, mainly testing protective effects against metals and their compounds: cadmium (Li et al., 2015), mercury (Guo, 2016), cis-platinum (Guerrero-Beltrán et al., 2010; 2012) and calcium oxalate (Liu et al., 2012). The toxic effects induced by these metals at the renal level caused mainly renal failure, glomerulopathies, and tubulopathies. All the studies highlight a common mechanism of action: SFN restores the levels of urinary blood urea nitrogen (BUN) and restores the enzymatic activities of NAG, LDH, ALP, SOD, and GSH-Px. Additionally, because of its antioxidant effects, it is able to decrease the high levels of MDA produced by toxic substances.

Cardioprotective effects of sulforaphane

Table 4 presents a synthesis of the cardioprotective effects of SFN, including evidence derived from both *in vitro* and *in vivo* models. Table 4 consolidates the key findings from the selected studies to provide a comprehensive overview of SFN's impact on cardiovascular health. These effects are mainly ascribed to the antioxidant activity of SFN, demonstrating its efficacy in preventing damage to the heart and aorta.

There is only one *in vitro* study using primary cultured cells from rat. A mechanism of action against H_2O_2-produced oxidative stress has been demonstrated in which the presence of antioxidant enzymes is increased when cells are treated with SFN and estradiol (E2) (Angeloni et al., 2017). All the *in vivo* studies have been conducted in C57BL/6 J mice. The toxic substances assayed were as follows: cuprizone (CPZ), methamphetamine (MA) and angiotensin II (Ang II) to induce hypertension.

Table 2

Overview of studies reporting the protective effects of SFN in neuronal.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
6-hydroxydopamine (6-OHDA)	5 mg/kg	4 groups of mice: 2 received a 6-OHDA injection in the left striatum; the other 2 received saline solution. After brain lesions, SFN was administered (5 mg/kg) or vehicle in both lesioned and sham mice. Injections were made twice a week for 4 weeks. The groups are: 6-OHDA/VH; 6-OHDA/SFN; Sham/VH; Sham/SFN.	Male C57BL/6 J mice	6-OHDA-induced rotations and deficits in motor coordination that were ameliorated by SFN. In addition, SFN protected 6-OHDA-induced apoptosis by blocking DNA fragmentation and caspase-3 activation. These results were further supported by immunohistochemical findings in the substantia nigra that showed that SFN protected neurons from neurotoxic effects of 6-OHDA. SFN increased the GSH content in mice with 6-OHDA lesion compared to 6-OHDA/VH mice, and GST (41 %) and GR (69 %) activities. SFN treatment could dramatically down-regulate ERK1 / 2 phosphorylation induced by 6-OHDA.	Morroni et al., 2013
Amyloid-beta (Aβ) and Aβ$_{25-35}$ fibrils	1 or 10 μM	Primary cells: treatment with different concentrations of either Aβ, or 50 μM Aβ together with 1 μM SFN for 3, 6, 12, or 24 h. BV-2 cells: 50 μM Aβ with & without 10 μM SFN for 30 h.	Primary microglia and primary cortical neurones from 1-day-old C57BL/6 J mice. Mouse microglial cell line (BV-2)	SFN indirectly attenuated microglia-mediated neurotoxicity. SFN inhibited the activation of cytostatic autophagy and the NLRP3 inflammasome, as well as the production of pro-inflammatory cytokines and the polarisation of M1. This is mainly related to the decrease in intracellular ROS in Aβ-activated microglia.	Yang et al., 2023
Chlorpyrifos (CPF)	0.5 – 5 μM	SFN 6 h before exposure to 100 μM chlorpyrifos for 3 or 24 h	SH-SY5Y cells	SFN prevented CPF-induced mitochondrial dysfunction and redox impairment. Anti-inflammatory effects stabilising IL-1β levels. HMOX-1 mediates mitochondrial protection.	Brasil et al., 2023
Cis-platinum	2 mg/kg	The SFN was intranasally administered for 5 days followed by a single i.p. dose of 12 mg/kg/week of Cis-platinum on the sixth day, followed by 3 successive days of intranasally administration of SFN.	Wistar rats	The increase in AchE activity caused by Cis-platinum was inhibited by SFN which decreased LPO, NO, and GSH in the brain. Regressing lesions were found in the cerebral cortex of rats in which only sporadic necrosis of neurons was found.	Fouad et al., 2022
Ethanol	1 μM	NCCs were pretreated with or without 1 μM SFN for 24 h, followed by concurrent exposure to 1 μM SFN and 50 or 100 mM ethanol.	Neural crest cells (NCCs)	SFN significantly reduced ethanol-induced apoptosis in NCC cells. SFN also decreased ethanol-induced changes in the expression of E-cadherin and vimentin, and restored EMT in NCCs exposed NCCs. SFN decreased ethanol-induced reduction of H3K4me3 in the promoter regions of the Snail1 gene, restored the expression of Snail1 and down-regulated Snail1 gene E-cadherin.	Li et al., 2019
Ethanol	5 mg/kg	SFN was i.p. administered (5 mg / kg / day, 5 consecutive days), and then 20 % EtOH v / v (1.75 g/kg for ataxia, 3 g/kg for hypothermia and 4.0 g/kg for LORR) was injected i.p.	Male C57BL/6 J mice	SFN significantly shortened the duration of alcohol-induced LORR in mice but did not affect the latency. It reduced the alcohol-induced temperature change, while it did not affect the basal core temperature of the mice. In the ataxia test, SFN increased the latency of falling from the rotarod dowel after alcohol injection. However, it did not affect the basal coordination of the mice. SFN reversed CUS-potentiated alcohol-induced hypothermia and rescued the reduced alcohol induced ataxia. It increased CAT activity in the hippocampus of CUS mice.	Xu et al., 2020
Hydrogen peroxide (H$_2$O$_2$)	1- 10 μM	SFN: briefly (1–4 h), constantly (24 h) or repeatedly (4 h daily, up to 4 days) and 50–75 μM H$_2$O$_2$: (30 min-5 h)	Primary astrocyte cultures from Sprague-Dawley rats	Elevate levels of GSH. Prolonged induction of NQO1. Attenuation of Nrf2-dependent HMOX1 induction. NQO1 accumulation and continuous induction of GSH after daily SFN stimulations, resulting in prolonged protection against superoxides.	Bergstrom et al., 2011
Hydrogen peroxide (H$_2$O$_2$)	5 μM	Cells were treated with SFN and other compounds (OPZ, D3T) in	Murine BV-2 microgial cells	Induction of the expression of antioxidant detoxification proteins by SFN (and other	Konwinski et al., 2004

Table 2 (*continued*)

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Hydrogen peroxide (H_2O_2)	Up to 2.50 μM	DMSO for 24 h. Cells were treated with H_2O_2 (400 μM, 4 h. Furthermore, cells were treated with SF, OPZ or D3T 5 μM and LPS (80 ng/ml, 24 h) Cells were treated with SFN for 18 h-20 h followed by graded concentrations of H_2O_2 for additional 18 h.	Neuro2A murine neuroblastoma cells	compounds, OPZ, D3T) was correlated with increased resistance of cells to H_2O_2-induced toxicity, as well as elimination of intracellular ROS in response to LPS treatment, during microgial cell activation. SFN enhanced the 26 proteasome expression of the catalytic subunits, as well as proteasomal peptidase activities in the cells. SFN protected cells against H_2O_2-mediated cytotoxicity in a manner dependent on proteosomal function. Inhibition of proteasome activities decreased the protective effects of SFN, as well as protein oxidation.	Kwak et al., 2007
Hydrogen peroxide (H_2O_2) and paraquat	0–10 μM	Cultures were incubated in the presence or absence of SFN, 6-HITC, or BSO for 24 h. Cultures were exposed to H_2O_2 or paraquat for 24 h, and then cell viability was determined by LDH release assay.	Primary neuronal cultures of rat striatum	Pretreatment with SFN and 6-HITC protected against H_2O_2- and paraquat-induced cytotoxicity in a concentration-dependent manner. SFN and 6-HITC induced Nrf2 translocation into the nucleus and increased the expression of γ-GCS, a rate-limiting enzyme in GSH synthesis and content. Exposure to BSO, an irreversible inhibitor of γ-GCS, suppressed the protective effects of both compounds. On the contrary, SFN and 6-HITC increased the expression of heme oxygenase-1 (HO-1) in neurons.	Mizuno et al., 2011
LiCl + pilocarpine	5 mg/kg	SFN was administered in two doses, 48 and 24 h before the administration of 127 mg/kg of LiCl i.p. 35 mg/kg of Pilocarpine was administered i.p. 24 h later.	Wistar rats	Pretreatment with SFN did not change latency, character, duration, or severity of seizures and mortality. Complete prevention of the increase in hippocampal fields and the dentate gyrus was found.	Folbergrová et al., 2023
Lipopolysaccharide (*Escherichia coli* serotype 0111:B4)	5 and 50 mg / kg i.p.	Mice were exposed to 5 mg/kg and 50 mg/kg of SFN dissolved in corn oil by injection 24 h prior to exposure to 0.5 mg/kg of LPS.	Wild-type (WT) C57BL/6 J mice and Nfe2l2tm1Ywk knockout (Nrf2-KO) mice.	SFN may reduce cerebral inflammation in sepsis produced by LPS by mediating its anti-inflammatory and antioxidant effects through Keap1/Nrf2 transcriptional activation of the antioxidant system and via the NFκB pathway.	Holloway et al., 2016
Lipopolysaccharide (LPS)	*In vitro* 0–40 μM SFN *In vivo* 1 mg/kg/day SFN	*In vitro* Cells were pre-incubated with SFN or 1 % DMSO for 1 h followed by post-incubation with LPS for 24 h. *In vivo* Three groups: control group, LPS (1 mg/kg/day LPS i.p.) group; and LPS +SFN group (1 mg/kg/day SFN 30 min before injection of LPS). All treatments were held for 5 days.	*In vitro* BV2 cells *In vivo* SPF mice	*In vitro* The modulation of TRAF6 and RIPK1 ubiquitination by Cezanne played a key role in the mechanism of SFN inhibiting NF-κB pathway. *In vivo* SFN improved LPS-induced neurocognitive dysfunction in rats. It inhibited neuroinflammation by suppressing NF-κB signalling activation induced by LPS.	Wang et al., 2020
Prion protein (PrP)	1–10 μM	Cells were treated with several concentrations of SFN and PrP (106–126 sequence) for up to 36 h.	SH-SY5Y cells	SFN prevented prion-mediated cell apoptosis by decreasing the previous increase in PrP-positive annexin V cells by PrP, and by decreasing LDH release levels. The TUNEL assay also revealed that SFN inhibited PrP -induced apoptosis. SFN induced autophagy, decreasing PeP-induced neurotoxicity. ATG% eliminates the blocked neuroprotective effects of SFN. Activation of the AMPK pathway regulated the neuroprotective effects of SFN-induced autophagy.	Lee et al., 2014
Rotenone	*In vitro:* 10 μM *In vivo:* 50 mg/kg	*In vitro*: cells were treated with SFN for 2 h and then exposed to rotenone (0.5 and 1 μM) for 24 h. *In vivo*: control group; SFN group injected i.p. every other day; Rotenone group 30 mg/kg orally & daily for 60 days and Rotenone+SFN group in a similar way	*In vitro*: SH-SY5Y *In vivo*: Male C57BL/6 J mice	SFN exerted a neuroprotective effect that involved Nrf2-dependent reductions in oxidative stress, mTOR-dependent inhibition of neuronal apoptosis, and restoration of normal autophagy. Thus, SFN inhibited locomotor activity deficiency and dopaminergic neuronal loss, inhibited ROS production, MDA accumulation, and increased GSH levels. It also increased the expression of Nrf2, HO-1 and NQO1. It inhibited the mTOR-mediated signalling pathway of p70S6K	Zhou et al., 2016

