Toxicology Reports 12 (2024) 148–157

Contents lists available at ScienceDirect

Toxicology Reports

journal homepage: www.elsevier.com/locate/toxrep

# Carbamate compounds induced toxic effects by affecting Nrf2 signaling pathways

Mohammadbagher Nasrabadi [a], Maryam Nazarian [a], Majid Darroudi [b,c], Somayeh Marouzi [c,d], Mohammad Sadra Harifi-Mood [a], Saeed Samarghandian [e,*], Tahereh Farkhondeh [f,*]

[a] Student Research Committee, Birjand University of Medical Sciences, Birjand, Iran
[b] Nuclear Medicine Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Department of Basic Sciences, Neyshabur University of Medical Sciences, Neyshabur 9318614139, Iran
[d] Department of Medical Biotechnology and Nanotechnology, Faculty of Medicine, Mashhad, Iran
[e] Healthy Ageing Research Centre, Neyshabur University of Medical Sciences, Neyshabur 9318614139, Iran
[f] Department of Toxicology and Pharmacology, School of Pharmacy, Birjand University of Medical Sciences, Birjand, Iran

ARTICLE INFO

Handling Editor: Prof. L.H. Lash

*Keywords:*
Nrf2 pathway
Carbamates
Oxidative stress
Toxicity

ABSTRACT

Carbamate (CBs) is a class of insecticides which is being known as an important cause of intentional or accidental poisoning. CBs, cause carbamylation of acetylcholinesterase at neuronal synapses and neuromuscular junction. Exposure to CBs through skin contact, inhalation, or ingestion can result in significant cholinergic toxicity. This is due to the elevation of acetylcholine levels at ganglionic synapses found in both the sympathetic and parasympathetic nervous systems, as well as muscarinic receptors located in target organs of the parasympathetic nervous system, nicotinic receptors situated in skeletal muscle tissue, and the central nervous system. The association between human illnesses and environmental exposures to CBs have been extensively studied in several studies. Although CBs-triggered toxicity leads to overproduction of reactive oxygen species (ROS), the detailed association between the toxicity under CBs exposure and NFE2-related factor 2 (Nrf2) signaling pathways has not been completely clarified. In this review we aimed to summarize the latest findings on the functional interrelationship between carbamates compounds and Nrf2 signaling.

## 1. Introduction

Carbamates (CBs) are a N-methyl group of organic compounds derived from carbamic acid (NH2COOH)[1]. They formed by replacing one or more of the hydrogen atoms by other organic functional groups. Although carbamic acids are unstable, different types of CBs (covalent or ionic) are stable and many divalent carbamate groups create polymers [2]. They are using in medicine for the treatment of glaucoma, myasthenia gravis, and Alzheimer's disease (echothiophate, pyridostigmine, tacrine, and donepezil) as well as the reversal of neuromuscular blockade (neostigmine, pyridostigmine, edrophonium) [3]. CBs have been used as insecticides worldwide for more than 20 years [4]. These insecticides contain carbon, hydrogen, oxygen, and nitrogen without chlorine or phosphorus, and can enter the body through inhalation, skin and digestion (Fig. 1) [5].

These compounds offer multiple advantages to society, such as safeguarding and enhancing agricultural productivity, and protecting humans and animals from illnesses transmitted through insect vectors [6]. CBs reversibly bind to acetylcholinesterase and have a similar toxicological presentation to organophosphates poisonings [7]. Despite

*List of abbreviations:* AChE, Acetylcholinesterase; ARE, Antioxidant response element; Bach1, BTB and CNC homology 1; BHBA, Bis (2-hydroxybenzylidene) acetone; BTB, Broad complex, Tramtrack, and Bric-a-Brac; bZIP, Basic leucine zipper; CAT, Catalase; CBP, CREB-binding protein; CBs, Carbamates; CBZ, Carbendazim; CNS, Central nervous system; CS-NPs, Chitosan nanoparticles; CTR, C-terminal domain; CTR, C-terminal region; Cul3, Cullin-based E3 ligase; DC, Double Clap; DGR, Double glycine repeat or Kelch repeat; EPA, Environmental Protection Agency's; GPx, Glutathione peroxidase; GR, Glutathione reductase; GSH, Glutathione; GSHPx, Glutathione peroxidase.; GSSG, Glutathione disulfide; GST, Glutathione-S transferases; iNOS, Inducible nitric oxide synthetase; IVR, Intermediate variable region; Keap1, Kelch-like ECH-associated protein 1; MDA, Malondialdehyde; MMP, Mitochondrial membrane potential; Neh, Nrf2-ECH homology; Nrf2, NFE2-related factor 2; NTR, N-terminal domain; ROS, Reactive oxygen species; sMaf, Small musculoaponeurotic fibrosarcoma; SOD, Superoxide dismutase; Srx, Sulfiredoxin; TAC, Total antioxidant capacity; TBARS, Thiobarbituric acid reactive substances.

* Corresponding authors.
*E-mail addresses:* samarghandians1@nums.ac.ir (S. Samarghandian), farkhondeh2324@gmail.com (T. Farkhondeh).

https://doi.org/10.1016/j.toxrep.2023.12.004
Received 6 June 2023; Received in revised form 7 December 2023; Accepted 11 December 2023
Available online 6 January 2024

their limited bioaccumulation potential and short-term toxicity, they classified as known endocrine disruptor chemicals and are on the United States Environmental Protection Agency's (EPA) priority list [8]. Many carbamates act as neurotoxins, the duration of their toxicity is less than 24 h, and reduce the amount of cholinesterase enzyme[9,10]. For instance carbaryl (1-naphthyl methylcarbamate), as an insecticide, is a member of the carbamate family. It is a white crystalline solid first introduced in 1958 [11]. Carbaryl has been widely used in public health and agricultural programs as a pesticide. It can be used in the form of a spray to fight against adult mosquitoes, and since its toxicity to mammals is negligible, it is also used to fight against fleas and lice [12]. The association between human illnesses and environmental exposures to CBs have been extensively studied in several studies.Fig. 2.

Carbamate toxicity is caused following elevated acetylcholine levels at "ganglionic synapses of the nervous systems; the muscarinic receptors on parasympathetic nervous system target organs; the central nervous system; and nicotinic receptors in skeletal muscle tissue". Our understanding of the toxicological impact of CBs has primarily focused on the cholinergic pathways and the resulting elevation of acetylcholine levels in various systems [13–15]. However, the specific mechanisms linking CBs exposure to Nuclear factor erythroid2-related factor (Nrf2) activation and its downstream effects on oxidative stress are areas that require further exploration.

