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Detoxification Mechanisms in Silkworms: A Comprehensive Review of Molecular Responses to Xenobiotic Stress

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Silkworms, particularly *Bombyx mori*, have long been recognized for their economic importance in sericulture, yet their molecular resilience to environmental xenobiotics remains a growing field of interest. This review comprehensively summarizes the current understanding of detoxification mechanisms in silkworms, emphasizing the roles of key enzyme families such as cytochrome P450 monooxygenases (CYPs), carboxylesterases (CarbEs), glutathione S-transferases (GSTs), UDP-glucuronosyltransferases (UGTs), and ATP-binding cassette (ABC) transporters. The activation of

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these detoxification genes in response to insecticides, heavy metals, and plant allelochemicals reveals the silkworm's dynamic molecular adaptations. We also highlight tissue-specific and developmental expression patterns, regulatory pathways including transcription factors and miRNAs, and the interplay between oxidative stress and xenobiotic metabolism. Beyond the domesticated silkworm, comparative insights from wild species such as *Antheraea pernyi* suggest conserved detoxification frameworks across Lepidoptera. The integration of transcriptomics and functional genomics is unraveling complex detoxification networks and aiding the development of pesticide-resistant silkworm strains. This synthesis offers a foundation for future genetic and biotechnological advancements in silkworm breeding, environmental risk assessment, and pest management strategies.

Keywords: Silkworm detoxification; xenobiotic metabolism; Cytochrome P450 monooxygenases (CYPs); Carboxylesterases (CarbEs); Glutathione S-transferases (GSTs); ATP-binding cassette (ABC) transporters; insecticide resistance; transcriptomic response.

1. INTRODUCTION

Bombyx mori Linnaeus, (Lepidoptera: Bombycidae) s a pivotal species in sericulture and insect physiology research, with a fully sequenced genome that lays the foundation for studying detoxification pathways (Xia et al., 2014). Throughout its lifecycle, silkworms face diverse xenobiotics including organophosphate insecticides like phoxim, heavy metals such as lead, and plant metabolites that impair growth, metabolic function, and silk yield when detoxification systems are overwhelmed (Wang et al*.,* 2013; Gu et al., 2014).

Detoxification proceeds through the classical three-phase model**:** Phase I functionalization by cytochrome P450sand carboxylesterases; Phase II conjugation via *glutathione S-transferases* (GSTs); and Phase III efflux facilitated by *ATP-binding cassette (ABC) transporters.* Studies show phoxim exposure markedly increases activities and gene expression of *CYP6ae22*, *CYP9a21*, *GSTo1*, and *BmCC* in the midgut and fat body (Wang *et al.,* 2013); and microarray/qRT-PCR profiling further confirmed induction of multiple CYP family genes in fat body tissues (Li et al*.,* 2015). Protein–protein interaction analysis by Xin & Zhang (2021) demonstrates that P450s, GSTs, and ABC transporters serve as central hubs in silkworm detox networks. Recent transcriptomic investigations into heavy-metal stress revealed over 1,265 differentially expressed genes in midgut tissue and many of them associated with oxidative stress, antioxidant defenses, and detoxification pathways highlighting the broader systemic roles of detox enzymes (Lead exposure transcriptome) (Ye et al*.,* 2024).

Parallel to host metabolism, the gut microbiota of *B. mori* performs remarkable xenobiotic transformations. Yuan*et al.,* (2023) showed that gut bacteria gluocosylate prenylated isoflavones (PIFs) into nontoxic derivatives (GPIFs), enhancing growth when silkworms feed on suboptimal plant sources; probiotic supplementation further boosted performance. Similarly, Chenet al*.,* (2023a) found that cadmium-contaminated mulberry leaves significantly disrupted the gut microbiota composition in *B. mori*, increasing α-diversity, altering dominant bacterial genera in the midgut, and associating with detoxification-related metabolomic shifts and immune modulation. Chen et al*.,* (2024) demonstrated that artificial diet feeding in *B. mori* significantly altered gut microbiota composition and reduced diversity, which was accompanied by differential expression of detoxification-related genes highlighting the link between diet, microbiota, and host detox physiology.

Despite the availability of numerous studies focusing individually on enzyme activation, stress-responsive gene expression, and microbial detoxification in *B. mori*, there remains a lack of comprehensive reviews that holistically integrate these detoxification strategies. Therefore, this review aims to systematically consolidate current knowledge on the structural, transcriptomic, enzymatic, and microbiome-mediated mechanisms involved in xenobiotic detoxification in *B. mori*. It further seeks to elucidate the molecular regulatory pathways such as PI3K/Akt–CncC, FoxO–Keap1, and antioxidant defense systems that govern detoxification responses. Additionally, it highlights underexplored yet critical components like UDP-glucuronosyltransferase (UGT) activity, ATP-binding cassette (ABC) transporter

regulation, and host–microbiota immune interactions. Importantly, while *B. mori* has been the principal model for detoxification studies in sericulture, research on detoxification mechanisms in non-mulberry silkworms such as *Antheraea pernyi* Gue´rin-Me´neville (Lepidoptera: Saturniidae), *Antheraea assamensis* Helfer (Lepidoptera: Saturniidae, and *Samia ricini* Donovan (Lepidoptera: Saturniidae) remains sparse and fragmented, leaving significant gaps in our understanding of their physiological resilience to environmental toxicants. By bridging these multidimensional insights, this review aspires to provide a scientific foundation for advanced strategies in selective silkworm breeding, probiotic supplementation, and sustainable sericulture practices, especially in light of growing environmental and agrochemical stresses.

2. MAJOR CLASSES OF DETOXIFICATION ENZYMES IN SILKWORMS AND THEIR ROLES

2.1 Cytochrome P450 Monooxygenases (CYPs)

Cytochrome P450 monooxygenases (CYPs) are a superfamily of heme-thiolate enzymes (Hrycay and Bandiera, 2012; Zanger and Schwab, 2013) that play a fundamental role in phase I detoxification. These enzymes introduce reactive or polar groups to lipophilic xenobiotics, facilitating their conversion into more water-soluble derivatives, which can then be further processed or excreted. In silkworms such as *B. mori* and *A. pernyi*, CYPs are crucial mediators of insecticide metabolism, plant secondary compound detoxification, and oxidative stress responses.