Table 2 (*continued*)

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Scopolamine (SCOP)	*Ex vivo*: 1, 10, and 100 μM, *In vivo*: 15 mg/kg.	- The hippocampal slices were treated with SFN (1, 10, and 100 μM), SCOP (300 μM) or SFN + SCOP [SFN (10 μM) + SCOP (300 μM)], dissolved in artificial cerebrospinal fluid (aCSF), 10 min after recording. - After 5 days of adaptation, rats were treated with SFN (15 mg / kg, ip for 14 days. Treatment with SCOP (1.5 mg / kg, i.p.m) was started on the 11th day and continued for 9 days, and behavioural tests were performed from the 11th day to the 18th day. On day 19, rats were sacrificed, and hippocampi were removed for molecular analysis.	Organotypic Cultured rat hippocampal tissues (OHSCs) from rats (*ex vivo*) and *in vivo* rats	and 4E-BP1. SFN might have a role in preventing Parkinson's disease. SFN increased total fEPSP in a dose-dependent manner after high-frequency stimulation and attenuated SCOP-induced interference of the fEPSP in the CA1 area. SFN also restored cognitive function and inhibited memory impairment as indicated by the alleviation of the negative neurological effects of SCOP, that is, a lower ratio of spontaneous alternation in the Y-maze, a reduced latency in the passive avoidance test, and an increased navigation time in the Morris water maze.	Park et al., 2021

4E-BP1: Eukaryotic translation initiation factor 4E binding protein 1; 6-OHDA: 6hydroxydopamine; 6-HITC: 6-(methylsulifnyl) hexyl isotiocyanate; Ache: acetyl-cholinesterase; AMPK: protein kinase activated with adenosine 5′-monophosphate (AMP)-activated protein kinase; BSO: l-buthionine-sulfoximine; CPF: Chlorpyrifos; D3T: 3-H-1,2.dithiole-3-thione; EMT: epithelial-mesenchymal transition; ERK1/2: extracellular signal-regulated kinases 1 and 2; IL: interleukin;; fEPSP: total field excitatory postsynaptic potential; GR: glutathione reductase; GSH: glutathione; GSK-3b: glycogen synthase kinase 3 beta; GST: glutathione-S-transferase; H3k4me3: Trimethylation of histone H3 lysine 4; HMOX-1: heme oxygenase 1 gene; LDH: Lactate dehydrogenase; LPS: bacterial lipopolysaccharide; LPO: lipid peroxidation; LORR: Loss of the righting reflex; NCCs: Neural Crest Cells; NLRP3: pyrin domain-containing 3; NF-kB: nuclear factor kappa-light-chain enhancer of activated B cells; NQO1: NAD(P)H dehydrogenase [quinone] 1; NO: nitric oxide; MDA: malondialdehyde; mTOR: mammalian target of rapamycin; OPZ: Oltipraz; ROS: reactive oxygen species; SCOP: scopolamine; SH-SY5Y: neuroblastoma cell line; SNAI1: snail family transcriptional repressor 1; TRAF6: Tumour necrosis factor receptor associated factor 6; RIPK1: Receptor-interacting serine / threonine protein kinase 1; P70S6K: Ribosomal protein S6 kinase beta-1 protein; VCAM-1: Vascular cell adhesion protein 1; γ-GCL: γ Glutamate–cysteine ligase.
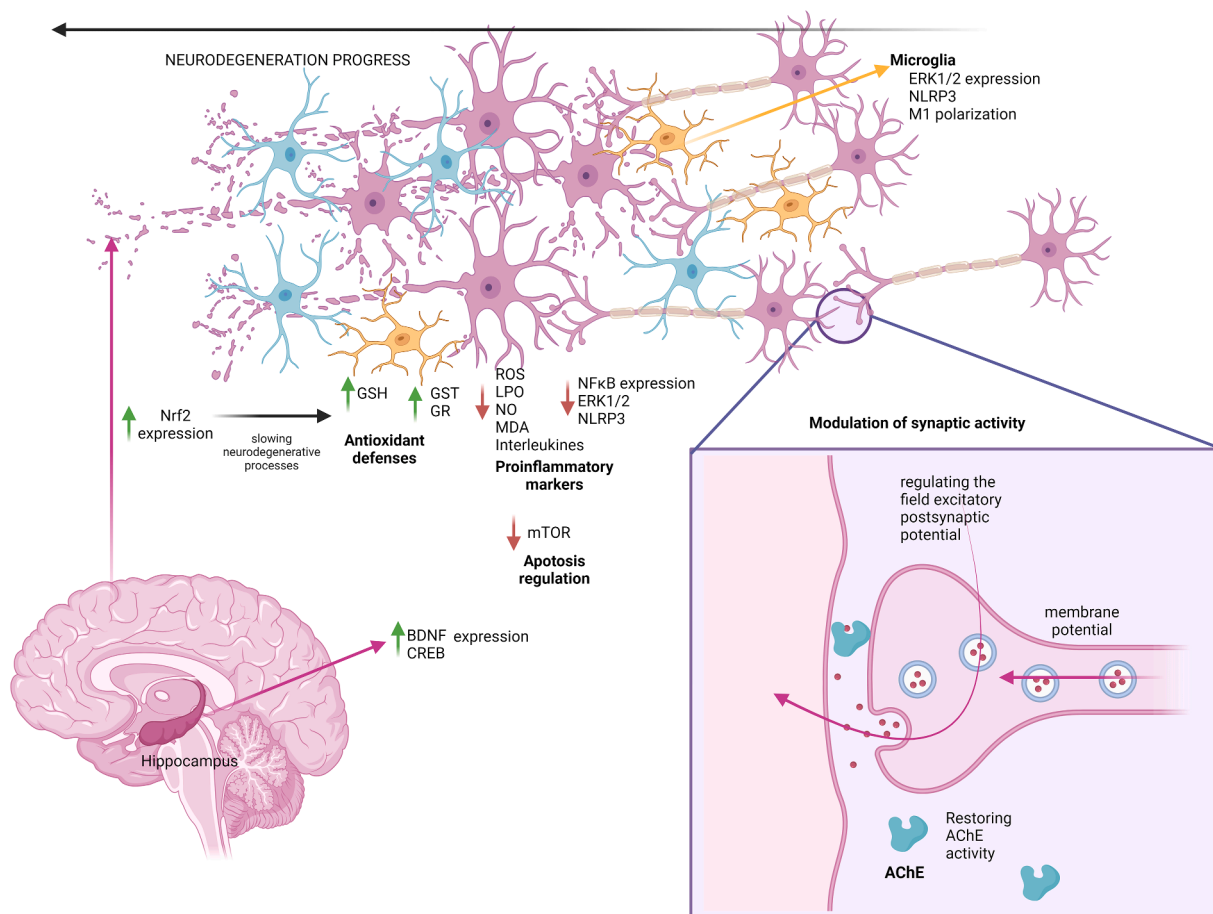

Fig. 5. Mechanism of action of the protective effects of SFN in the nervous system. Created with BioRender.com.