Although CBs-triggered toxicity leads to overproduction of reactive oxygen species (ROS), the detailed association between the toxicity under CBs exposure and Nrf2 signaling pathways has not been completely clarified. Although CBs-triggered toxicity is well-documented and is associated with the overproduction of ROS, the intricate relationship between CBs exposure and the Nrf2 signaling pathways remains incompletely elucidated [16–18]. This review aims to bridge this knowledge gap by summarizing the latest findings on the functional interrelationship between carbamate compounds and Nrf2 signaling. This review study was performed to show the findings of the latest investigations related to the role of Nrf2 signaling in CBs toxicity and also the activating of the Nrf2 signaling to prevent CBs-induced toxicity.

## 2. Nrf2 signaling

### 2.1. Canonical mechanisms of Nrf2 activation

Nrf2 is a transcription factor that belongs to the Cap'n'collar (CNC) family proteins which are a group of the basic leucine zipper (bZIP) type transcription factors (TFs) that have essential roles in cell fate decisions and cellular responses to environmental stresses and stimulations.

It is encoded by the NFE2L2 gene and consists of 605 amino acids, with a molecular weight of approximately 66 kDa. Nrf2 plays a critical role in regulating the expression of genes involved in cellular defense against oxidative stress and inflammation. When activated, it can bind to antioxidant response elements (ARE) sequences in the promoter region of target genes, leading to their upregulation and increased production of antioxidant and detoxification enzymes [19].

## 3. Neh domains

Nrf2 has seven functional domains, Neh (Nrf2-ECH homology) 1 to Neh7[20]. Nrf2 forms heterodimers with small musculoaponeurotic fibrosarcoma (sMaf) proteins, which belong to the Maf family of transcription factors. The Neh1 domain of Nrf2 is responsible for this interaction with sMaf proteins, and together they bind to AREs in DNA to regulate gene expression. In addition to sMaf proteins, Nrf2 also interacts with other transcription partners, such as coactivators and co-repressors, to fine-tune its regulatory activities. For example, Nrf2 can interact with p300/CBP and CREB-binding protein (CBP), which are