In response to insecticide stress, particularly organophosphates like phoxim and novel agents such as chlorfenapyr, significant transcriptional upregulation of several CYP gene families has been reported. For example, *CYP6AE22, CYP9A21*, and *CYP4M5* were significantly upregulated in the fat body and midgut following phoxim exposure, suggesting a tissue-specific adaptive detoxification response (Wang et al., 2013; Hu et al., 2018). Xin and Zhang (2021) further confirmed that P450s act as central regulatory hubs in protein–protein interaction networks associated with detoxification in *B. mori*, supporting their systemic role in metabolic defense.

RNA sequencing (RNA-Seq) based studies on chlorfenapyr exposure demonstrated broad transcriptional modulation in silkworm larvae, where multiple CYP genes exhibited differential expression patterns, reinforcing their role in detoxification cascades (Shao et al., 2021). Additionally, functional studies indicated that the knockdown of these upregulated CYP genes increased larval mortality under insecticide challenge, confirming their protective function.

Despite the abundance of detoxification research on *B. mori*, studies exploring these mechanisms in non-mulberry silkworms like *A. pernyi* remain sparse. To address this gap, recent work has provided a genome-wide inventory of detoxification gene families in *A. pernyi*, revealing a significant expansion in the repertoire of detoxification-related genes- 104 CYPs, 32 GSTs, 48 ABC transporters, and 97 CarbEs exceeding the numbers found in *B. mori* (Chen et al., 2023b). Transcriptome analysis following exposure to coumaphos, an organophosphate, identified four significantly upregulated genes (*ABCB1*, *ABCB3*, *ABCG11*, and *ae43*) and one downregulated P450 (CYP6AE9), linking these specific genes to the detoxification response of *A. pernyi* (Chen et al., 2023b). This emerging evidence establishes that *A. pernyi* not only possesses a broader detox gene set than domesticated silkworms but also activates specific genes under xenobiotic challenge, underscoring conserved yet species-specific detoxification strategies across lepidopterans.

Detoxification via CYPs is not only reactive but also regulated by intricate molecular signaling pathways. Hu et al., (2018) revealed that exposure to phoxim activates the PI3K/Akt–CncC/Keap1 axis, which in turn upregulates downstream CYP genes such as *CYP4M5*, *CYP6AE2*, and *CYP9G3*. These findings emphasize a tightly controlled transcriptional response governed by oxidative stress signaling. The expression of CYPs is further influenced by diet. Liu et al., (2023) demonstrated that artificial diets rich in anti-nutritional factors stimulate CYP activity in Malpighian tubules, suggesting that dietary xenobiotics also trigger detoxification gene activation in non-classical tissues. These adaptations highlight the dynamic nature of CYP-mediated responses based on both environmental and dietary inputs.

Collectively, these studies confirm that cytochrome P450 monooxygenases constitute a dynamic and inducible detoxification system in

silkworms. Their gene expression is tightly regulated by external stimuli such as insecticides and heavy metals, internal signals like oxidative stress, and even nutritional inputs. The coordinated action of multiple CYP isoforms enables silkworms to survive in chemically challenging environments, making these enzymes crucial for both survival and silk productivity in modern sericulture.

2.2 Glutathione S-Transferases (GSTs)

Glutathione S-transferases (GSTs) are a major class of Phase II detoxification enzymes in insects, including silkworms, and function primarily by catalyzing the conjugation of reduced glutathione (GSH) to a wide range of electrophilic xenobiotic substrates. This reaction significantly enhances the water solubility and excretability of toxic compounds, rendering them less harmful to host cellular components (Enayati et al*.,* 2005). In *B. mori*, several GST classes such as delta, epsilon, omega, theta, and sigma have been identified, each with distinct substrate affinities and tissue-specific expression patterns (Yu et al*.,* 2008). Exposure to organophosphate pesticides such as phoxim induces the expression of specific GST isoforms (*GSTo1*) in the silkworm midgut and fat body, suggesting their direct involvement in xenobiotic metabolism (Wang et al*.,* 2013). Transcriptomic and enzymatic analyses have shown that exposure to acetamiprid, a commonly used neonicotinoid pesticide, induces significant upregulation of glutathione S-transferase isoforms in *B. mori*. In particular, *GSTe3* and *GSTd1* were significantly upregulated in the midgut following sublethal exposure, with corresponding increases in enzymatic GST activity. These changes were linked to the activation of transcription factors such as *FoxO*, *CncC*, and *Keap1*, underscoring the integral role of GSTs in both detoxification and oxidative stress mitigation (Wang et al*.,* 2020).

Dietary supplementation with quercetin, a naturally occurring flavonoid found in mulberry leaves (Ding et al*.,* 2021), has been shown to significantly induce the activity of detoxification enzymes in *B. mori*, with a pronounced effect on glutathione S-transferases (GSTs). In a study by Zhang et al*.,* (2012), exposure of silkworms to a 1% quercetin-enriched diet for seven days led to a marked increase in GST activity within the midgut tissues. Transcriptional profiling through real-time RT-PCR further revealed substantial upregulation of specific GST genes, notably *GSTe8*, which showed over a seven-fold increase in expression. These findings highlight the responsiveness of GSTs to dietary flavonoids and underscore their crucial role in the detoxification of plant-derived secondary metabolites in silkworms. The results also support the concept that GSTs possess a high degree of regulatory plasticity, enabling *B. mori* to adapt to variable phytochemical exposures encountered during feeding.

Exposure to xenobiotic stress in *B. mori* triggers a coordinated antioxidant and detoxification response, in which glutathione S-transferases (GSTs) play a central role. For example, Muthusamy & Rajakumar (2016) demonstrated that dietary exposure to the organophosphate insecticide dichlorvos significantly increased midgut enzymatic activities of GST, superoxide dismutase (*SOD*), and glutathione peroxidase (*GPx*), whereas catalase (*CAT*) activity decreased, indicating a complex response aimed at countering oxidative damage. Similarly, Ye et al*.,* (2024) conducted transcriptomic analyses in lead-exposed larvae and identified over 1,265 differentially expressed genes in the midgut, including upregulated *GSTd3* and *GSTt1*, along with enhanced expression of *SOD* and *CAT* genes, highlighting the integration of GST activity within broader antioxidant defense networks during heavy metal stress.