Table 3

Overview of studies reporting the protective effects of SFN on kidney.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Cadmium chloride (CdCl$_2$)	1 mg/kg	Rats were treated with 1 mg/kg SFN i.p. three times a week. Subsequently, two hours later, three groups received i.p. injections of CdCl$_2$ at doses of 3 µmol/kg, 6 µmol/kg, and 9 µmol/kg, respectively, five times a week.	Wistar rats	The high dose of cadmium exhibited significant increases in urinary protein and BUN levels, along with elevated NAG, LDH, and ALP in urine, as well as higher levels of MDA in the renal cortex. At the same time, there was a notable decrease in the content of GSH and the activities of SOD and GSH-Px in the renal cortex. However, in rats pretreated with SFN, these changes showed significant improvement.	Li, 2015
Calcium oxalate	0.2 mg/kg	Rats with kidney stone model were injected with 0.2 mg/kg of SFN.	Wistar rats	Urine levels of oxalic acid, Ca2+, and MDA were significantly lower compared to the model group; consequently, the formation of calcium oxalate kidney stones was inhibited.	Liu et al., 2020
Cisplatin	0.5–5 µM for 24 h 500 µg/kg	Cells were treated with SFN (0–5 µM) for 24 h and then 40 µM of cisplatin was added for another 24 h. Rats were exposed to a single dose of cisplatin by injection (7.5 mg/kg). SFN (500 µg/kg i.v.) was administered twice: 24 h before exposure and also 24 h after exposure to toxicants and the animals were killed three days after injection of cisplatin.	LLC-PK1 cells Wistar rats	Protection against mitochondrial alterations produced by cisplatin in both LLC-PK1 cells (loss of membrane potential) and isolated mitochondria (inhibition of mitochondrial calcium uptake, cytochrome c release of cytochrome c, and decrease in GSH content, aconitase activity, adenosine triphosphate (ATP) content, and oxygen consumption). Prevention of cisplatin-induced increase in ROS production and decrease in NQO1 and γ-GCL activities.	Guerrero-Beltrán et al., 2010
Cisplatin	500 µg/kg	Rats were exposed to a single dose of cisplatin by injection (7.5 mg/kg). SFN (500 µg/kg i.v.) was administered twice: 24 h before exposure and also 24 h after exposure to toxicants and the animals were killed three days after injection of cisplatin.	Wistar rats	SFN prevented cisplatin-induced renal injury by modulating the activation of various cell death and pro-inflammatory pathways (p53, JNK, p38-α, TNF-α and NF-κB) and impairments of key pro-survival signaling mechanisms (ERK and p38-β).	Guerrero-Beltrán et al., 2012
Mercury chloride (HgCl$_2$)	2 mg/kg	Rats received 2.2, 4.4 and 8.8 mg/kg of HgCl$_2$ and 2 mg/kg of SFN in the intervention groups.	Wistar rats	Urinary protein and BUN levels, along with kidney cortical GSH and MDA levels, were notably higher compared to the SFN intervention group. On the contrary, the SFN group exhibited significant increases in urinary LDH, ALP, NAG, as well as kidney cortical SOD and GSH-Px activities.	Guo, 2016

ALT: alanine aminotransferase; GPx: glutathione peroxidase; GSH: glutathione; JNK: Mitogen-Activated Pro-tein Kinases; BUN: Blood Ureic Nitrogen; ERK1/2: extracellular signal-regulated kinases 1 and 2; LDH: lactate dehydro-genase; LLC-PK1: Lilly Laboratories Culture-Porcine Kidney 1; NAG: N-acetylglutamate synthase; NF-kB: nuclear factor of activated B cells; NQO1: NAD(P)H dehydrogenase [quinone] 1; p38: tumour protein; p53: tumour protein; SOD: Superoxide dismutase; TNFα: tumour necrosis factor alpha; γ-GCL: γ Glutamate–cysteine ligase.

In these studies, as in the in *vitro* studies, there is an increase in the activity of antioxidant defenses mediated mainly by the activation of Nrf2 factor (Xin et al., 2018; Wang et al., 2022b; Fouad, 2023; Yu et al., 2023).

Pulmonary protection of sulforaphane

Table 5 provides a summary of the main findings regarding the pulmonary protective effects of SFN, encompassing the most significant results from the selected studies. Again, the effects are predominantly attributed to the antioxidant and anti-inflammatory activities of SFN, showing its effectiveness in preventing pulmonary system injuries.

In vitro studies have employed only two types of experimental models: bronchial epithelial cell lines (Wang et al., 2018; Gasparello et al., 2021; Quin et al., 2021) and alveolar epithelial cells (Lv et al., 2020). However, there is a variety of substances tested *in vitro*: proteins such as the SARS-COV-2 protein (Gasparello et al., 2021), metals such as cadmium (Wang et al., 2018), potassium dichromate (Lv et al., 2020) and particulate matter (PM.5) (Quin et al., 2021). On the other hand, *in vivo* studies in rat and female mice (CD1 strain) have been applied against the detrimental effects of potassium dichromate and benzo(a) pyrene, respectively. Most of these toxins induce an inflammatory state and cause oxidative stress. The antioxidant efficacy of SFN has been demonstrated by its ability to increase Nrf2 and HO-1 (Wang et al.,

2018), to decrease MDA and ROS (Quin et al., 2021) and therefore increase the capacity of antioxidant enzymes (Kalpana Deepa Priya et al., 2011). Furthermore, the anti-inflammatory effect has been demonstrated by decreasing the expression of proinflammatory interleukins (IL-6 and IL-8) and other markers (Lv et al., 2020; Gasparello et al., 2021).

Gastrointestinal protection induced by sulforaphane

Table 6 provides a detailed summary of the findings, highlighting the significant protective effects of SFN on the gastrointestinal system as evidenced by the selected *in vivo* studies. In these studies, Sprague Dawley and Wistar rats have been chosen as experimental models, together with mice of the C57BL/6 J strain and Syrian hamsters. In this type of studies, the following substances have been used to induce models of gastrointestinal disease in animals: acetic acid (Alattar et al., 2022) or sodium dextran sodium sulfate (DSS) (Wagner et al., 2013; Wu et al., 2023; Holman et al., 2023; Zhang et al., 2023) to produce intestinal colitis, acetylsalicylic acid (ASA) (Zeren et al., 2016) to produce gastric ulcers and N-nitroso-bis(2-oxopropyl) amine (BOP) (Kuroiwa et al., 2006) to produce pancreatic cancer.

SFN has exerted a protective effect against all these compounds and in all disease models assayed. The effects of SFN in colitis are mainly due to its anti-inflammatory and protective action on the intestinal

Table 4
Overview of studies reporting the protective effects of SFN on heart.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Angiotensin II (Ang II)	0.5 mg / kg b.w. SFN	Four groups: control, SFN, Ang II, and Ang II + SFN (Ang II/SFN). Mice were treated subcutaneously with Ang II (0.5 mg/kg b. w.) or an equivalent volume of 0.9% physiological stroke saline solution every other day for 2 months, with or without SFN treatment 5 days a week for 3 months.	Wild-type (WT) C57BL/6 J and Nrf2-knockout (Nrf2-KO) mice	SFN treatment prevented aortic damage through Nrf2 activation in WT mice. However, the protective effect of SFN on Ang II-induced aortic damage and up-regulation of genes downstream of Nrf2 was not observed in Nrf2-KO mice. SFN induced up-regulation of aortic Nrf2 and inhibited the accumulation of ERK, GSK-3β, and Fyn in the nuclei.	Wang et al., 2022b
Angiotensin II (Ang II)	5 mg/kg	Exp1. Mice were injected with 0.5 mg / kg of Ang II every other day for 2 months, with / without injection of SFN 5 days a week for 3 months. In the end some mice were euthanized and others kept for additional 3 months without SFN. Exp2. Nrf2-KO and WT in a similar way but suppressing the last 3 months of treatment. Exp3. Ang II or vehicle for 2 months and then 1 month without treatment.	Male C57BL/6 J mice: Nrf2-TG, Nrf2-KO and wild type	SFN can prevent Ang II-induced cardiomyopathy by activating Nrf2-mediated exogenous antioxidant defences, and that up-regulation and activation of Nrf2 by SFN are achieved partially through the Akt/GSK-3β/Fyn pathway.	Xin et al., 2018
Cuprizone (CPZ)	2 mg/kg/day for two weeks	Rats were fed a CPZ-contained diet (0.2 %) for four weeks after which they were SFN i.p. administrated (2 mg/kg/day) for two weeks.	Wistar rats	SFN resulted in a reduction in LPO and an enhancement of total antioxidant capacity levels and CAT activities in rats pretreated with CPZ, thus alleviating CPZ-induced oxidative stress in cardiac tissues.	Fouad, 2023
Hydrogen peroxide H_2O_2	0.1–0.5 μM	SFN for 24 in the absence or presence of physiological concentrations of E2 (10–50 nM) and 100 μM H_2O_2 for 30 min.	Primary cardiomyocytes cultures from Sprague–Dawley rats	The protective effect of cotreatment is not mediated by ER. Cotreatment of SFN and E2 synergistically up-regulated phase II enzymes and activated pro-survival signaling pathways. The simultaneous presence of ERK1/2 and Akt inhibitors leads to a significant reduction in cardiomyocyte viability with respect to SFN + E2-treated cells before H2O2-induced damage. SFN reduced LDH release.	Angeloni et al., 2017
Methamphetamine (MA)	10 mg/kg	Mice received SFN i.p. once a day or SFN + 2 mg/kg MA i.p. twice a day for 5 days.	Adult male C57BL/6 J mice	Nrf2 attenuated MA-induced myocardial injury by regulating oxidative stress and apoptosis, thus playing a protective role. In particular, MA effects (MDA, SOD, protein expression levels of Caspase-3 and Bax, protein expression levels of Bcl-2) were reversed by Nrf2 activation.	Yu et al., 2023

Akt: serine/threonine protein kinase; ALT: alanine aminotransferase; AST: aspartate aminotransferase; b.w.: body weight; CPZ: cuprizone; E2: 17-β-estradiol; ERK1/2: extracellular signal-regulated kinases 1 and 2; Fyn: tyrosine specific phospho-transferase; GSK-3b: glycogen synthase; Nrf2: nuclear factor; LDH: Lactate dehydrogenase; T-AOC: total antioxidant capacity; P13K: fosfatidilinositol 3 kinasa.

microbiota, decreasing the expression and production of interleukins and other inflammatory markers (Wagner et al., 2013; Wu et al., 2023). Furthermore, this anti-inflammatory effect is also responsible for the reduction of gastric ulcers together with its antioxidant effect, increasing the activity of enzymes such as SOD and GPX and decreasing the expression of inflammatory markers such as NO and NF-κB gene (Zeren et al., 2016). Finally, a time-dependent anticancer effect ameliorating pancreatic hyperplasia has been demonstrated (Kuroiwa et al., 2006).