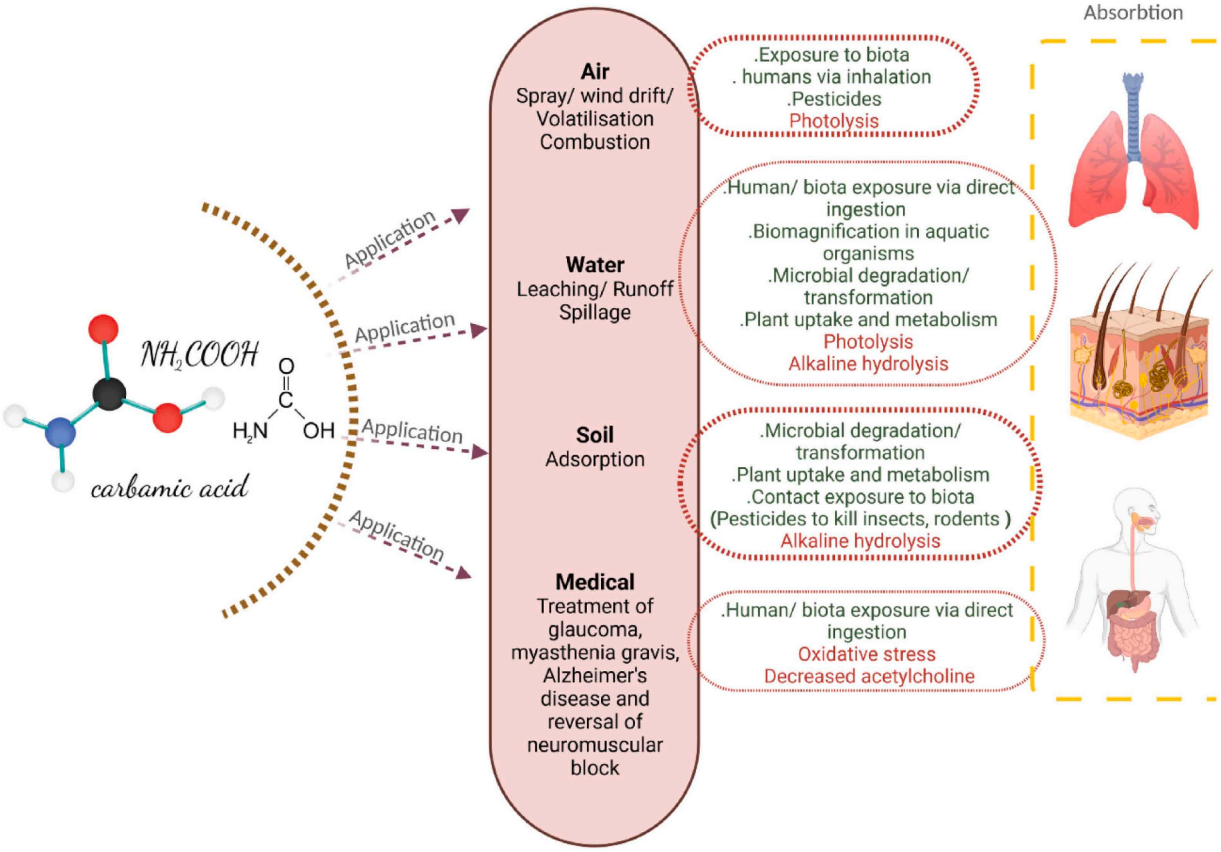


**Fig. 1.** The Human exposure routs to CBs.

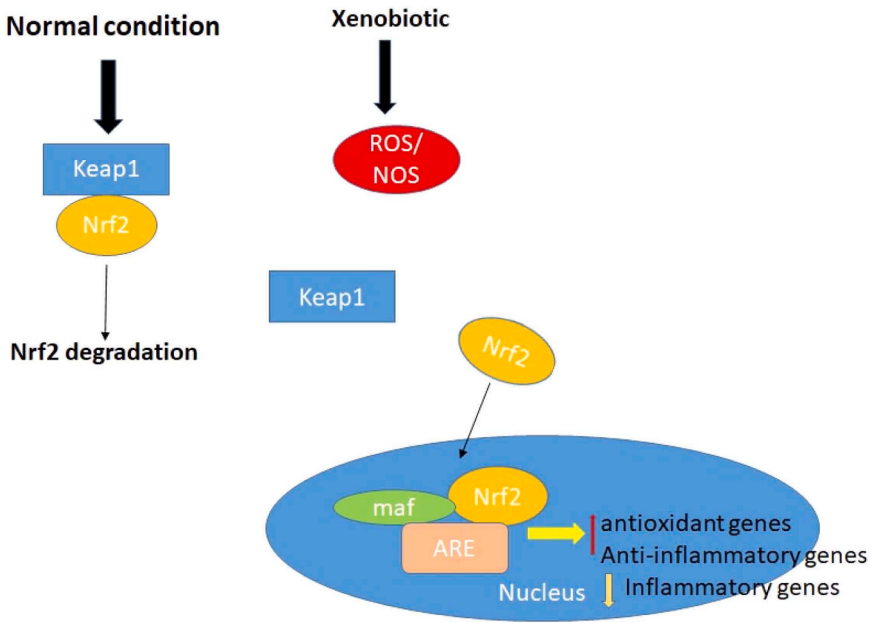


**Fig. 2.** indicates the Nrf2-ARE signaling pathway under normal conditions and/or under xenobiotic stress, and related inflammation and detoxification.

histone acetyltransferases that promote gene transcription by modifying chromatin structure.

Neh4 and Neh5 synergistically recruit CBP molecule [27], leading to Nrf2 acetylation, and increase its activity [28]. Phosphorylation of DSGIS and DSAPGS in motifs Neh6 leads a phosphodegron for Nrf2 ubiquitination by Cul1-β-TrCP complex [29]. In addition, Neh7 domain can modulate the activity of Nrf2 by binding to retinoic acid receptor α.

On the other hand, Nrf2 can also interact with BTB and CNC homology 1 (Bach1), a repressor protein that competes with Nrf2 for binding to AREs and suppresses Nrf2-mediated gene expression [21,22]. Neh1 domain has a CNC-bZIP domain in which DNA binds the sMaf proteins as Nrf2 dimerization partners[23]. Neh2 is in the N terminus of Nrf2 and via interaction with acytoplasmic protein Keap1 regulates cellular stress response[24]. The Neh3 region of Nrf2 is situated towards the end of its protein chain, at the C-terminal. Meanwhile, the Neh4 and Neh5 domains serve as transactivation domains that activate gene transcription by binding with CBP (cAMP-response-element-binding protein-binding protein) [25–27].

The Neh2 domain possess two motifs with peptide bonds (ETGE and DLG) that increase binding of Nrf2 with different proteins. Also, these motifs regulate the stability of Neh2 [28]. Keap1 (Kelch-like ECH-associated protein 1), with its 624 amino acids, recruits Neh2 through recognizing ETGE and DLG motifs and correctly orientate the lysine residues of the Neh2 domain to help ubiquitination for protein turnover [22, 28–30].

## 4. Keap1 functional domains

There are five functional domains that Keap1 possesses, which include the BTB (Broad complex, Tramtrack, and Bric-a-Brac) domain, the IVR (intermediate variable region) domain in the central part, the DGR (double glycine repeat or Kelch repeat) domain, the CTR (C-terminal domain), and the NTR (N-terminal domain) [31]. The BTB domain forms nuclear heterodimers with sMaf as well as contributes to the binding of Keap1 with cullin-based E3 ligase (Cul3), leading to the formation of the E3 complex of Keap1-Cul3-RBX1 ligase (ring box protein 1)[32–35]. The IVR (intervening) region is a flexible, extended helical linker that connects the BTB domain and the DC (double Clap) domain or β-propeller substrate-binding domain. This region contributes to the flexibility of the Cul3-based E3 ubiquitin ligase complex and allows it to bind to a diverse array of substrates. The IVR region also plays an important role in regulating the activity of the E3 ligase complex by modulating the distance between the BTB and DC domains [36]. The Neh2 domain of Nrf2 interacts with the double glycine repeat or Kelch repeat (DGR/Kelch) domain and the C-terminal region (CTR) of Cul3 to facilitate ubiquitination and degradation of Nrf2. The DGR/Kelch domain forms a six-bladed β-propeller structure and serves as the substrate recognition module by binding to specific motifs on Nrf2, while the CTR serves as a docking site for the Neh2 domain. This interaction between Cul3 and Nrf2 is critical for the regulation of cellular oxidative stress responses and maintaining redox homeostasis [31]. The cysteine residues, namely C151, C273, and C288, are extremely sensitive and reactive to covalent modifications caused by electrophiles such as ROS, RNA, H2S, etc. Thus, S-sulfenylation, S-nitrosylation, and S-sulfhydration of these residues contribute to conformational changes of Keap1 that, which in turn, inhibit the stabilization of Nrf2 [37–39]. Nrf2 can be regulated at different levels (from transcription to epigenetic modifications), and it is highly activated in several process including oxidative stress, inflammation, after stimulation with growth factors [40]. Furthermore, Nrf2 signaling is involved in redox signaling, xenobiotic metabolism, metabolism of carbohydrates, lipids and iron, antioxidant responses, and anti-inflammatory responses [41]. On the other hand, NF-κB is a transcription factor that is regulated by redox and it plays a role in controlling both inflammatory responses and cellular damage.

## 5. Non-canonical mechanisms of Nrf2 activation

A group of proteins including DPP3, p62, WTX, PALB2, Prothymosin α, BRCA1 and p21, can directly bind to Keap1 or Nrf2 and dissociate the Keap1-Nrf2 by direct interaction, preventing Nrf2 proteasomal degradation and elevating in the nuclear translocation and activation of Nrf2. The most studied mechanism of the non-canonical pathway activation of Nrf2 is regulated by SQSTM1/p62 protein. p62 can interact with Keap1 induce a dependent autophagy degradation of Keap1 and subsequent Nrf2 stabilization and activation in MEF and HEK293 cells [42].