Recent genome-wide analysis in the wild silkworm *A. pernyi* has revealed that glutathione S-transferases (GSTs), although less characterized compared to their counterparts in *B. mori*, play a potentially vital role in the species' detoxification processes. Chen et al*.,* (2023b) identified a total of 32 GST genes in the *A. pernyi* genome, indicating a broader detoxification capacity than previously assumed. Under coumaphos exposure- an organophosphate pesticide, transcriptomic profiling showed that several GST were significantly upregulated. This upregulation was accompanied by increased expression of ATP-binding cassette (ABC) transporters such as *ABCB1*, *ABCB3*, and *ABCG11*, suggesting that GSTs may function synergistically with transporters in conjugation and efflux of toxic metabolites. Functional enrichment analysis further supported the involvement of these enzymes in xenobiotic response pathways, particularly those associated with protein processing in the endoplasmic reticulum. These findings emphasize the adaptive detoxification mechanisms in *A. pernyi* and highlight GSTs as critical components in

managing organophosphate-induced stress (Chen et al., 2023b). These insights collectively highlight GSTs as versatile and inducible enzymes central to silkworm detoxification, linking chemical defense with oxidative stress regulation. Their diverse expression profiles and regulatory responsiveness make them key players in xenobiotic adaptation and valuable targets for enhancing silkworm resilience through molecular or dietary interventions.

2.3 Carboxylesterases (CarbEs)

Carboxylesterases (CarbEs) constitute a vital family of Phase I detoxification enzymes that hydrolyze ester-containing xenobiotics into less toxic and more excretable compounds. In silkworms, these enzymes have been widely implicated in the metabolism of various agrochemicals, notably organophosphate and pyrethroid insecticides. Their detoxification role lies primarily in the cleavage of ester bonds, thereby preventing the accumulation of neurotoxic metabolites and supporting insect survival under chemical stress (Wheelock et al., 2005).

Carboxylesterases (CarbEs) are vital Phase I detoxification enzymes in *B. mori*, responsible for hydrolyzing ester bonds in xenobiotics such as phoxim. Feng et al., (2012) cloned and characterized the CarE gene *Bmae33*, demonstrating its significant upregulation in larval head and adult antennae following phoxim exposure. This tissue-specific induction suggests a protective role in regions directly exposed to environmental chemicals. Although oxidative stress markers were not measured, the pronounced transcriptional response of *Bmae33* underscores the enzyme's importance in silkworm detoxification. These findings highlight CarEs as critical components of a coordinated enzymatic defense system alongside cytochrome P450s and GSTs.

Under chemical stress, CarEs in *B. mori* exhibit notable expansion and tissue-specific expression, underlining their pivotal role in detoxification. According to Yu et al., (2009), the *B. mori* genome encodes approximately 76 carboxylesterase genes, many of which are expressed in detox-relevant tissues such as the midgut, head, integument, and silk glands. Functional annotation suggests that midgut-specific CarEs help detoxify both mulberry allelochemicals and dietary insecticide residues, while head- and integument-expressed esterases are likely involved in environmental sensing and odorant degradation. This genomic architecture highlights the adaptive versatility of silkworm CarEs and their centrality in detoxifying xenobiotics ingested during feeding or contacted during environmental exposure.

Beyond *B. mori*, evidence from wild silkworms such as *A. pernyi* also supports a conserved role for CarEs. The study by Chen et al., (2023b) identified 97 carboxylesterase (COE) genes in the genome of *A. pernyi*, classifying them into eight subfamilies, with the α-esterase (ae) subfamily being the most expanded. Transcriptomic analysis revealed that the ae43 gene was significantly upregulated in larvae exposed to coumaphos, suggesting a potential role in detoxification. This aligns with the known function of COEs in metabolizing organophosphorus compounds. The expansion of COE genes, particularly in the *ae*, *gli* (gliotactin), and *ie*(integument esterase) subfamilies, highlights their evolutionary significance in detoxification pathways, reinforcing their conserved role across Lepidoptera, including both wild and domesticated species.

The intricate diversity, tissue specificity, and inducibility of CarE genes in silkworms reflect an evolutionarily optimized response system against a spectrum of xenobiotic pressures. As environmental pesticide loads increase, especially in sericultural zones, the molecular characterization and functional validation of key CarEs offer a promising avenue for developing insecticide-resilient silkworm strains. Furthermore, leveraging these detoxification signatures through genetic selection, CRISPR-based editing, or enzyme biomarkers could lead to more sustainable pest management and improved silkworm health under agrochemical exposure.

2.4 ATP-Binding Cassette (ABC) Transporters

ATP-binding cassette (ABC) transporters are essential membrane proteins that function in Phase III detoxification by actively exporting xenobiotics and their metabolites out of cells, thus preventing intracellular accumulation and cellular damage. In insects, ABC transporters have been extensively studied for their roles in insecticide resistance, stress tolerance, and toxicant clearance (Dermauw & Van, 2014).

In *B. mori*, genome-wide identification of ATP-binding cassette (ABC) transporter genes has revealed 53 members, classified into eight subfamilies (ABCA–ABCH) with distinct tissue-specific and inducible expression patterns (Xie et al., 2012). Notably, among these, members of the *ABCB*, *ABCC*, and *ABCG* subfamilies exhibit high expression in detoxification-related tissues such as the midgut and Malpighian tubules, with some ABCG transporters such as *BmABC005226*, demonstrating 20-hydroxyecdysone-responsive regulation, suggesting their involvement in both development and xenobiotic efflux (Liu et al., 2011).

Transcriptomic analysis of *B. mori* midgut under lead stress has revealed profound molecular alterations, notably including transporter activity pathways. Specifically, Bian et al., (2025) identified 1,418 differentially expressed genes (DEGs), with significant enrichment observed in ABC transporter, glutathione metabolism, and oxidative stress response categories consistent with coordinated detoxification and cellular defense mechanisms in silkworm tissues (Bian et al., 2025). These findings support the concept that ABC transporters operate in concert with antioxidant systems, facilitating reactive oxygen species (ROS) scavenging and xenobiotic efflux to maintain physiological homeostasis under heavy metal exposure.