Inmunoprotective effects of sulforaphane

Table 7 provides a summary of the main findings regarding the immunoprotective effects of SFN, including the most significant results from the selected *in vitro* and *in vivo* studies. In general, the immunoprotective mechanism of action consists mainly of a synergy between antioxidant effects and inflammatory marker-reducing effects (Xu et al., 2024). On the one hand, the activation of the Nrf2 factor, which through keap1 reduces ROS and cell apoptosis. On the other hand, it inhibits the gene expression of genes related to inflammatory factors such as VCAM-1, E-selectin. This in turn contributes to the reduction of proinflammatory cytokines and NO release. SFN produces a down-regulation of genes related to carcinogenic processes such as miR-19a, miR-19b,

PTEN and p21. At the genetic level, it has also been shown to reduce the % of micronuclei. Thus, the mechanism of action of SFN on the immune system consists mainly in the reduction of inflammatory cytokines and NO (Holloway et al., 2016; Ruhee et al., 2019). Moreover, antioxidant effects have been also described, although to a lesser degree. In the case of immune system models used, there is an imbalance between *in vitro* (*n* = 6) and *in vivo* (*n* = 1) assays. *In vitro* assays have mainly used human lymphocytes and monocytes from volunteers, but they have been also performed on cell lines such as MCF-7, RAW 264.7, GLC and HBMEC-3. The protective effects of SFN against metals such as cadmium, inflammatory chemicals such as LPS or hydrogen peroxide, plasticizers such as butylbenzyl phthalate, and physical agents such as gamma radiation have been studied. On the other hand, there is only one *in vivo* study performed in C57BL/6 J mice exposed to LPS (Holloway et al., 2016).

Other protective effects of sulforaphane

Table 8 presents a thorough overview of other protective effects of SFN different from those mentioned above. SFN has protective activity against numerous chemical and physical agents and on many different tissues such as the skin, endocrine system, reproductive system, eyes, bones, and muscles. As shown above, the models used are very varied,

Table 5

Overview of the studies reporting the protective effects of SFN on toxicants with pulmonary effects.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Arsenic	10 mg/kg	The animals were exposed to 4.8 mg/m^3 of synthetic dust (with 10 % arsenic trioxide (2–3 μm) for 30 min/day. SFN was injected every other day until the end of the experiment (14 days).	Nrf2-WT and Nrf2-KO mice	Exposure to inhaled arsenic resulted in pathological alterations, oxidative DNA damage, and mild apoptotic cell death in the lung; all of which were blocked by SFN in a Nrf2-dependent manner.	Zheng et al., 2012
Benzo(a) pyrene (BaP)	9 μmol/mice/day	Animals were exposed to the toxic (100 mg/kg of BaP i.p.) thrice a week for 20 weeks. Moreover, two animal groups were asigned for SFN treatment and BaP: Pre-treatment group was administrated with SFN alternate days for two weeks before BaP and Post-treatment group was exposed to SFN alternate days from the 12th week to the 20th week of BaP treatment.	Swiss albino CD1 female mice	SFN attenuated the oxidative stress caused by BaP modulating SOD, CAT, GR, GPx, and G6PDH activity, as well as attenuating the GSH and LPO content in the lung. SFN restored the enzymatic activities in charge of the electronic transport of mitochondria in lung. Post-treated SFN animals shows highly reduced immunoreactivity for the Bcl2 protein.	Kalpana Deepa Priya et al., 2011
Cadmium (Cd)	0, 2.5, 5 and 10 μM SFN	Cells were transformed by exposure to Cd for 5 months. Furthermore, both transformed and untransformed cells were exposed to SFN for 24 h.	BEAS-2BR cells	In cadmium-transformed BEAS-2BR cells, SFN restored autophagy, decreased Nrf2, and decreased apoptosis resistance. In untransformed cells, SFN induced Nrf2 to decrease ROS and possibly malignant cell transformation. They concluded that SFN protected against Cd-induced lung carcinogenesis.	Wang et al., 2018
K$_2$Cr$_2$O$_7$	4 mg/kg	*In vivo* rats were divided in 4 groups: control; SFN, K2Cr2O7, and K$_2$Cr$_2$O$_7$ + SF. Rats in SFN group were subcutaneously injected with SFN solution and i.p. injected with saline solution every day for 35 d Rats in the chromium group were i.p. injected with K$_2$Cr$_2$O$_7$ solution (4 mg/kg) and subcutaneously with sterile saline solution. Rats in the K$_2$Cr$_2$O$_7$ * $_{SFN}$ group were i.p. injected with the chromium salt and sub. injected with SF. *In vitro* MLE-12 cells were treated with K$_2$Cr$_2$O$_7$ (1 μg/ml) for 24 h after pre-treatment with 0.1 μM for 30 min.	*In vivo* Wistar rats *In vitro* mouse alveolar type II epithelial cell line (MLE-12).	SFN prevented oxidative stress, histopathological lesions, inflammation, apoptosis, and changes in Akt and GSK-3b levels *in vivo* and *in vitro*. However, SFN cannot play the protective effect against K$_2$Cr$_2$O$_7$-induced cell injury after treatment with an Akt-specific inhibitor (MK-2206 2HCl) in MLE-12 cells.	Lv et al., 2020
Particulate matter (PM.5)	(0.25–16 μM)	After incubation for 24 h, cells were washed and incubated with PM.5 (100–400 μg/ml) and/or SFN (0.25–16 μM) for 24, 48 and 72 h.	Human bronchial epithelial (HBE) cells	PM2.5 generated ROS and inflammatory responses. Pretreatment with SFN alleviated these negative effects: levels of MDA and ROS decreased, LDH release decreased, and cell apoptosis reducing the apoptosis ratio in the cells. SFN decreased inflammation by lowering the secretion of some inflammatory cytokines (IL-6, IL-8). Pre-incubation with SFN increased Nrf2 and HO-1.	Qin et al., 2021
S-protein of SARS-CoV-2	5 and 10 μM for 24 h.	IB3–1 cells were treated with 5, 15 o 50 nM S-protein in the presence of 5 and 10 μM SFN for 24 h.	Bronchial epithelial IB3–1 cells	Treatment with SFN reversed the up-regulation of IL-6 and IL-8 induced by SARS-CoV-2 Spike protein in IB3–1 cells. Furthermore, SFN-mediated inhibitory effects were also observed for PDGF, IL-9, G-CSF, GM-CSF, IFN-γ, MCP-1 and MIP-1β	Gasparello et al., 2021

CAT: catalase; G6PDH: Glucose-6-phosphate de-hydrogenase; GCLM: glutamate cysteine ligase modifier subunit; G-CSF: granulocyte colony stimulating factor; GM-CSF: granulocyte-macrophage colony-stimulating factor; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GSK-3b: glycogen synthase; HMOX1: heme oxygen-ase-1; IL: interleukin; MLE-12: mouse alveolar type II epithelial cell line; LDH: Lactate dehydrogenase; LPO: lipid peroxidation; MDA: malondialdehyde; SARS-CoV-2: coronavirus 2 of severe acute respiratory syndrome coronavirus 2; PM.5: particulate matter.

both *in vitro* and *in vivo*. *In vitro*, cell lines, primary cultures, and zebrafish larvae have been used. On the other hand, the experimental *in vivo* models are Wistar rats and mice of several strains (CD1, C5BL/6 J, B6129SF1 and ICR). In addition, non-rodent species such as *Oreochromis niloticus, Drosophila melanogaster* and *Marsuperanus japonicus* have been also employed.

In relation to protective effects on the skin, SFN shows antioxidant, anti-inflammatory and anticarcinogenic effects against radiation and irritants (Abel et al., 2011; Klesczynski et al., 2013; Mathew et al., 2014) *in vitro*. Similarly, in assays using eye-related models, SFN is also capable of protecting against irradiating and oxidizing agents. Its mechanism of action is based on enhancing Nrf2-mediated antioxidant and anti-inflammatory activity, demonstrated only *in vitro* models (Chang et al., 2020; Yang et al., 2021). Regarding muscle and bone tissues, antioxidant activity has been demonstrated using both *in vitro* and *in vivo* assays. In addition, in muscle, SFN has the ability to restore protein synthesis and increase regenerative capacity (Hoon Son et al., 2017). In the endocrine system, SFN has masculinization ability to restore the development in mice exposed to vinclozolin (Amato et al., 2022). However, the mechanism of action by which it protects against endocrine disruption is not clear or established. SFN also exhibits the ability to restore metabolic problems in the pancreas (Song et al., 2009) and lipid metabolism through inhibition of LKB1/AMPK by increasing lipophagy and mitochondrial fatty acid metabolism (Zhang et al., 2014).