As ARE sequences are present in the p62 promoter, which it creates a positive feedback loop, p62 expression is regulated by Nrf2. [43]There are some compounds that can regulate the activations of Nrf2 by p62 signaling pathway. As an example, LPS in RAW cells and overexpression

of Sens2 protein in HEK293 and MEF cells can increase the Nrf2 activation [44,45]. However, impairment in autophagy and increase in p62 phosphorylation which can activate Nrf2 sustained, promotes antineoplastic drug chemoresistance to doxorubicin, cisplatin, erastin, sorafenib, buthionine sulfoxamine and carfilzomib which it leads to cancer cell proliferation; although mutation in KIR domain in p62 that prevents Keap1-p62 interaction, is associated with ROS increase and the etiology of amyotrophic lateral sclerosis[35, 42, 46–51]. Overexpression of dipeptidyl peptidase III (DPP3) as a zinc aminopeptidase decreases Nrf2 ubiquitination and increases Nrf2 nuclear migration and activation, while it does not increase Nrf2 levels in HEK293 T cells lead to inhibiting cellular death by decreasing ROS levels; however, in breast cancer cells, an increase in DPP3, is associated with metastasis and drug resistance [52, 53, 54].

WTX is a protein codified by Wilms tumor gene in chromosome X which is mutated in 30% of cases of Wilms tumor. WTX negatively regulates WTN/β-catenin pathway through degradation of β-catenin protein and it is also balance oxidative stress via Nrf2 activation. It is reported that an enhanced level of WTX decreases Nrf2 ubiquitination and activates Nrf2 transcriptional, in an independent way of WNT/β-catenin pathway and electrophilic compounds such as tBHQ [55].

Prothymosin α (ProTα) is another nuclear protein which is associated with cellular proliferation and protection against apoptosis. The interaction between ProTα and Keap1 increases Nrf2 activation and HO-1 expression. Further, this interaction is carried out in the nucleus of HeLa cells and increases in the presence of oxidative stress induced by diethyl maleate [56,57].

The protein partner and localizer of BRCA2 (PALB2) is a nuclear protein that interacts with interact with the Kelch domain of Keap1 through the 91ETGE94 sequence, lead to activation of Nrf2 [58]. Overexpression of Breast cancer type 1 susceptibility protein (BRCA1) as a tumor suppressor protein can increase Nrf2 transcriptional level which it leads to increase antioxidant enzyme expression and protects cells against oxidative stress [59–61].

p21 is a cyclin-dependent kinase inhibitor that is able to inhibit cyclin/CDK complex. P21 interacts with the DLG motif in Nrf2 through the 154KRR156 sequence in its structure, leading to prevents Nrf2 ubiquitination and increasing Nrf2 stabilization [62]. Moreover, overexpression of p21 increases Nrf2 activation and HO-1 and NQO1 expression which it decreases ROS levels and protects JB6P + cells and mouse epidermis against oxidative stress [63]. The non-canonical Nrf2 activation through p21 prevents skin carcinogenesis and the inflammatory response [63]; however, this mechanism has been also reported in cancer cell proliferation and doxorubicin, camptothecin [87] and cisplatin resistance [64]. So, it can support the idea that Nrf2 has dual function in early and longer carcinogenic times. In early carcinogenic processes times, Nrf2 is able to protect cells against the carcinogenic process through the decrease of ROS levels, whereas at longer times, Nrf2 promotes cancer cell proliferation and chemo-resistance [65].

The inhibitor of nuclear factor kappa-B kinase subunit beta (IKKβ) as a serine/threonine kinase is a part of the IKK complex, which phosphorylates the inhibitor of NF-κB family proteins and activates the NF-κB signaling pathway [66]. Phosphoglycerate mutase 5 (PGAM5) is another serine/threonine phosphatase that interacts with external mitochondrial membrane [67]. PGAM5 and IKKβ have 77NXE(S/T)GE82 [68] and 34NQETGE39[69] sequences, respectively, which both of them are able to interact with Keap1 through the Kelch domain. However, to date, there is no evidence which is able to indicate that Keap1 and PGAM5 or Keap1 and IKKβ interaction can increase Nrf2 transcriptional activation; in fact, the enhanced level of PGAM5 prevents Nrf2 activation [67]. In addition to Keap1 dependent Nrf2 ubiquitination and degradation, IKKβ is also a target for Keap1. The interaction between these two proteins targets IKKβ to ubiquitinate and degrade 26 S proteasomal, increase NF-κB cytoplasmic repression by IκBα and decrease nuclear translocation and activation of NF-κB. it can indirectly increase Nrf2 transcriptional activity as NF-κB represses Nrf2 by CBP competition. However, to date, there is no evidence supporting this notion [66, 69, 70].

## 6. Mechanisms of carbamate toxicity

Pesticides primarily cause harm by blocking the action of acetylcholinesterase (AChE) (Fig. 3), which is responsible for breaking down acetylcholine (ACh). ACh is a crucial neurotransmitter in the central nervous system (CNS) of humans, rodents, and insects [71].

Toxic exposures to CBs may occur through ingestion, inhalation, or skin. There is a variable range of CBs toxicity. CBs have moderate ($LD_{50}$ > 200 mg/kg) to highly ($LD_{50}$ <50 mg/kg) toxic activity in rodents (Table 1)[72]. The most frequent causes of CBs poisoning are intentional oral consumption or occupational cutaneous exposure [73]. Large epidemics caused by contaminated food and crops have been documented in the third world countries [74]. After working in regions that have recently been sprayed or fogged with pesticides, exposure can occur via mixed cutaneous and inhalational exposure. It is estimated that more than 3000,000 people are exposed to organophosphate or CBs each year and up to 300,000 death caused by them [75,76]. In toxicity with CBs, ACh accumulates in the body and parasympathetic nerve activity increased. In CBs toxicity, due to the interruption of cholinesterase, ACh is not metabolized naturally and the increase of ACh causes an increase in parasympathetic actions. CBs inhibit cholinesterase enzyme, as a result, several symptoms including slow pulse, diarrhea, vomiting, muscle contractions, and increased body secretions, etc. are observed after toxicity. In acute stage, signs and symptoms appear after 30 to 60 min and reach their maximum intensity within 2 to 8 h. Patient with CBs toxicity may experience various symptoms including anorexia, headache, sleepiness, weakness, hypersalivation, lacrimation, GI distress, bronchorrhea, diaphoresis, nausea, increased secretion of saliva, abdominal cramps, vomiting, sweat, and diarrhea. Generally, poisoning symptoms can be divided into 4 categories as follows: 1-Muscarinic effects: including tears, increased salivation, nausea, vomiting, diarrhea, abdominal pain, acute lung edema due to the secretion of mucus, excessive urination and frequent urination, miosis, bradycardia and hypotension, weakness, lethargy, dizziness, paleness and shock; 2-Nicotinic effects: tremor, convulsion, tachycardia, and hypertension; 3-CNS effects: decreased consciousness, drowsiness, and coma; 4-late symptoms: In some patients, neural damage such as peripheral polyneuropathy may occur with muscle weakness, numb or tingly hands and feet [77]. Table 1 indicates some in vivo studies evaluating main CBs effects. Fig. 4 indicates the effect of CBs on AchE in mammals and insects.