Research in *Spodoptera exigua* has provided compelling evidence on the role of ABC transporters in Bt toxin resistance. Pinos et al., (2019) demonstrated that the *SeABCC2* transporter acts as a functional receptor for the Cry1A family of Bt toxins, including Cry1Ca. Using ligand-binding assays, heterologous expression, and RNA interference, they showed that loss of *SeABCC2* function significantly reduces Cry1A toxicity in larval cells and organisms, establishing *ABCC2* as a critical determinant of Cry toxin susceptibility. While such detailed studies have not yet been performed in *B. mori*, the conserved nature of *ABCC2* across lepidopteran species implies a potentially similar function in silkworm detoxification and immune response.

Given their inducibility and specificity, ABC transporters represent a crucial interface between cellular detoxification and environmental adaptation. Their integration into hormonal and xenobiotic signaling frameworks points to a broader physiological significance that extends beyond classical transport roles. Focused functional studies will be key to decoding their regulatory plasticity and potential application in stress-resilient silkworm strains.

3. KEY REGULATORY PATHWAYS

3.1 PI3K/Akt/CncC Pathway

The PI3K/Akt/CncC signaling axis is a critical regulator of xenobiotic detoxification and oxidative stress response in lepidopteran insects. In *Bombyx mori*, activation of this pathway under pesticide exposure leads to transcriptional upregulation of key detoxification genes, including members of the cytochrome P450 family and glutathione S-transferases. PI3K activation initiates a phosphorylation cascade through Akt, promoting the nuclear translocation of the transcription factor CncC, a homolog of mammalian Nrf2, which binds to antioxidant response elements (AREs) and enhances detox gene expression. This molecular mechanism is increasingly recognized as central to the adaptive response of silkworms to environmental toxicants.

Several studies have demonstrated that the expression of detoxification-related enzymes such as glutathione S-transferases (GSTs), cytochrome P450 monooxygenases (P450s), and carboxylesterases (CarEs) is modulated by the CncC/Keap1 signaling axis, which is itself subject to upstream regulation by the PI3K/Akt pathway (Wilding, 2018). Phosphatidylinositol 3-kinase (PI3K) functions as an intracellular lipid kinase, and its downstream effector, protein kinase B (Akt), plays a central role in signal transduction following PI3K activation (Vara et al., 2004). The PI3K/Akt signaling pathway is involved in regulating diverse physiological processes, including cell growth, differentiation, and protein synthesis (Hietakangas and Cohen, 2009). Downstream effectors such as p70S6K and phosphoinositide-dependent kinase (PDK) facilitate translation by activating factors like eIF-4E and repressing 4E-BPs, thereby promoting protein synthesis (Quevedo et al., 2002). Additionally, PI3K/Akt signaling modulates the CncC/Keap1 axis, further influencing detoxification responses (Hao et al., 2015). Phoxim exposure, in particular, has been shown to trigger this pathway in *B. mori*, leading to the upregulation of multiple detoxification genes and enhancing the organism's defense against xenobiotic stress (Sykiotis and Bohmann, 2008).

In a study by Zhao et al., (2020a), the effects of pyriproxyfen, a juvenile hormone analog, were analyzed in the fat body of *B. mori*, with a prime focus on the PI3K/Akt/CncC signaling cascade. Pyriproxyfen exposure disrupted larval development and fat body structure, inducing histopathological damage and reducing cocooning rates. Molecular analyses revealed significant upregulation of PI3K, Akt, PDK, p70S6K, CncC, and Keap1, with peak expression observed between 48 and 72 hours. This activation enhanced the expression of downstream detoxification genes such as *CYP9a19*, *CYP9a20*, *GSTd1*, *GSTo3*, *GSTs1*, and *CarE10*, supported by increased enzymatic activity of P450s, GSTs, and CarEs confirming a functional detoxification response.

Tian et al., (2017) further emphasized the role of the PI3K/Akt pathway in regulating protein synthesis and detoxification in response to external stimuli such as TiO_2 nanoparticles and phoxim. TiO_2 NPs upregulated Akt, p70S6K, and moderately elevated 4EBP2 and S6, promoting growth through enhanced nutrient metabolism. In contrast, phoxim induced stronger activation of the pathway, coupled with pronounced expression of detoxification enzymes like P450, GST, and CarE2, reflecting a stress-induced adaptive response. Interestingly, TiO_2 pre-treatment attenuated phoxim's toxic effects, highlighting the pathway's role in differential stress modulation.

A related study by Mao et al., (2019) explored the impact of chlorantraniliprole (CAP) on the fat body of *B. mori*, again emphasizing the PI3K/Akt/CncC signaling axis. CAP exposure significantly upregulated PI3K, PDK, Akt, Keap1, and CncC, with PI3K and CncC showing the highest induction at 72 hours. While CAP inhibited Akt phosphorylation, it concurrently led to reduced Keap1 and enhanced nuclear accumulation of CncC, ultimately upregulating downstream detox genes including P450s, GSTs, and CarEs. These molecular events reaffirm the pathway's central role in xenobiotic stress adaptation.

Cheng et al., (2018) specifically evaluated phoxim-induced responses in the silk gland of *B. mori*, providing direct evidence of PI3K/Akt/CncC pathway activation in this organ. They observed significant transcriptional upregulation of Akt, Tor1, p70S6K, and 4e-bp, alongside downstream effectors CncC and Keap1, particularly at 48 hours post-exposure. This signaling activation was associated with increased expression and enzymatic activity of P450s, GSTs, and CarEs, confirming role of the pathway in silk gland detoxification and maintaining glandular integrity under pesticide stress.

Although the PI3K/Akt/CncC signaling pathway has been extensively studied in *B. mori*, its role in non-mulberry silkworms such as *A. mylitta*, *A. assamensis*, *A. pernyi*, and *S. ricini* remains largely unexplored. These wild sericigenous species are frequently exposed to agrochemicals in forest-based or semi-domesticated environments, yet the molecular basis of their detoxification response is poorly understood. Investigating the operation of this signaling axis in non-mulberry silkworms could uncover critical species-specific mechanisms of stress resilience. Such studies are vital for enhancing the adaptability and sustainability of non-mulberry sericulture under escalating environmental pressures.

3.2 FoxO/CncC/Keap1 Axis

The sublethal exposure of *B. mori* to acetamiprid, a widely used neonicotinoid insecticide, has raised significant concerns regarding its physiological impacts on economically important non-target insects. Central to the insect's adaptive defense mechanisms against xenobiotic stressors is the FoxO/CncC/Keap1 signaling axis, a conserved regulatory pathway that orchestrates detoxification and oxidative stress responses. This pathway regulates the transcription of key detoxifying enzymes and antioxidant genes, enabling insects to maintain cellular homeostasis under pesticide-induced challenges. Recent findings have revealed that even low concentrations of acetamiprid can provoke significant transcriptional and enzymatic responses mediated by this pathway across various silkworm tissues.