Table 6
Overview of studies reporting the protective effects of SFN on toxicants with gastrointestinal effects.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Acetic acid	15 mg/kg	Intracolonic single injection of 2 ml of 4 % acetic acid and SFN by oral gavage daily for two weeks.	Sprague Dawley rats	All morphological changes in the colon were improved by treating with SFN. Significantly increased expression of PGC-1, TFAM, Nrf2, and HO-1 associated with a reduction in the expression of mTOR, cyclin D1, and PCNA in the liver.	Alattar et al., 2022
Acetylsalicylic acid (ASA)	5 mg/kg	Induction of gastric ulcer (GU) was undertaken using 1-ml ASA (150 mg / kg of body weight, i.g.). SFN was administered by orogastric gavage 1 h before induction of GU.	Male Wistar Albino rats	SFN reduced the number of TUNEL positive cells and iNOS-positive cells. It decreased TOS and OSI, as well as increased TAS and TT levels. It increased the activities of SOD and GPX. It decreased TNF-α and ADMA levels and increased NO levels. Increased mRNA expression of DDAH-1 and DDAH-2 and reduced NF-kB mRNA in comparison with those of the ASA group.	Zeren et al., 2016
Dextran sodium sulphate (DSS)	25 mg / kg b.w. SFN	Mice orally received phosphate buffered saline as control or SFN for 7 days. Acute colitis was induced by ingestion of 4 % DSS through drinking water for 5 days. Subsequently, both groups of animals received PBS for a further 7 days.	C57BL/6 J mice	Pretreatment with SFN before inducing colitis significantly minimised both body weight loss and disease activity index when compared to control mice. Furthermore, SFN-pretreated mice exhibited significantly longer colon lengths than control mice. Both macroscopic and microscopic examinations of the colon showed reduced inflammation in SFN pretreated animals. mRNA analysis of distal colon samples confirmed decreased expression of inflammatory markers and increased expression of Nrf2-dependent genes in SFN pretreated mice.	Wagner et al., 2013
Dextran sodium sulphate (DSS)	Broccoli seed extract (BSE): 212.0 ± 41.0 nmol/ g SFN (concentration detected in mice colonic contents)	The first week: 0.2 ml of skim milk and the second week: 0.2 ml of skim milk + 2.5 % DSS or 0.2 ml of 370 mg/kg BSE-dissolved skim milk + 2.5 % DSS	C57BL/6 J mice	The administration of broccoli seed extract demonstrated a potential to alleviate the development of colitis in mice subjected to DSS treatment. In particular, the BSE intervention led to a significant reduction in the production of inflammatory markers, including IL-6, IL-1β, and TNF-α, within the colon. Furthermore, BSE treatment diminished MDA activity. Furthermore, the intervention with broccoli seed extract improved colon injury, as evidenced by the reduction of tissue edema and substantial inflammatory cell infiltration within the mucous and submucous layers. Furthermore, it mitigated colonic structural damage, including crypt disruption, goblet cell depletion, and intestinal epithelial cells. Interestingly, Broccoli seed extract contributed to the restoration of intestinal barrier function, as indicated by enhanced expression of ZO-1 and claudin-1 proteins.	Wu et al., 2023
Dextran sodium sulfate (DSS)	Broccoli extract (nonstandardised) The authors attribute the effects to the SFN	Mice were fed a 10 % steamed broccoli sprout diet and a three-cycle regimen of 2.5 % DSS in drinking water over a 34-day experiment to simulate chronic relapsing ulcerative colitis.	C57BL/6 J mice	Recovery of intestinal microbiota levels.	Holman et al., 2023
Dextran sulphate sodium (DSS)	Steamed broccoli sprout diet (5 %) containing a low level of SFN (<25 µg/g diet) and a high level of glucoraphanin	Prepared diet for 2 weeks and then treated with 2 % DSS	C57BL/6 J female and male mice	The broccoli sprouts reduced chemically induced colitis. This protective effect was dependent on the presence of an intact microbiota.	Zhang et al., 2023
N- nitrosobis(2-oxopropyl) amine (BOP)	80 ppm	The hamsters were s.c. injected with BOP (10 mg/kgb.w) 4 times a week, and fed a diet supplemented with 80 ppm SFN during the initiation (3 weeks including 1 week before and after the injection of BPO) or post initiation stages (basal diet after the last BOP ingestion and fed for 14 weeks with SFN)	Male 6-week-old Syriam hamsters	SFN blocked the initiation of BOP of hamster pancreatic carcinogens. SFN decreased atypical hyperplasias in pancreatic ducts, and multiplicity of adenocarcinomas in the initiation stage but not in the post-initiation stage.	Kuroiwa et al., 2006

ADMA: asymmetric dimethylarginine; ASA: Acetylsalicylic acid; BOP: N-nitrosobis(2-oxopropyl)amine; DDAH: dime-thylaminohydrolase; DSS: Dextran sodium sulphate; GPx: glutathione peroxidase; HMOX1: heme oxygenase-1 (HO-1H3K4me3: histone H3 lysine 4; IL: interleukin; iNOS: inducible nitric oxide sinthase; MDA: malondialdehyde; mTOR: mammalian target of rapamycin; NF-kB: nuclear factor kappa light chain enhancer of activated B cells; Nrf2: nuclear factor erythroid 2-related factor 2; NO: nitric oxide; OSI: oxidative stress index; PCNA: proliferating cell nuclear antigen; PGC-1: peroxisome proliferator activated receptor-gamma coactivator; s.c.: subcutaneous; SOD: Superoxide dismutase; TAS: total antioxidant status; TFAM: mitochondrial transcription factor A; TNFα: tumour necrosis factor alpha; TOS: total oxidative stress; TUNEL: Terminal deoxynucleotidyl transferase dUTP nick end labelling.

Table 7
Overview of the studies reporting the protective effects of SFN against toxicants with immunitary effects.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Butylbenzyl Phthalate (BBP)	5 μM	10^{-5} M of BBP + SFN (the medium was replaced every two days during 4-day treatment)	MCF-7 cells	The growth promoting effect of BBP could be mitigated by SFN, accompanied by a reversal of altered expression of miR-19a, miR-19b, PTEN, and p21. SFN also suppressed the binding of upregulated miR-19 with PTEN induced by BBP.	Cao et al., 2023
Cd	20–100 μM	24 h with IC_{10}, IC_{25}, IC_{50} of Cd (data not shown) and SFN for 24 and 48 h.	Human lymphocytes and monocytes	SFN decreased Cd-induced cytotoxicity in a concentration- and time-dependent manner	Alkharashi et al., 2017
Co-60 γ-radiation, doxorubicin or bleomycin	400 nM	Whole blood or lymphocytes were exposed to 0–2 Gy Co-60 gamma ray radiation or to carcinogen (doxorubcin or bleomycin) for 1 h before PHA stimulation. Subsequently, 400 nM of SFN was added in phase G0 or G1 of the cell cycle (2 or 20 h after PHA stimulation, respectively).	Blood from volunteers	Reduction of MN% (up to 70 %) in peripheral blood lymphocytes of individuals exposed to γ- and β-radiation, doxorubicin or bleomycin. SFN reduces both acute and late effects related to radiation-induced genomic damage.	Katoch et al., 2013
Hydrogen peroxide (H_2O_2)	10 μM SFN for 24 h	Cells were exposed for 22 h to SFN and then 2 h to 200 μM of H_2O_2 for inducing oxidative stress.	Granulosa-lutein cells (GLCs) were collected from women 20 to 38 years old with normal menstrual cycles with 25–35-day periods	Intracellular ROS and apoptosis rose dramatically in GLC with enhanced oxidative stress. SFN therapy decreased ROS and apoptosis levels and increased overexpression of AMPK, AKT, and NRF2 genes and proteins.	Taheri et al., 2022
Lipopolysaccharide (*E. coli* serotype 0111:B4)	5 and 50 mg / kg i.p. 1, 10 and 100 μM	*In vivo:* Mice were exposed to 5 mg/kg and 50 mg/kg of SFN dissolved in corn oil 24 h by injection prior to exposure to 0.5 mg/kg of LPS. *In vitro:* Cells were treated with SFN (1, 10 and 100 μM) 24 h before adding LPS (500 ng/ml and 10 μg/ml) for 4 h.	Wild-type (WT) C57BL/6 J mice and Nfe2l2tm1Ywk knockout (Nrf2-KO) mice. HBMEC-3 cell line	*In vivo,* SFN reduced increased LPS in brain and serum inflammatory cytokines (IFN-γ, MCP-1 and TNF-α) whilst also increasing IL-10 expression and reduced MPO levels. *In vitro* SFN reduced neutrophil recruitment through down-regulation of E-selectin and vascular cell adhesion molecule 1 (VCAM-1) and decreased ROS induced by LPS. SFN may reduce cerebral inflammation in sepsis produced by LPS by mediating its anti-inflammatory and antioxidant effects through Keap1/Nrf2 transcriptional activation of the antioxidant system and via the NFκB pathway.	Holloway et al., 2016
Lipopolysaccharide (LPS)	0–50 μM	Macrophages were preincubated for 6 h with and without SFN and then treated with LPS for 24 h.	Raw 264.7 murine macrophages	LPS significantly increased the expression of inflammatory cytokines and the concentration of NO in nontreated cells. SFN prevented the expression of NO and cytokines by regulating inflammatory enzyme iNOS and activating the Nrf2 / HO-1 signal transduction pathway.	Ruhee et al., 2019

Akt: serine/threonine protein kinase; AMPK: adenosine 5′-monophosphate (AMP)-activated protein kinase; GLC cells: Granulosa-lutein cell line; GU: Gastric Ulcer; HBMEC-3: Human Brain Microvascular Endothelial Cells; HMOX1: heme oxygenase-1 (HO-1H3K4me3: histone H3 lysine 4; IFN-γ: Interferon gamma; iNOS: óxido ntrico sintasa indicible; Keap1: Kelch-like ECH-associated protein 1; LPS: bacterial lipopolysaccharide; MCF-7: Breast Cancer Cells; MCP-1: Monocyte chemoattractant protein-1; miR-19A: MicroRNA 19a; miR-19b: MicroRNA 19b; MN: micronuclei; NSAID: Non-steroidal anti-inflammatory drugs; Nrf2: erythroid 2-related factor 2; NO: nitric oxide; p21: tumour protein; PTEN: phosphatidylinositol-3,4,5-trisphosphate 3-phosphatase; ROS: reactive oxygen species; TNFα: tumour necrosis factor alpha; VCAM-1: Vascular cell adhesion protein 1.

Some studies also demonstrated the protective effects of SFN during pregnancy in embryos exposed to 2-amino-1-methyl-6-phenylimid-azo [4,5-b] pyrimidine (Zhang et al., 2021) and on placental cell lines against the inflammatory effect of TNF-α (Cox et al., 2019). The safety of SFN and broccoli sprout supplements during pregnancy is warranted given the commercial availability. Furthermore, no effects on the incidence of sperm abnormalities were reported in mice treated with up to 10 g/kg b.w. (Zhou et al., 2015). However, it is important to note that

Table 8
Overview of the other studies reporting the protective effects of SFN against toxic substances in several models.