## 7. Oxidative stress indices in carbamates toxicity

CBs are classified as the second most commonly used pesticides in Europe and the US. New research has indicated that CBs may cause toxicity by generating an excess of ROS. Oxidative stress can be triggered by various factors such as xenobiotics, drugs, heavy metals, and ionizing radiation [78]. Oxidative stress contributes to the formation of ROS and electrophiles. ROS include both free radicals (superoxide anion and the hydroxyl radical) and oxidants (hydrogen peroxide). Free radicals and oxidants can cause several diseases such as cancer, cardiovascular complications, inflammatory diseases, neurodegenerative diseases, premature aging, and lower survival rate. Therefore, assessing the changes caused by CBs in oxidative processes can be valuable indicators of exposure to environmental carbamates. This can help in identifying the early toxicity of carbamate pesticides [79]. In a study, Leomanni et al. exposed land snails to the carbamate pesticide carbaryl, at 1 μM for 1 h, and evaluated antioxidant and oxidative stress related responses in snails. The researchers observed various enzymatic antioxidant reactions, ranging from the quick activation of catalase, glutathione peroxidase, and glutathione reductase, to the slower activation of superoxide dismutase. Additionally, they found that there was an

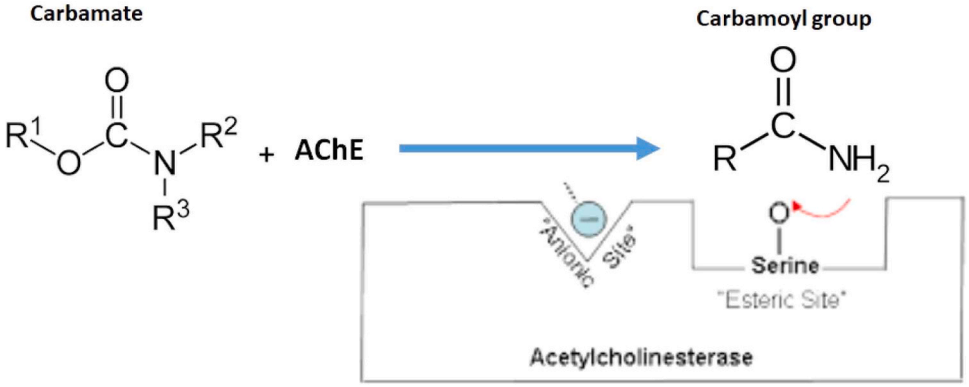


**Fig. 3.** The interaction between CBs and AchE.

**Table 1**
Some in vivo studies evaluating main CBs effects. BW=body weight; IP = intraperitoneally.

| Type of Carbamate | Animal | Dosing and body organ | Results | Reference |
|---|---|---|---|---|
| Bendiocarb | Rabbit | Orally 5 mg/kg/ bw, Testis | Toxicity of lymphoid organs, Hemorrhagic effects on liver and renal parenchyma | [40] |
| Carbaryl | Rat | 10, 30 mg/kg via IP, 2 mL/kg via oral gavage, Brain, plasma, liver, Testis, Blood | Increase in LH and FSH, decrease in testosterone and germ cells | [41–43] |
| Aminocarb | Rat | Orally, 10, 20 and 40 mg/kg bw for 14 days. Blood, liver and kidney | Decrease of testicular weight and profound changes in parenchyma and Leydig cells | [40] |
| Thiodicarb | Rat | 2.9-5.8 mg/kg daily, Liver and heart | Dramatic elevation in liver enzymes after seven day. Inhibiting AChE. No side effects on liver and heart | [44] |
| Pirimicarb | Mouse | Oral gavage 2.14, and 10.7 mg/kg/ day pirimicarb, and dichlorvos plus pirimicarb daily for 30 consecutive days | Dramatic elevation in liver enzymes after seven day. Inhibiting AChE. No side effects on liver and heart | [45] |

unfavorable relationship between the capability of enzymatic antioxidant defense and the intensity of oxidative stress [79]. Salazar-Flores et al. evaluated oxidative markers [glutathione (GSH), glutathione disulfide (GSSG), carbonyl groups, nitric oxide metabolites and lipid peroxides] in farmers who had daily exposure with CBs. Their results showed increased levels of oxidative stress in occupationally exposed farmers compared with the control group [80]. Another study, Maran et al. evaluated the effects of four CBs (aldicarb, aldicarb sulfone, aldicarb sulfoxide, and propoxur) on glutathione content and the activity of antioxidants after 24-h exposure. CBs exposure significantly reduced GSH, no change was observed in GSSG, and GSH/GSSG ratio and glutathione peroxidase (GPx) activity were decreased [81]. Furthermore, SOBEKOVÁ and colleagues analyzed the impact of bendiocarb on superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSHPx), glutathione reductase (GR), glutathione-S transferases (GST), and the levels of thiobarbituric acid reactive substances (TBARS)- in the liver and kidney of rabbits that were exposed to the substance. Bendiocarb significantly decreased GSHPx- ten days after the experiment.