Wang et al., (2020) demonstrated that continuous exposure to a sublethal dose of acetamiprid (0.15 mg/L) in the 5th instar larvae significantly disrupted detoxification dynamics in the midgut. Their study revealed an initial upregulation in cytochrome P450 monooxygenases (*CYP4M5* and *CYP6AB4*) and glutathione S-transferases (*GSTe3* and *GSTd1*), indicating an active detoxification response. Notably, GST activity markedly increased from 48 to 96 hours, while carboxylesterase (CarE) genes and enzyme activity were consistently

suppressed. Concurrently, the expression of FoxO, CncC, and Keap1 showed a parallel pattern with GST genes, peaking at 24 hours and declining thereafter. These results strongly indicate that the detoxification process, particularly GST-mediated metabolism, is transcriptionally regulated through the FoxO/CncC/Keap1 axis. The transient upregulation of this pathway suggests an early adaptive attempt to counteract pesticide toxicity, which later diminishes likely due to metabolic burden or accumulated physiological stress.

Complementing these findings, Lu et al*.,* (2021) reported that low-dose acetamiprid exposure induced oxidative stress and developmental impairments in the posterior silk glands (PSGs) of *B. mori*. The treatment resulted in elevated levels of oxidative biomarkers such as hydrogen peroxide (H_2O_2) and malondialdehyde (MDA), accompanied by significant increases in the activities and gene expression of key antioxidant enzymes, including SOD, CAT, GPX, and TPX. Importantly, the study identified consistent and significant upregulation of FoxO, CncC, Keap1, HO-1, NQO1, and sMaf transcripts throughout the exposure period, peaking at 72 hours. This persistent activation of the FoxO/CncC/Keap1 signaling pathway underscores its central role in mediating antioxidant defenses in silk gland tissues. Despite these protective responses, the physiological indicators such as silk gland index and cocooning rate were severely diminished, highlighting the inability of the pathway to fully mitigate long-term oxidative damage at the tissue level.

Collectively, these studies provide compelling evidence that the FoxO/CncC/Keap1 pathway plays an indispensable role in the silkworm's response to acetamiprid-induced stress. While this signaling cascade facilitates both detoxification and antioxidant defense, its efficacy appears time-limited and tissue-dependent. In the midgut, it orchestrates GST-mediated detoxification, whereas in the silk gland, it regulates oxidative stress enzymes to preserve cellular integrity. These insights not only deepen our understanding of insect stress physiology but also emphasize the ecological risks posed by persistent pesticide residues, especially in sericulture environments where silkworm health directly influences economic productivity.

4. TISSUE-SPECIFIC RESPONSES TO XENOBIOTICS

4.1 Midgut

The midgut of the silkworm plays a dual role in both nutrient digestion and absorption, as well as in the defense against chemical pesticides and microbial pathogens (Zhang et al*.,* 2011). As the primary interface with ingested xenobiotics, it acts as a critical barrier and metabolic hub, orchestrating detoxification responses to environmental challenges. The insect midgut is known to produce an array of detoxification enzymes that facilitate adaptation to toxic compounds, including plant allelochemicals and synthetic insecticides (Zhu et al*.,* 2011).

Among these enzymes, cytochrome P450 monooxygenases (CYPs) represent a large, evolutionarily conserved family of multifunctional proteins involved in phase I detoxification processes. They catalyze oxidative reactions, transforming lipophilic xenobiotics into more polar, excretable metabolites (Schuler, 2011). In the silkworm, 17 P450 genes- primarily from the *CYP4*, *CYP6*, and *CYP9* families are reported to be highly expressed in the midgut, underscoring their central role in xenobiotic metabolism (Xia et al., 2007). Exposure to insecticides such as acetamiprid and phoxim significantly upregulates *CYP4M5*, *CYP6AB4*, and other P450 genes in the midgut, indicating their role in silkworm defense and detoxification (Zhou et al*.,* 2012;Gu et al*.,* 2014; Wang et al*.,* 2020).

Carboxylesterases (CarEs) constitute another major class of detoxifying enzymes involved in the hydrolytic degradation of ester-containing compounds (Montella et al*.,* 2012; Hatfield et al*.,* 2016). While CarEs generally contribute to organophosphate detoxification, their expression and activity can be influenced by the type and dose of xenobiotic exposure. For instance, low-dose acetamiprid and chlorantraniliprole were found to inhibit CarE expression and enzymatic activity in silkworms, indicating a potentially suppressive effect on hydrolytic detoxification pathways (Mao et al*.,* 2019). Glutathione S-transferases (GSTs)**,** as phase II enzymes, catalyze the conjugation of glutathione (GSH) to electrophilic xenobiotic substrates, thereby promoting detoxification and reducing oxidative stress (Mannervik et al*.,* 1985; Hayes et al*.,* 2005). Elevated midgut expression of GST genes, such as *GSTe3*, *GSTd1*, and *GSTe8*, has been reported in response to both acetamiprid

and dietary flavonoids like quercetin (Zhang et al., 2012; Wang et al., 2020), further highlighting their protective role against oxidative damage.

4.2 Fat Body

The fat body of *B. mori*, functionally analogous to the mammalian liver, is a central metabolic and detoxification organ responsible for nutrient storage, hormonal regulation, immune defense, and systemic xenobiotic clearance (Tojo et al., 1981). It harbors major detoxification enzymes, including cytochrome P450 monooxygenases (CYPs), glutathione S-transferases (GSTs), and carboxylesterases (CarEs), all of which are inducible in response to chemical stressors (Wang et al., 2013). CYPs, a diverse family of heme-thiolate monooxygenases, mediate oxidative biotransformation of lipophilic xenobiotics, enhancing their solubility and elimination (Hrycay & Bandiera, 2012; Zanger & Schwab, 2013). GSTs catalyze the conjugation of reduced glutathione (GSH) with electrophilic compounds, playing vital roles in cellular protection from oxidative stress and DNA damage (Hayes et al., 2005; Chatterjee & Gupta, 2018). CarEs contribute to phase I detoxification by hydrolyzing ester-containing pesticides and are considered crucial for detoxifying organophosphates and pyrethroids (Montella et al., 2012; Hatfield et al., 2016).