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
2-Amino-1-methyl-6-phenylimidazo[4,5-b] pyrimidine (PhIP)	5 µM Nano-SFN	200 µM PhIP + 5 µM Nano-SFN for 36h	Early chick culture (embryos)	Nano-SFN can effectively alleviate the PhIP microenvironment-induced abnormal development of the embryonic nervous system and has a protective effect on embryonic development. Thus, it reversed PhIP-inhibited neural cell differentiation in the neural tube. Improved generation and migration.	Zhang et al., 2021
2-chloroethyl ethyl sulfide	5 µM	48 h with SFN and 5 min with 2 mM of 2-chloroethyl ethyl sulfide	NCTC2544 human keratinocytes	SFN restored toxicity by up-regulating the gene expression of GCLM and Nrf2. Moreover, SFN increased the GHS content.	Abel et al., 2011
4-hydroxy-2-nonenal (4-(HNE)	5.0 mg/kg	Mice were administered SFN or carnosic acid (CA) (1 mg / kg) (or respective vehicles) 48 h before cortical mitochondria. The purified mitochondria were then exposed *ex vivo* to 4-HNE and incubated for 15 min at 37 °C. The samples were analyzed for mitochondrial bioenergetics.	Male CF-1 mice	Administration of either compound (SFN or CA) significantly increased heme oxygenase-1 mRNA in cortical tissue 48 h after administration, verifying that both were capable of inducing the Nrf2–ARE pathway. Furthermore, the prior *in vivo* administration of SFP and CA attenuated 4-HNE-induced inhibition of mitochondrial respiration for complex I, but only CA acted to protect complex II. SFN and CA reduced the amount of 4-HNE bound to mitochondrial proteins determined by Western blot.	Miller et al., 2013
5-Fluorouracil	2 and 20 mg/kg	Negative control group, solvent control, 5-Fu+SFN-l (2 mg/kg) and 5-Fu+SFN—H (20 mg/kg). Exposure by gavage for 11 days. Intestinal mucositis was induced in the 5-Fu, SFN-l and SFN—H groups by injection of 5-Fu (300 mg/kg) on sixth day.	Male Balb/c mice	SFN was able to attenuate 5-Fu-induced intestinal injury by restricting related clinical symptoms, regulating inflammatory homeostasis, and maintained intestinal permeability. SFN at high doses was more effective in enhancing antioxidant defence and tight junction in jejunum segment at the molecular level.	Wei et al., 2020
Benzo (a) pyrene (BaP)	9 mM/mice/d	Mice were treated with BaP (100 mg / kg b.w. i.p.) three times a week and kept for 20 weeks. Previously, animals were administered orally with SFN (9 mM/mice/d) on alternate days for two weeks prior to first dose of BaP and continuously treated until the 12th week and sacrificed later.	Swiss Albino mice	SFN had inhibitory effects on B[a]P-induced aryl hydrocarbon receptor (AHR) activation resulting in decreased Phase I enzyme activities in mice. SFN enhanced Nrf2 transcription together with phase II enzymes resulting in reduced carcinogen-induced stress.	Kalpana Deepa Priya et al., 2011
Benzo(a)pyren (BaP)	0.55 mg / kg of SFN or 30 % dry broccoli (substituting alfalfa)	The fish were fed over 30 days with three different diets, one containing broccoli, another containing SFN, and controls containing alfalfa instead. Subsequently, 100 µg/g b.w. BaP was injected i.p. to all fish being sacrificed at different times (0, 12, 24, 36, 48 and 72 h).	Tilapia fish (*Oreochromis niloticus*)	The presence of broccoli or SFN significantly increased the basal levels of CYP450 activity, which could improve detoxification capacity. After exposure to BaP, less aromatic metabolites were found in bile in fish fed with diets enriched with broccoli or SFN compared with control fish. Thus, these diets induce changes in the enzymatic systems involved in the detoxification metabolism of fish.	Villa-Cruz et al., 2009
Blue light (400 nm)	5–25 µM	Cells were pretreated with SFN for 3 h and then exposed to blue light at intensity of 2000 ± 500 lx for 24h	Human RPE cell line ARPE-19	SFN protected against blue light–induced oxidative injury, inflammation, and apoptosis. Molecular mechanisms may involve increased antioxidative, autophagy, and PGC-1α related mitoprotective properties. It maintains the Nrf2-related redox state and upregulates the expression and autophagy of SIRT1 and PGC-1α expression and autophagy.	Yang et al., 2021

Table 8 (*continued*)

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
Cadmium (Cd)	20–100 μM	24 h with 6, 13.65 and 39.8 μM of Cd and SFN for 24 and 48 h.	Human mesenchymal stem cells (hMSCs)	Reduced necrotic cells. Alleviated the acidified cytoplasm. Attenuated the decrease in MMP. The expression pattern of the POR, TNFRSF1A and TNFSF10 genes was significantly stabilized.	Alkharashi et al., 2019
Cadmium (Cd)	5 μg/g b.w.	Shrimps were injected with Nrf2 targeting sdRNA (4 μg/g b.w.) or SFN (5 μg/g b.w.) and then exposed to 40 mg / l of Cd Cl$_2$ for 48 h.	*Marsupenarus japonicus*	SFN decreased the number of apoptotic cells and alleviated the oxidative stress: increased T-AOC, SOD activity, SOD mRNA expression, and reduced MDA content. Detoxified enzymes, such as GST, Erod, and their corresponding gene expressions, were elevated in the SFN group.	Ren et al., 2021
Cadmium (Cd)	50 μM SFN	Zebrafish larvae were incubated with SFN or 0.01 % DMSO control for 24 h at 28 °C. Subsequently, they were exposed to 5 and 25 μM Cd.	Zebrafish larvae (EKW strain).	Pre-incubation of zebrafish larvae with SFN increased gst pi, gclc, and prdx1 expression levels, which were associated with protection against Cd-mediated damage to the olfactory epithelium, supporting Nrf2′s protective role against Cd-induced oxidative stress. Furthermore, SFN pre-incubation afforded protection against cell damage, thus supporting a protective role for an Nrf2-regulated cellular.	Wang and Gallagher, 2013
Cisplatin	0.5–5 μM for 24 h 500 μg/kg	Cells were treated with SFN (0–5 μM) for 24 h and then 40 μM of cisplatin was added for another 24 h. Rats were exposed to a single dose of cisplatin by injection (7.5 mg/kg). SFN (500 μg/kg i.v.) was administered twice: 24 h before exposure and also 24 h after toxicant exposure and the animals were sacrificed three days after injection of cisplatin.	LLC-PK1 cells Wistar rats	SFN prevented cisplatin-induced cell death. SFN improved renal dysfunction, structural damage, oxidative/nitrosative stress, GSH depletion, increased urinary excretion of hydrogen peroxide, and decreased antioxidant enzymes (CAT, GPx, and GST) activities.	Guerrero-Beltrán et al., 2010
Dexamethasone (DEX)	5 μM	The C2C12 myoblasts were treated with 5 μM SFN in the presence of 5 μM DEX for 24 h.	C2C12 myoblasts	SFN treatment improved protein synthesis by activating Akt and prevented DEX-mediated upregulations of the myostatin and Atrogin-1 mRNA, increasing the mRNA level of MyoD and reducing protein degradation.	Son et al., 2017
Dibenzo[def,p]chrysene (DBC)	400 ppm SFN alone 600 ppm SFN + 500 ppm indole-3-carbinol (I3C)	Pregnant mice were exposed to different diets from day 9 of gestation until weaning (postnatal day 21). They were exposed to SFN alone or in combination with I3C.	Female mice B6129SF1	Purified SFN, incorporated into the maternal diet at 400 ppm, decreased the latency of DBC-dependent morbidity. However, when I3C and SFN were administered in equimolar combination, no protective effect was observed. SFN metabolites measured in the plasma of newborns were positively correlated with exposure levels through the maternal diet but not with offspring mortality.	Shorey et al., 2013
H$_2$O$_2$	7 μM	The OACs were incubated with 200 μM H$_2$O$_2$ for 8 h. Subsequently, the OAC and osteochondral composites were treated with SFN for 72 h.	Osteoarthritic articular chondrocytes (OACs)	SFN ameliorates H$_2$O$_2$-induced oxidative stress and cartilage matrix through suppressing inflammatory cytokines and activating the Keap1 / Nrf2 pathway.	Yang et al., 2020
High-fat and high-fructose (HFHFr) diet	15 and 30 mg/kg	Control group, HFHFr group, HFHFr + low-dose SFN and HFHFr + high-dose SFN for 12 weeks. SFN was administered to mice every two days by gavage.	Male C57BL/6 mice	Long-term SFN supplementation improved the intestinal microbial composition in HFHFr diet-induced NAFLD mice. SFN improved tight junction proteins in the colon, inhibited the LPS/TLR4 signalling pathway and endoplasmic reticulum stress in the intestine, and improves intestinal inflammation. As a consequence, it maintained	Xu et al., 2023

Table 8 (*continued*)