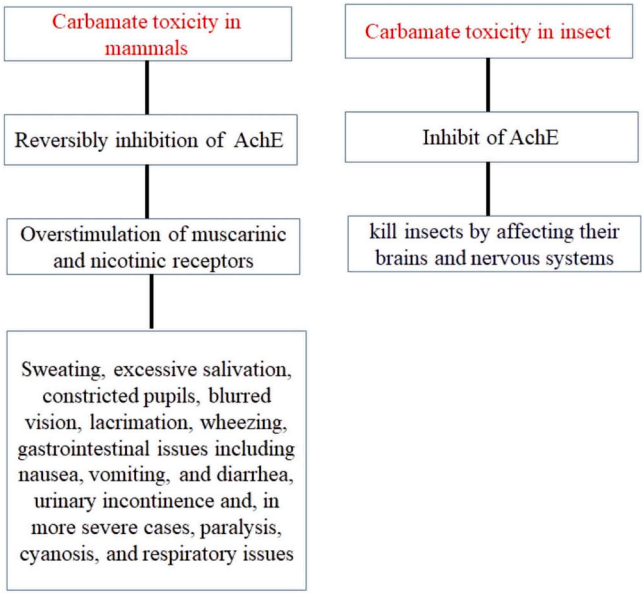


**Fig. 4.** The effect of CBs on AchE in mammals and insects.

Moreover, the GST activity was significantly increased after nine days of being exposed. The study found that in the kidney, SOD activity was notably enhanced, while CAT and GSHPx-H2O2 were significantly reduced in the experimental groups. The findings suggest that organs can respond differently to CBs, depending on the type of damage and their protective capabilities [82]. A.Mecdad et al. investigated and compared the levels of GST, GR, **malondialdehyde** (**MDA**), and total antioxidant capacity (TAC), and immunomodulatory effects (IgG, IgM, TNF-α) of CBs exposure in blood samples of agricultural workers. Their result showed decreased TAC, IgM and IgG while, MDA and TNF-α levels showed significant increase in CBs-exposed workers compared with the control group[83]. In summary, the data indicates that the harmful effects of CBs may result from oxidative stress, which leads to an increase and buildup of ROS. Consequently, controlling oxidative stress through enhanced antioxidant defense (such as through the consumption of fruits and vegetables) can be a potential therapeutic approach for mitigating CBs-induced toxicity [84]. For instance, Li et al. evaluated protective effects of mulberry fruit against ethyl carbamate-induced cytotoxicity and oxidative stress. Mulberry through reduction of ROS showed potent antioxidant capacity and protected human liver HepG2 cells from ethyl carbamate-induced cytotoxicity. Ethyl carbamate increased intracellular GSH depletion and caused mitochondrial membrane potential (MMP) collapse, whereas Mulberry considerably inhibited GSH depletion and restored the mitochondrial membrane

function [84].

## 8. Carbamates and Nrf2 signaling

Maintaining homeostasis and the redox balance in cells and tissues, as well as protecting against oxidative stress, are other responsibilities of Nrf2 signaling pathway. In fact, the Nrf2 pathway is considered the most important mechanism of defense against oxidative damage [85]. Nrf2 and its inhibitor, Keap1, are intrinsic cellular defense systems that are present in all organisms and play a crucial role in combating oxidative stress. The function of Nrf2 is to maintain the balance of redox by regulating genes such as GSS, GPX4, GCLC, and SLC7A11, in addition to its role as a transcription factor. Studies have investigated the impact of various common CBs, such as aldicarb, carbofuran, carbaryl, ethinenocarb, fenobucarb, oxamyl, methomyl, pirimicarb, propoxur, and trimethacarb on this system [1]. In this context, one study has demonstrated that blocking the Nrf2 signaling pathway can trigger ferroptosis, a type of cell death. CBs could activate cell death by inhibiting the Nrf2 signaling pathway. It was discovered that ethyl carbamate reduced cell viability, GSH, GPX4, and ferritin levels, resulting in ferroptosis in liver cells. Moreover, ethyl carbamate led to liver dysfunction, inflammation, oxidative stress, and reduced Nrf2 signaling in Balb/c mice [18]. Mancozeb (MZ) is a widespread carbamate fungicide caused developmental change in *Drosophila melanogaster*. It was reported that MZ caused phenotype and behavioral changes in young flies, due to induction of oxidative stress, resulted in deregulation of genes involved in metabolism, growth and sleep. MZ could alter glucose, proteins, and triglycerides content by inhibiting oxidative phosphorylation at complex I. MZ was able to inhibit the expression of genes that are responsive to oxidative stress, and metabolism including Nrf2 [17].

Shen et al. found a curcumin derivative that via activation of the Nrf2 signaling inhibited vinyl carbamate-induced lung cancer. They found that bis(2-hydroxybenzylidene)acetone (BHBA) activated the Nrf2 pathway in the canonical Keap1-Cys151-dependent manner and significantly decreased lung cancer [86]. Yates and colleagues found that the loss of Nrf2 signaling in mice leads to increased vulnerability to acute toxicity, inflammation, and carcinogenesis due to the inability to respond adaptively [87]. On the other hand, disrupting Keap1 can provide protection against these stresses in mice. However, recent research has identified inactivating mutations in Keap1 in some human cancers [87–89]. Mice that lack the Nrf2 gene are more vulnerable to various types of cancer caused by chemicals. In one experiment, Nrf2-deficient mice developed only half as many lung tumors induced by urethane (ethyl carbamate) compared to normal mice [90]. Chu et al. found Tetrastigma hemsleyanum vines (TVP, a polysaccharide) inhibited cytotoxicity and genotoxicity, attenuate oxidative damage and mitochondrial dysfunction induced by CBs in Caco-2 cells [91]. Moreover, TVP could ameliorate oxidative stress and reduce toxicity via upregulation of Sirt1-FoxO1 and Nrf2-Keap1 signaling pathways [91]. Staab et al. found that Aldicarb causes acetylcholine to accumulate in synaptic clefts, leading to oxidative stress, resulting in muscle contraction and eventual paralysis [92]. Under these conditions, the activation of Nrf2 may impact cellular processes of a distant tissue through hormonal signaling. This could potentially protect the nervous system from harm caused by oxidative stress [92]. Kim and Sieburth conducted an analysis to identify suppressors of aldicarb resistance caused by arsenite, which activates SKN-1 (a homolog of Nrf2). They discovered two receptor tyrosine kinases, DAF-2 and EGL-15, that regulate neuromuscular junctions in response to stress independently of SKN-1 and SPHK-1. The study found that the regulation of NMJ function in response to oxidative stress requires the EGL-15 ligand EGL-17 FGF and canonical EGL-15 effectors [93,94]. In another investigation, Khan et al. examined the effects of carbofuran-induced oxidative stress on cellular autophagy and senescence. Their research indicated that exposure to carbofuran down-regulates Nrf2, and this down-regulation accelerates cellular autophagy through enervation of the Nrf2 signaling pathway [95].