Numerous studies have shown that exposure to insecticides significantly alters the transcription and enzymatic activity of these detoxification systems in the fat body. In response to diverse pesticides including chlorantraniliprole (CAP), pyriproxyfen, phoxim, and λ-cyhalothrin, the fat body upregulates genes such as *CYP4M5*, *CYP6AB4*, *CYP6A8*, *CYP9A19*, *CYP9G3*, *CarE2*, *CarE5*, *CarE10*, *GSTe1*, *GSTe3*, *GSTd1*, and *GSTo3* (Tian et al., 2017; Zhao et al., 2011, 2020a; Hu et al., 2016; Mao et al., 2019). CAP and pyriproxyfen have been shown to activate the PI3K/Akt/CncC signaling pathway, which regulates antioxidant and detoxification responses. Specifically, Mao et al., (2019) reported a dose-dependent induction of PI3K, Akt, CncC, and downstream genes such as *CYP306A1* and *GSTe3*, reflecting a dynamic response to xenobiotic burden. Zhao et al., (2020b) further demonstrated that pyriproxyfen similarly modulated CncC/Keap1 transcription and elevated detox enzyme activity, suggesting conserved stress-responsive pathways.

In addition, phoxim, a potent organophosphate induces transcription of key detox signaling components (PI3K, Akt, CncC, Keap1, Tor1, p70s6k, and 4e-bp) in the fat body, alongside increased CYP, GST, and CarE activities (Hu et al., 2016). Hu et al., (2016) observed that phoxim exposure upregulated *BmCncC* and Akt, while *BmKeap1* was downregulated, implying that detoxification is regulated by redox-sensitive and nutrient-sensing signaling pathways. This response has been associated with increased insecticide tolerance, survival, and cellular resilience. Moreover, phoxim is known to inhibit acetylcholinesterase (AChE) activity in the brain, fat body, and silk gland, potentially exacerbating neurotoxic effects (Nath & Kumar, 1999). Transcriptomic analyses also confirm time-dependent increases in detox gene expression and enzyme activities under prolonged exposure to λ-cyhalothrin. Bian et al., (2022) demonstrated that silkworms raised on artificial diets (AD) showed impaired P450 enzyme activity and pronounced histopathological damage in the fat body such as nuclear shrinkage, mitochondrial swelling, and ER dilation highlighting how nutritional status modulates detoxification efficiency.

In addition to pesticide detoxification, the fat body also responds to heavy metal stress, particularly lead (Pb). Pb exposure results in oxidative damage through activation of the mitochondrial apoptotic pathway (via Bax/Bcl-2 modulation) and DNA damage response (via p53 signaling), leading to increased expression of CYPs and GSTs (Amer et al., 2022; Aathmanathan et al., 2018; Hou et al., 2025). These findings underline the broad-spectrum detoxification capacity of the fat body against both organic and inorganic toxicants.

Comparative studies in the wild silkworm *A. pernyi* reveal a more robust detoxification repertoire. Elevated GST activity in the fat body of *A. pernyi* is correlated with enhanced tolerance to coumaphos, an organophosphate pesticide, indicating interspecific variation in detoxification potential (Li et al., 2015). This evolutionary divergence presents an opportunity for genetic improvement of domestic strains. Despite its inducibility, the baseline activity of detoxification enzymes in *B. mori* remains considerably lower than in generalist herbivores like *Helicoverpa armigera* (Wang et al., 2013). This reduced detox capacity is likely a result of long-term domestication and reduced exposure to plant secondary metabolites or environmental

xenobiotics. As such, understanding the fat body's biochemical responses offers a critical pathway for improving silkworm resistance via breeding, diet modification, or biotechnological intervention.

4.3 Malpighian Tubules

The Malpighian tubules of *B. mori* are critical excretory and osmoregulatory organs analogous to the vertebrate kidney. Beyond their classical role in waste elimination, these tubules function as a vital interface for xenobiotic clearance, facilitating phase I, II, and III detoxification processes. They serve as a major site for the biotransformation and excretion of both endogenous metabolic byproducts and exogenous toxicants.

Recent transcriptomic studies have emphasized the responsiveness of Malpighian tubules to dietary and environmental stressors. Notably, silkworms reared on artificial diets (ADs) exhibit marked upregulation of detoxification-associated genes in the Malpighian tubules, reflecting an adaptive response to nutritional and chemical imbalances. Liu et al*.,* (2023) reported significant induction of multiple gene families involved in xenobiotic metabolism, including cytochrome P450 monooxygenases (CYPs), glutathione S-transferases (GSTs), UDP-glycosyltransferases (UGTs), and ATP-binding cassette (ABC) transporters. These gene classes represent the core enzymatic systems underpinning the three-phase detoxification cascade—oxidation, conjugation, and excretion. Activity assays confirmed increased CYP and GST levels in artificial diet-fed larvae, and transcriptome analyses identified over 2400 DEGs, many involved in detoxification pathways (Liu et al*.,* 2023).

4.4 Silk Glands

Although the silk gland of *B. mori* is primarily recognized for its role in silk protein synthesis, emerging evidence suggests that it also participates in localized detoxification responses under chemical stress. This gland is responsible for the production of fibroin and sericin, key proteins determining cocoon yield and quality making it a critical organ for sericultural productivity (Li et al*.,* 2013; 2014). However, xenobiotic exposure, particularly to organophosphorus (OP) insecticides such as phoxim and diamides like chlorantraniliprole (CAP), has been shown to compromise silk gland integrity and function.

Phoxim accumulation within the silk gland persists for up to 24 hours post-exposure, with residue levels surpassing those in hemolymph, indicating inefficient systemic clearance and gland-specific sequestration (Cheng et al*.,* 2018). This accumulation leads to significant physiological disruptions, including inhibition of fibroin synthesis, apoptosis of glandular epithelial cells, and eventual failure of cocoon formation (Ma et al*.,* 2013; Cheng et al*.,* 2018). Pathological assessments have revealed progressive cellular damage with increased phoxim exposure duration, characterized by structural degeneration of secretory regions (Gu et al*.,* 2014).