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
				intestinal barrier integrity, reduced intestinal-derived LPS, and inhibited the liver LPS/TLR4 signalling pathway to improve liver steatosis and steatohepatitis.	
Ionizing radiation	0–30 μM	Single treatment: cells were incubated 4 h with SFN. Repeated treatment: cells were treated for 4 h with SFN for 3 consecutive days prior to radiation exposure.	Primary human skin fibroblasts	Fibroblasts exposed to repeated SFN showed a more pronounced dose-dependent induction of Nrf2-regulated mRNA and a reduced amount of radiation-induced free radicals compared to cells treated once with SFN. Cellular protection from ionizing radiation measured by the EdU assay was increased, specifically in cells exposed to repeated SFN treatment. SFN was unable to protect Nrf2-knockout mouse embryonic fibroblasts, indicating that radioprotection was Nrf2-dependent. Furthermore, radioprotection by repeated SFN treatment was dose dependent with an optimal effect at 10 uM,	Mathew et al., 2014
Maleic acid	1 mg/kg	1 dose of 400 mg / kg maleic acid and SFN each day for four days	Male Wistar rats	SFN prevented proteinuria, increased urinary excretion of N-acetyl-β-ᴅ-glucosaminidase, and decreased plasma GPx activity, renal blood flow, and oxygenation and perfusion of the renal cortex.	Briones-Herrera et al., 2018
Methyl methanesulfonate (MMS), urethane (URE), 4-NQO and H_2O_2	0.14, 0.28, 0.56 mM (corresponding to 25, 50, 100 % lyophilized broccoli) in a DMSO/Tw80–EtOH mixture (DTE)	The third instar larvae were fed SFN (0.14, 0.28, 0.56 mM)/DTE plus mutagens to give a final concentration of MMS (0.5 mM), URE (20 mM), 4-NQO (2 mM) or H_2O_2 (20 mM) in triplicate.	Standard and high bioactivation (HB) crosses of *Drosophila melanogaster*	SFN did not show clear protective effects against the genotoxicity of the compounds tested. Although SFN showed a tendency to reduce the genotoxicity produced by MMS, this effect could be due to the action of the solvent (DTE mixture). Additionally, SFN showed *per se* genotoxic effects and increased the standard cross of genotoxicity of H_2O_2 in the *D. melanogaster* and HB cross at 0.28 mM and 0.56 mM, respectively. The results were inconclusive for the protective effects of SFN against the genotoxicity of MMS, URE, 4-NQO, and H_2O_2.	Dueñas-García et al., 2012
Paraquat	1 μM	22–24 h of cotreatment with 250 μM of paraquat and SF	Bovine *in vitro*-matured oocytes	Inhibition of increased levels of ROS content. Rescue of GSH and T-SOD levels. Cotreatment with SFN elevated the low ratio of matured oocytes induced by paraquat. The application of SFN markedly facilitated the transcriptions of GCLC, GCLM, HO-1, NQO1, and TXN1 under paraquat exposure, but the expressions of the mRNA TXNRD1 and PRDX1 mRNA had no marked difference.	Feng et al., 2023
Quinolinic acid (QUIN)	5 mg/kg	Rats received i.p. SFN 24 h and 5 min before the intrastriatal infusion of QUIN.	Male Wistar rats	SFN enhanced reduced GSH levels and increased GPx and GR activities. SFN also prevented QUIN-induced oxidative stress (measured by oxidised proteins levels), histological damage, and the circling behaviour.	Santana-Martínez et al., 2014
Sodium arsenite	40 μM	Pretreatment with SFN (40 mM) for 12 h was performed in larvae for 12 h before arsenite treatment (2 mM from 3.5 to 4 days after fertilisation), and SFN was then withdrawn by exchanging E3+ medium	Zebrafish larvae	Pretreatment with SFN improved the survival of zebrafish larvae after arsenic exposure.	Fuse et al., 2016

Table 8 (*continued*)

Toxic substance	Sulforaphane Concentration or dose	Exposure conditions	Experimental model	Main results	Reference
		containing arsenite. The solutions were changed every 2 days during the tests.			
Streptozotocin (STZ)	40 μg/kg	Mice received i.p. injections of 40 μg/kg SFN daily for 3 days prior to the administration of 80 mg of STZ / kg b.w. (to induce diabetes). Apart from that group, there were 3 more: one nontreated group and two only receiving SFN or STZ. Five days after the first STZ injection, all animals were sacrificed.	Male ICR mice	Pretreatment with SFN blocked STZ-induced islet destruction and restored the number of islet cells secreting insulin to the level of the control.	Song et al., 2009
Tert-butyl hydroperoxide	0.5 μM	24 h of SFN pretreatment and 24 h of 0.1, 0.5, 1, 5, or 10 mM tert-butyl hydroperoxide	ARPE-19 cells	SFN attenuated ROS production, decreased mitochondrial dysfunction, activated phase II detoxification enzymes, and suppressed pro-inflammatory mediators (cytokines ICAM-1 and MCP-1).	2020
TNFα	5–20 μM	SFN and 1–100 ng/ml of TNFα for 24h	Placentae, umbilical cords, and serum. HUVEC cells.	Reduced TNF-α mediated HUVEC secretion of endothelin-1, VCAM-1, ICAM-1, and E-selectin, and prevented increased endothelial permeability. In placental explants, reduced the secretion of soluble Flt-1, soluble endoglin, and activin A.	Cox et al., 2019
Type 2 *diabetes mellitus* model induced by a high-fat diet for 3 months and streptozotocin	0.5 mg/kg	4-month SFN given s.c. daily five days a week	Male C57BL/6 J mice	SFN may prevent inhibition of the LKB1/AMPK pathway DM-derived oxidative stress of the LKB1 / AMPK pathway, and subsequently influence the master regulators of lipid metabolism, including the degradation of intracellular LDs to fatty acids by lipophagy, and the increase in mitochondrial fatty acid uptake and oxidation by upregulated CPT-1 and PGC-1α. This results in the prevention of T2DM-induced lipotoxicity and, hence, cardiomyopathy.	Zhang et al., 2014
UV irradiation (UVR)	5, 10, 25 μM	Cells were pre-incubated with 5, 10 or 25 μM of SFN for 24 h before UV irradiation (300 mJ/cm^2).	HaCaT cell line (keratinocytes)	SFN, mainly at 10 μM up-regulated the gene for the enzyme CAT and the Nrf2 target genes, γ-GCS, HO-1 and NQO1 in UV irradiated skin cells. SFN reduced UVR-induced structural damage in the epidermis 48 h after UV-irradiation in a concentration-dependent manner (5 and 10 μM). However, the higher concentration of SFN (25 μM) led to a significant increase in sunburn cells compared to non-SF treated skin.	Kleszczyński et al., 2013
Vinclozolin	45 mg/kg	One dose of 125 mg/kg vinclozolin and one dose of SFN 48 h later	CD-1 mice	SFN produced a dose-dependent rescue (increase) in masculinisation of anogenital distance.	Amato et al., 2022
X-ray Irradiation	0.5 mg/kg b.w.	Control, SFN, IR, and IR+SFN groups. SFN and IR+SFN groups were injected i.v. once, 5 times a week for 4 weeks. Later, in the IR and IR-SFN groups, the left thigh of mice was treated with a single X-ray irradiation of 40 Gy at a dose rate of 200 cGy/min. The mice were sacrificed after 1 month of feeding.	SPF C5BL/6 mice	SFN prevented radiation-induced muscle fibrosis. It inhibited the expression of components of the IR-induced extracellular matrix and oxidative stress in the skeletal muscle. Also, it increased Nrf2 expression and function through AKT/GSK-3β/Fyn pathway, and downregulated TGF-β1/Smad pathway.	Wang et al., 2022c

5-Fu: 5-Fluorouracil; Akt: serine/threonine protein kinase; AHR: aryl hydrocarbon receptor; AMPK: adenosine 5′-monophosphate (AMP)-activated protein kinase; ARPE-19: spontaneously arising retinal pigment epithelia; BaP: Benzo (a) pyrene; CA: carnosic acid; DBC: Dibenzo[def,p]chrysene; DEX: Dexamethasone; EdU: 5-ethynyl 2′-deoxyuridine; Flt-1: vascular endothelial growth factor receptor 1; Fyn: tyrosine-specific phospho-transferase; GCLM: glutamate cysteine ligase modifier subunit; GPx: glutathione peroxidase; GR: glutathione reductase; GSH: glutathione; GSK-3b: glycogen synthase kinase 3 beta; HaCaT cell: keratinocytes; hMSCs: human mesenchymal stem cells; HNE: 4-hydroxy-2-nonenal; HMOX1: heme oxygenase-1 (HO-1H3K4me3: histone H3 lysine 4; ICAM-1: Intercellular Adhesion Molecule 1; I3C: Indole-3-Carbino; i.v.: intravenous; LLC-PK1: Lilly Laboratories Culture-Porcine Kidney 1; HUVEC: EndoGRO Hu-man Umbilical Vein Endothelial Cells; LPS: bacterial lipopolysaccharide; NAFLD: Non-alcoholic fatty liver disease; NCCs: Neural Crest Cells; NCTC2544: human keratinocytes; Nrf2: nuclear factor erythroid 2-related

factor 2; MCP-1: Monocyte chemoattractant protein-1; MMP: Ma-trix metalloproteinases; MMS: Methyl methanesulfonate; MyoD: myogenic differentiation protein 1; PGC-1α: peroxisome proliferator activated receptor-alpha coactivator; PhIP: 2-Amino-1-methyl-6-phenylimid-azo[4,5-b]pyrimidine; POR: Cytochrome P450 Oxido-reductase; s.c.: subcutaneously; Smad: mothers against decapentaplegic; T-AOC: total antioxidant capacity; TGF-β1: transforming growth factor beta 1; TLR4: toll-like receptor 4; TNFα: tumour necrosis factor alpha; TNFRSF1A: Tumour necrosis factor receptor superfamily member 1 A; TNFSF10: Tumour necrosis factor ligand su-perfamily member 10; TrxR-1: thioredoxin reductase-1; TXR1: human thioredoxin; PRDX1: Peroxiredoxin 1; SOD: Total Superoxide Dismutase; URE: urethane; VCAM-1: Vascular cell adhesion protein 1; γ-GCL: γ Glutamate–cysteine ligase.

the concentrations used in these studies far exceed what would be achieved with typical consumption of fresh vegetables or recommended doses of available supplements (Shorey et al., 2013).