Leomanni et al. found that 1 μM Carbaryl increased lipid peroxidation, increased activities of CAT, SOD, GPx, and GR, and decreased total oxyradical scavenging capacity. Moreover, the higher levels of oxidative stress caused cell apoptosis through downregulation of Keap1/Nrf2/ARE [79]. Mishra et al. found that cigarette smoke and urethane through the activation of Nrf2 significantly stimulated the expression of sulfiredoxin (Srx) levels in cultured normal lung epithelial cells and Srx led to a significantly higher number and larger size of lung tumors [96]. Carbendazim (CBZ, methyl N-(1 H-benzimidazol-2-yl) carbamate) is a systemic fungicide that is commonly used in agriculture to combat fungal infections. In a study, Mo et al. investigated how modified chitosan nanoparticles (CS-NPs) could potentially reduce the liver and kidney damage caused by CBZ in rats [97]. The study discovered that rats receiving CBZ had severe tissue damage in their liver and kidney sections, including cell death and inflammation. These rats also had significantly higher levels of ALT, AST, urea, creatinine, and MDA, and lower levels of TAC, inducible nitric oxide synthetase (iNOS), and caspase-3 protein. Additionally, the study found that CBZ increased oxidative stress, upregulated the Keap1 gene, down-regulated Nrf2, and caused hepatorenal toxicity [97]. Propoxur is a carbamate insecticide called N-methylcarbamate ester (2-isopropoxyphenyl N-methylcarbamate) that has multiple uses. In a study conducted by Shi et al., the effects of propoxur on tumor cell migration and invasion were examined in two human breast cancer cell lines, MCF-7 and MDA-MB-231 cells [98]. The results indicated that propoxur increased MMP-2 expression, thereby promoting tumor cell migration and invasion. This process was facilitated through the up-regulation of the ERK/Nrf2 signaling pathway [98].

The study conducted by Saraiva et al. (2018) indicated that MZ was able to induce Nrf2 in time and dose dependent manner. They found that exposure of flies to MZ at 5 and 10 mg/mL for 2 weeks augmented the expression of Nrf2 to provide cytoprotection temporary against overproduction of ROS and lipid peroxidation. The increase of Nrf2 was associated with increase in the activities of catalase and glutathione S-transferase and decrease in nitric oxide. The findings indicated the adaptative response of antioxidant systemduring MZ exposure [99].

Nrf2 signaling is a main pathway in modulating antioxidant balance, during exposure to carbamates. However, there is several conflicting data about the carbamates effect on Nrf2 signaling. While some data show that carbamates can induce Nrf2 activation by Keap1-depended mechanisms, suggesting protection effect against their toxicity, other studies indicate that the Nrf2–ARE defense pathway is inhibited under exposure to carbamates. However, the final outcome of the Nrf2 activation process was dependent on the time of the exposure. In this context, several studies showed that short exposure time to carbamates significantly potential the antioxidant defense. However, chronic exposure to carbamates could cause Nrf2 hyperactivation in cancer cells and inhibition of Nrf2 in normal cells. Therefore, Nrf2 signaling is considered as a double-edged sword during exposure to carbamates.

Carbamate compounds, widely used as pesticides and insecticides, have been implicated in eliciting oxidative stress and disrupting cellular redox balance. To comprehend the intricate dynamics, it is crucial to scrutinize how carbamates modulate the Nrf2 signaling pathway, a central regulator of cellular defense against oxidative damage. Recent studies have unveiled the dual nature of this interaction, indicating both activating and inhibitory effects on Nrf2, depending on various factors such as the specific carbamate compound, exposure duration, and cell type.

One key aspect highlighted in our extended discussion is the direct modulation of Nrf2 activation by carbamates through dynamic interactions with Keap1 or Nrf2. The ability of carbamates to directly bind to these key players in the Nrf2 pathway disrupts the Keap1-Nrf2 complex, preventing Nrf2 degradation. This disruption enables Nrf2 to translocate into the nucleus, where it activates a battery of antioxidant and detoxification genes.

Several investigations have demonstrated that certain carbamates,

including aldicarb, carbofuran, carbaryl, and propoxur, can directly influence the Nrf2 system. This influence is exemplified by carbamates' ability to bind to Keap1 or Nrf2 directly, resulting in the dissociation of the Keap1-Nrf2 complex. Such interactions prevent Nrf2 proteasomal degradation, facilitating its nuclear translocation and subsequent activation. However, the temporal aspect of exposure plays a pivotal role in determining the overall impact on Nrf2 activation. Short-term exposure to carbamates has been shown to potentiate antioxidant defense mechanisms through Nrf2 activation, whereas chronic exposure may lead to Nrf2 hyperactivation in cancer cells and inhibition in normal cells.

Furthermore, it is noteworthy to mention that the activation or inhibition of Nrf2 signaling appears to have context-specific outcomes. For instance, short exposures to carbamates can significantly enhance antioxidant defense, providing a protective effect against oxidative stress. In contrast, chronic exposure may result in Nrf2 hyperactivation in cancer cells, contributing to cancer cell growth and chemoresistance, while inhibiting Nrf2 in normal cells. It has been explored that the temporal dimension of carbamate exposure in shaping the dynamics of Nrf2 activation. Short-term exposure to carbamates is associated with a rapid and robust activation of Nrf2, leading to enhanced cellular antioxidant defenses. In contrast, chronic exposure scenarios reveal a nuanced response, with the potential for Nrf2 hyperactivation observed in cancer cells and, intriguingly, a possible inhibition in normal cells.

This nuanced discussion enriches our understanding of how carbamates influence Nrf2 signaling, shedding light on the molecular intricacies that define their interaction. It also underscored that the dual role of Nrf2 in response to carbamate exposure, emphasizing its importance in both safeguarding cells against oxidative stress and the potential pitfalls associated with hyperactivation in certain cellular contexts (Fig. 5).

## 9. Conclusion

Despite recent global efforts, the effects of CBs on body organs and environmental health still remains inconclusive. Studies have investigated the impacts of CBs such as thiodicarb on various biochemical parameters and blood enzymes in animal models. Overall, following administration, CBs did not significantly change the various biochemical profiles except inhibiting AChE. However, toxic effects of CBs on vital organs including renal, testis, thymus, spleen, and liver on rats have been well described. CBs are a group of insecticides that have been found to cause metabolic disorders, hyperglycemia and oxidative stress in both acute and chronic exposures. Studies have shown that CBs can disrupt glucose metabolism and insulin signaling pathways, leading to hyperglycemia and insulin resistance. This can increase the risk of developing type 2 diabetes and other metabolic disorders. CBs have also been found to induce oxidative stress by generating free radicals and depleting antioxidant defenses in the body. This can lead to damage to cells and tissues and contribute to the development of various diseases. It seems that CBs inhibit AChE and directly affect target organs. CBs induce cellular oxidative stress via overproduction of ROS, affect mitochondrial function, and disrupt neuronal and hormonal status of the body. In mice, enhancing the Nrf2 signaling pathway has been shown to reduce the likelihood of acute toxicity, inflammation, and carcinogenesis. Furthermore, activating the Nrf2 signaling pathway was observed to prevent CBs-induced lung cancer.