At the molecular level, the silk gland mounts a detoxification response by upregulating a suite of stress-responsive genes. Cheng et al*.,* (2018) and Wang et al*.,* (2013) reported the transcriptional induction of phase I, II, and III detoxification enzymes including *CYP6AB*, *GSTd1*, and *CarE2* following phoxim exposure. This enzymatic response is complemented by activation of the PI3K/Akt signaling pathway, which regulates cell growth, survival, and oxidative stress response. Upregulation of key pathway components such as Akt, Tor1, p70s6k, and 4e-bp reflects an intrinsic attempt to restore homeostasis and mitigate xenobiotic toxicity (Cheng et al*.,* 2018). Similarly, exposure to chlorantraniliprole induces endoplasmic reticulum (ER) stress within silk gland cells, marked by oxidative damage and morphological abnormalities such as ER dilation and mitochondrial swelling (Mao et al*.,* 2019). RNA-seq and enzyme activity assays confirmed the induction of detoxification enzymes, CYPs, GSTs, and CarEs suggesting that the silk gland is not a passive target but a responsive tissue capable of initiating a defense program under toxic insult.

Moreover, organophosphate insecticides like phoxim not only impair silk gland function but also interfere with broader physiological processes including neural transmission, growth, and reproduction (Li et al*.,* 2010; Nath & Kumar*,* 1999). Phoxim has been shown to inhibit acetylcholinesterase activity in the brain, fat body, and silk gland, exacerbating systemic toxicity (Nath & Kumar*,* 1999).

5. DETOXIFICATION IN WILD SILKWORM

The Chinese oak silkworm, *A. pernyi*, is one of the most prominent wild silkworm species and remains strictly oligophagous, feeding exclusively on oak (*Quercus*) leaves throughout its larval stage (Liu et al., 2010; Li et al., 2017, 2021). It has been commercially reared for silk production across China, India, Japan, and Korea for centuries (Liu et al., 2010). In sericultural practice, the organophosphorus insecticide coumaphos, known commercially as Miecanying in China, is used internally to eliminate parasitic fly larvae within *A. pernyi* without causing harm to the host at appropriate concentrations (Li et al., 2020). Coumaphos exerts its insecticidal activity by inhibiting acetylcholinesterase activity, thereby disrupting neural transmission (Li et al., 2015; Cheng et al., 2017; Pearce et al., 2017). However, at elevated doses, coumaphos can induce neurotoxic effects in *A. pernyi*, manifesting as physiological symptoms such as gastric regurgitation and failure to climb (Li et al., 2015).

Comparative genomics has revealed that *A. pernyi* harbors a considerably richer detoxification gene repertoire than *Bombyx mori*. A total of 281 detoxification-related genes have been identified across 46 chromosomes in *A. pernyi*, distributed among four major gene families: 104 cytochrome P450 monooxygenases (CYPs), 97 carboxylesterases (COEs), 48 ATP-binding cassette transporters (ABCs), and 32 glutathione S-transferases (GSTs) (Chen et al., 2023b). This expansion likely equips *A. pernyi* with superior adaptive capabilities for managing xenobiotic stress. Phylogenetic and Hidden Markov Model (HMMER) analyses support the classification of these genes and their putative roles in detoxification across multiple chemical classes. Moreover, this genomic enrichment is consistent with broader findings in Lepidoptera, where significant expansions in detoxification gene families have been attributed to transcriptomic and genomic plasticity in response to environmental challenges (Cheng et al., 2017; Kawamoto et al., 2019; Yu et al., 2009; Liu et al., 2011; Xie et al., 2012; Yu et al., 2008). These results collectively demonstrate how gene family expansion may enhance metabolic flexibility and pesticide resistance, especially for wild or semi-domesticated species such as *A. pernyi* that nevertheless face a variety of environmental pollutants in their native environments.

Transcriptome profiling of fat body tissues treated with coumaphos at two time points demonstrated significant differential expression in five detoxification-related genes: *ae43*, *CYP6AE9*, *ABCB1*, *ABCB3*, and *ABCG11* (Chen et al., 2023b). Notably, *ABCB1*, *ABCB3*, and *ABCG11* were upregulated in response to coumaphos, consistent with previous observations of ABC transporter-mediated detoxification in other insect species (Sun et al., 2017; Li et al., 2022). This suggests that ABC transporters, particularly from subfamilies B and G, may play pivotal roles in phase III detoxification and xenobiotic efflux in *A. pernyi*. In addition, GST activity in the fat body was significantly elevated following coumaphos exposure, supporting earlier reports of GST-mediated detoxification in this species (Li et al., 2015), as has been documented across various insect taxa (Ranson et al., 2002).

Despite this, both *A. pernyi* and *B. mori* possess fewer detoxification genes relative to generalist lepidopteran pests such as *Spodoptera litura*, *Spodoptera frugiperda*, *Helicoverpa armigera*, and *Helicoverpa zea*(Chen et al., 2023b). This discrepancy can be attributed to ecological and evolutionary constraints. Unlike polyphagous pests, domesticated or semi-domesticated silkworms exhibit highly specialized feeding behaviors and limited exposure to diverse phytochemicals or synthetic pesticides (Liu et al., 2010; Cheng et al., 2017; Pearce et al., 2017; Gouin et al., 2017). Their sedentary nature and historical artificial selection have further limited their detoxification gene evolution. Consequently, the detoxification gene families in silkworms evolve at a slower rate, and in some cases, may even exhibit signs of functional degeneration due to relaxed selection pressure (Cheng et al., 2017; Pearce et al., 2017; Gouin et al., 2017).

Taken together, *A. pernyi* exhibits a broader and potentially more efficient detoxification system than *B. mori*, underpinned by the expansion of key gene families and the inducibility of ABC transporters and GSTs in response to coumaphos. These species-specific molecular adaptations underscore the potential of *A. pernyi* for breeding programs aimed at enhancing xenobiotic resistance and improving the resilience of sericulture. However, it is important to note that while a considerable body of research has elucidated the detoxification mechanisms in *B. mori*, relatively fewer studies have explored these pathways in wild or semi-domesticated silkworm species such as *A. assamensis* (muga), *S. ricini* (eri), and *A. mylitta* (tropical tasar). These wild silkworms often

inhabit more chemically diverse environments and may possess unique, underexplored detoxification adaptations. Thus, future studies focusing on these non-mulberry silkworms are essential for a comprehensive understanding of detoxification biology across the sericigenous Lepidoptera.