Toxicity of sulforaphane

Although SFN has numerous beneficial and protective effects against toxins, no substance is exempt from having adverse or toxic effects at high doses. The toxicity of SFN has been tested both acutely and sub-chronically. Scola et al. (2017) observed severe toxicity, such as deep sedation, ataxia, ptosis, and tremors in mice acutely exposed to high doses of SFN intraperitoneally. In this study, doses of 300 mg SFN/kg resulted in death for all animals within 180 min. Similar symptoms were reported at doses of 250 mg/kg, with seven out of twelve mice dying within 240 min. At a dose of 200 mg/kg, sedation and ptosis were observed in all animals, with six out of twelve mice dying within the first night. At lower doses, such as 150 mg/kg, sedation persisted, but mor-tality was not observed. Additionally, authors determined the LD_{50} in 212.67 mg/kg.

On the other hand, Zhou et al. (2015) studied the effects of a glucoraphanin-rich broccoli extract in rats orally exposed for 30 days at doses of 3 g/kg/day (equivalent to 390 mg/kg/day of glucoraphanin, SFN precursor). During the 30-day feeding study, no mortality or treatment-related adverse clinical findings were observed. Animals in all groups displayed normal activities and growth. Body weights did not significantly differ between the SFN-treated and control groups, although there was a slight decrease in food consumption in high-dose males. However, this decrease was associated with an increase in food utilization rate, and body weight gain remained similar to control groups. Minor fluctuations in haematology and clinical chemistry pa-rameters were observed, but they fell within the historical control range and showed no clear dose-response relationships. Clinical chemistry parameters showed minimal changes within the historical control range of the testing laboratory, with no significant differences in liver or kid-ney toxicity indicators. Organ weights increased in high-dose males for the spleen and kidneys, while the absolute weight of the testes was slightly reduced. However, all organ weight values remained within the historical control range. Macroscopic evaluations at necropsy revealed no abnormalities, and histological evaluations of various tissues showed normal structure without observable abnormalities related to SFN treatment.

Additionally, mutagenicity/genotoxicity studies have been per-formed to evaluate the glucoraphanin-rich broccoli extract (GBE). Thus, three mutagenic/genotoxic experiments, including an Ames test, an *in vivo* mouse micronucleus, and an *in vivo* mouse sperm abnormality (Zhou et al., 015). In the Ames test, four *Salmonella typhimurium* histidine-deficient test strains (TA97, TA98, TA100 and TA102) were exposed up to 5000 μg SFN/plate in presence and absence of S9 meta-bolic activation system. In the two *in vivo* tests, mice were exposed up to 10 g/Kg b.w of BGE by oral gavage. The results showed no mutagenic activity in the Ames assay and no evidence of genotoxic potential in the *in vivo* assays at any of the doses tested.

In general, the toxic effects of SFN have been shown to occur at very high doses. However, there are few studies on this subject, and more investigations are needed to establish safe therapeutic doses of SFN.

Concluding remarks

The chemoprotective effects of SFN have gained toxicological

relevance due to the large number of xenobiotics against which it exerts protective properties, its contribution against the progression of different diseases, and its beneficial effects on health. Thus, several mechanisms of action have been reported for SFN such as antioxidant, anti-inflammatory, anticancer, immunomodulatory, metabolic regu-lator, or protective against endocrine disruption, as described in the present study.

The concentration used in the *in vitro* assays ranges between 0.5–160 μM based on cytotoxicity studies performed by some authors in previous assays, although the concentration most commonly used in these experimental models with a protective effect is 5 μM. On the other hand, *in vivo* studies, doses from 5 to 30 mg/kg have been used, 5 mg/kg being the dose usually chosen as effective. As outlined in the previous section, doses associated with toxic effects significantly exceed those involved in protective effects by a factor of approximately 50 (250 mg/kg *versus* 5 mg/kg). Consequently, a safe dosage range of SFN could be established in which only beneficial and protective effects are expected to occur.

In relation to kinetic studies, its distribution in most of the tissues has been demonstrated, while its potential uptake through the placental barrier remains to be elucidated. In general, the main mechanism of action of SFN against toxic agents is antioxidant, being a modulator of apoptosis and to have anticancer effects. SFN causes the activation of Nrf2 factor, which in turn increases the content of GSH and the activity of several antioxidant enzymes, CAT, SOD, GPx, and GR, which reduces biomarkers of oxidative stress (Santana-Martinez et al., 2014; Feng et al., 2023). Additionally, potentiation of the antioxidant response by SFN restores S-glutathionylation in the mitochondrial fraction (Aranda-R-ivero et al., 2023). Activation of Nrf2 also reduces inflammation by decreasing interleukin production (*i.e.* IL-1β) and pyrin domain 3 of the nucleotide-binding domain-like receptor family (Aranda-Rivero et al., 2023). Reduction of oxidative stress and inflammation avoid apoptosis by preventing caspase 3 cleavage and increased levels of B-cell lym-phoma 2 (Bcl2) (Kalpana Deepa Priya et al., 2011). Taken together, the present review shows that SFN decreases oxidative stress, prevents inflammation and cell death by apoptosis after exposure to toxic agents.

SFN has proven to be effective in different tissues and cellular types (liver, kidney, nervous system, etc.). In relation to the liver, one of the hepatoprotective effects of SFN involves its ability to shield the liver against carcinogens, thereby preventing tumour formation. An ideal chemopreventive agent is expected to exert minimal impact on normal cells while demonstrating potent inhibitory effects on cell proliferation and carcinogenic pathways in cancer cells. Despite numerous studies that investigated both the protective and cytotoxic effects of SFN, the data comparing its impact on normal cells *versus* cancer cells are still very scarce (Liu et al., 2019). Furthermore, ongoing research aims to explore the effects of SFN on lipogenic enzymes, transcription factors, cytokines such as TNF alpha, mitogen-activated kinases such as JNK, and mitochondrial dysfunction, representing future directions to improve our understanding of the protective role of SFN in the liver (Zhou et al., 2014). In addition, SFN has shown neuroprotector effects against substances capable of inducing neurodegenerative effects. This issue is of great interest due to the increase of this type of diseases and the great concern on the part of the population. However, additional research employing experimental animal models is necessary to assess the influence of SFN bioavailability on its potential mitochondrial pro-tection and exert anti-inflammatory actions in brain cells.

Globally, liver and nervous system are by far the most investigated target organs in which SFN protective effects have been studied (approximately 38 % of the studies). This could be due to its mechanism

of action, as it is well known that oxidative stress is a crucial factor in liver diseases (Cichoż-Lach and Michalak, 2014) and it is also a key modulator in many neurodegenerative diseases (Singh et al., 2019).

Numerous potential clinical applications in humans have been attributed to SFN. In this respect, it has demonstrated efficacy as a chemoprophylactic agent against various cancer types including stomach, breast, colon, and prostate cancers. Furthermore, SFN has shown to hold promise in mitigating hepatic insufficiency, as well as improving cognitive and locomotor functions. Moreover, SFN has been implicated in ameliorating complications associated with type II diabetes by regulating signalling pathways in organs such as the pancreas, kidney, heart, skeletal muscle, brain, and others. Additionally, there is an emerging interest in the chronic use of SFN as a novel therapeutic approach for preventing muscle damage in athletes undergoing daily high-intensity exercise (Sato et al., 2021).

Despite its potential clinical applications, there are not many studies that demonstrate the protective effect of SFN on the kidney, considering that it is a target organ of multiple toxic substances (Guerrero-Beltrán et al., 2012). Similarly, studies dealing with lungs, heart, or immune system are also limited. Moreover, although it has recently been demonstrated that SFN has beneficial effects on the intestinal microbiota (Marshall et al., 2023), the role of the microbiota in the mechanism of action of SFN against toxic gastrointestinal agents has not been extensively addressed, taking into account that the microbiota performs various crucial functions for the well-being of the organism (Marshall et al., 2023). It is worth noting the current interest to explore the beneficial effects of SFN at the endocrine level, particularly in the context of therapeutic, improving diabetes mellitus and metabolism complications induced by xenobiotics (Mthembu et al., 2023). These complications include diabetic cardiomyopathy, diabetic neuropathy, diabetic nephropathy, as well as other metabolic problems such as non-alcoholic fatty liver disease and skeletal muscle. However, similarly to previous instances, the precise mechanism of action is not yet understood. Some researchers propose that SFN may play an active role in activating the nuclear factor erythroid 2-related factor 2 or effectively modulating AMP-activated protein kinase to offer protection against diabetic complications (Mthembu et al., 2023).

A potential limitation of a study investigating the protective effects of SFN against toxic substances is the extrapolation of findings from experimental models to clinical settings. While the present *in vitro* and *in vivo* reported studies provide valuable insights into the mechanisms and efficacy of SFN, translating these results to human populations may pose challenges due to differences in metabolism, dosage requirements, and individual variability.

Regarding the evaluation of risk of bias, the present study has considered several variables, including the clarity of the objectives, the characterization of the product under investigation, the reproducibility of the assay, comparability, and the adequacy of statistical analysis. Out of the 87 studies included, only two exhibited a medium risk of bias across these parameters, while the remaining studies demonstrated a low risk. This indicates a generally robust methodological quality across the majority of the selected studies, enhancing the reliability of the findings.

Consequently, the potential SFN protection mechanism holds promise in countering the impact of new toxic agents and presents a great potential for therapeutic applications as an antioxidant, anti-inflammatory, antidiabetic, and anticancer substance. However, although there is a large body of research on liver and cerebral protective effects, there are still studies that do not clarify the mechanisms of action of SFN against metabolic disorders or endocrine disruption. Therefore, further research is needed to exploit the mechanisms of action of SFN and thus to broaden the potential safe therapeutic applications of this substance.

CRediT authorship contribution statement

Antonio Cascajosa-Lira: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ana I Prieto:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Silvia Pichardo:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Angeles Jos:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Ana M Cameán:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.phymed.2024.155731.

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