CBs can bind to Keap1 or Nrf2 directly and dissociate the Keap1-Nrf2 complex by direct interaction, preventing Nrf2 proteasomal degradation and inducing an increase in the nuclear translocation and activation of Nrf2 Overall, Nrf2 has a dual role in cancer and further exploration is needed to explore the true function of it in cancer signaling pathways. For the prevention of chronic diseases and cancer in which oxidative and inflammatory stress contributes to the pathogenesis, enhancing Nrf2 activity is still a traditional and effective approach. However, studies in

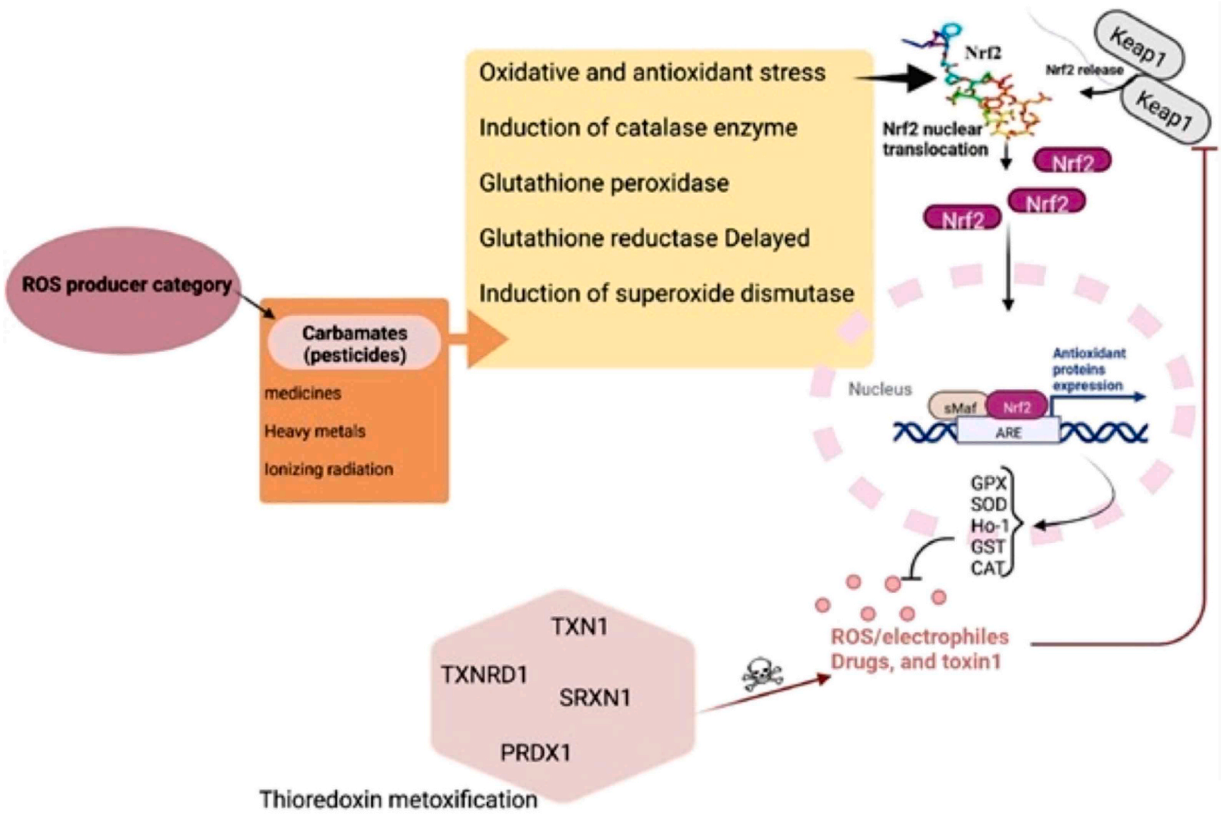


**Fig. 5.** The role of Nrf2 signaling in CBs toxicity.

the past few decades indicated that the overactivation of Nrf2 can promote cancer cell growth and proliferation, inhibit cell apoptosis, strengthen cancer cells self-renewal capacity, most importantly, enhance the chemoresistance and radioresistance of cancer cells. Hence, it is reasonable to consider Nrf2 inhibition in fully malignant cells that be an effective way for cancer prevention. Previous works provided a large number of Nrf2 inhibitors that can regulate Nrf2 at different levels which indicated preclinical effective anticancer results. However, currently, none of these inhibitors yielded strong and practicable results. Although, there are a few small molecules that have been discovered showing promising availabilities in Nrf2 inhibition, further investigations are still needed to be investigated and optimized. An ideal inhibitor for clinical application requires not only potent efficiency and specificity but also less toxicity, good bioactivity, and pharmacokinetics. An impressive strategy should focus on targeting Nrf2 directly and explore indirect methods such as the inhibition of upstream miRNAs or protein kinases. Further studies are needed to clarify inhibitory role of Nrf2 signaling pathway and its activators in CBs-induced toxicity.

Inclusion of these detailed insights elevates our manuscript by providing a comprehensive exploration of the interplay between carbamate compounds and Nrf2 signaling. This enhanced understanding contributes to the broader knowledge of cellular responses to carbamate exposure and establishes a foundation for future research in this critical area.

## Funding

None.

## CRediT authorship contribution statement

**Harifi-Mood Mohammad Sadra:** Writing – original draft. **Nazarian Maryam:** Investigation. **Marouzi Somayeh:** Investigation. **Darroudi Majid:** Writing – original draft. **Nasrabadi Mohammadbagher:** Writing – original draft. **Farkhondeh Tahereh:** Conceptualization, Investigation, Supervision, Writing – original draft, Writing – review & editing. **Samarghandian Saeed:** Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

## 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.

## Data Availability

No data was used for the research described in the article.

## Acknowledgements

Declared none.

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