6. EFFECTS OF ARTIFICIAL DIETS ON DETOXIFICATION PATHWAYS

The composition of silkworm diets plays a crucial role in shaping detoxification responses in *B. mori*. Artificial diets (ADs), developed as substitutes for fresh mulberry leaves, support silkworm development in off-season or indoor rearing conditions (Qin et al*.,* 2020). However, they introduce distinct chemical challenges, including plant-derived secondary metabolites, anti-nutritional factors, and food additives not typically encountered in the natural mulberry-based diet. These compounds can impose considerable metabolic and physiological burdens, necessitating compensatory detoxification responses (Dong et al*.,* 2017;2018).

A recent study by Liu et al., (2023) demonstrated that feeding *B. mori* on artificial diets significantly reprograms transcriptional and metabolic profiles, particularly in the Malpighian tubules-primary organs for xenobiotic excretion and homeostasis. Transcriptomic analysis revealed 2,436 differentially expressed genes (DEGs) and 245 differential metabolites between artificial diet-fed and mulberry-fed silkworms. These DEGs were enriched in pathways linked to phase I and phase II detoxification, transmembrane transport, and mitochondrial function, indicating a broad-spectrum molecular response to dietary xenobiotic stress.

The detoxification system in insects is generally categorized into three functional phases. Phase I involves oxidation, reduction, and hydrolysis reactions catalyzed primarily by cytochrome P450 monooxygenases (CYPs) and carboxylesterases (CarEs). Phase II consists of conjugation reactions via glutathione-S-transferases (GSTs) and UDP-glycosyltransferases (UGTs), increasing water solubility for excretion. Phase III entails active transport of modified toxins through ATP-binding cassette (ABC) transporters and solute carrier (SLC) proteins. Liu et al*.,* (2023) reported significant upregulation of genes across all three phases in AD-fed larvae, including *CYP6AE*, *CYP9*, *GSTd2_1*, *UGT33D6*, and *ABCB1_MDR49*. These were predominantly expressed in the upward curly region of the Malpighian tubules, suggesting anatomical specialization in detoxification. Functional assays corroborated transcriptomic data, showing increased enzymatic activities of CYPs and GSTs in the midgut, fat body, and Malpighian tubules of artificial diet-fed larvae (Liu et al*.,* 2023). Metabolomic profiling revealed that AD-fed larvae accumulated higher levels of plant-derived secondary metabolites such as terpenoids, flavonoids, alkaloids, and saponins, as well as organic acids and food preservatives, all of which likely contribute to the elevated detoxification demand. Notably, soybean anti-nutritional components such as isoflavones, tannins, oligosaccharides, and saponins—which are frequently found in artificial diets—have been linked to the stimulation of detoxification genes and the inhibition of development (Zhou et al*.,* 2008; Dong et al*.,* 2018; Liu et al*.,* 2023).

Although artificial diets stimulate baseline detoxification activity, their protective capacity appears compromised under acute chemical stress. Bian et al*.,* (2022) examined the impact of λ-cyhalothrin, a pyrethroid insecticide, on silkworms initially reared on artificial diets during early instars and subsequently transferred to mulberry leaves. This group exhibited a pronounced reduction (86.9%) in LC_{50} values compared to mulberry-fed controls, indicating heightened sensitivity. Histopathological analysis of the fat body revealed severe cellular damage in AD-fed larvae, including nuclear shrinkage, mitochondrial swelling, and dilation of the endoplasmic reticulum, indicative of oxidative stress and impaired metabolic function. Gene expression and enzymatic activity data supported these pathological findings. The expression of *CYP4M5*, *CYP6AB4*, *CarE2*, *CarE5*, *GSTe1*, and *GSTe3* increased post-exposure in a time-dependent manner. However, the induction of P450 activity in AD-fed larvae was delayed by 72 hours, suggesting reduced responsiveness or sluggish adaptation to insecticide challenge. Similar patterns were observed in CarE and GST activities, although their increases occurred earlier but were transient. These results corroborate other studies that demonstrated that B. mori is vulnerable to the upregulation of detoxification genes such as CYP6A8, CYP9A19, and GSTo3 with pesticide exposure (Zhao et al*.,* 2011; Hu et al*.,* 2016; Mao et al*.,* 2019).

Additionally, Liu et al., (2023) noted a marked increase in expression of mitochondrial function-related genes—including mitochondrial protein import complexes (TOM/TIM), chaperones (HSP60, PHB1/2), ribosomal proteins, and components of the electron transport chain in Artificial diet fed silkworms. These upregulations suggest elevated energy expenditure to sustain detoxification and metabolic demands. However, under xenobiotic pressure, these mitochondrial systems may become overtaxed, contributing to cellular injury and decreased resilience, as demonstrated by ultrastructural damage in the Bian et al., (2022) study. In summary, while artificial diets effectively activate detoxification mechanisms under normal conditions, they also impose a metabolic burden that may hinder rapid and effective response to acute toxic stress. Enhanced basal expression of detoxification enzymes does not necessarily translate to improved xenobiotic clearance under insecticide exposure. This underscores the need for refinement of artificial diet formulations not only to support nutritional requirements but also to enhance detoxification efficiency. Incorporating ingredients that support mitochondrial health and redox balance, such as antioxidants, trace minerals, or phytochemicals with protective roles, may bolster silkworm resilience against environmental and dietary stressors.

7. CONCLUSION

Silkworms possess a complex and efficient detoxification system involving key enzymes like cytochrome P450s, CarEs, GSTs, UGTs, and ABC transporters that collectively mitigate xenobiotic stress. These molecular defenses are activated in response to insecticides, heavy metals, and plant-derived toxins. Insights from both domesticated and wild silkworms reveal conserved mechanisms that can be leveraged for genetic improvement and pesticide resilience. Advancing our understanding of these pathways will strengthen sericulture and contribute to environmentally safe pest and chemical management strategies.

8. FURTHER RESEARCH

Future studies should focus on the detoxification capabilities of *vanya* silkworms, wild species indigenous to India such as *A. mylitta*, *A. assamensis*, and *S. ricini*. Exploring their gene expression profiles and stress tolerance could uncover novel detoxification genes and regulatory mechanisms, potentially informing cross-species resistance breeding and enhancing the sustainability of vanya silk production.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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