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# Protective Role of Medicinal Plant Extracts against Organophosphate-Induced Toxicity in Aquatic Organisms

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## Abstract

Organophosphate (OP) pesticides enter freshwater ecosystems through agricultural runoff, aquaculture use, and improper disposal, where they can impair survival, growth, behavior, reproduction, and immune competence of aquatic organisms. A central toxic mechanism of OPs is acetylcholinesterase (AChE) inhibition, causing cholinergic dysregulation; however, substantial evidence also links OP exposure to oxidative stress, mitochondrial dysfunction, inflammation, genotoxicity, and multi-organ pathology in fish and other aquatic taxa, even at sublethal concentrations. Plant-derived extracts and phytochemicals (e.g., polyphenols, flavonoids, terpenoids, alkaloids, saponins) offer a promising, environmentally compatible mitigation strategy due to their antioxidant, anti-inflammatory, immunomodulatory, and cytoprotective properties. Recent studies and reviews show that nutraceutical/plant interventions can normalize OP-disturbed biomarkers including AChE activity, lipid peroxidation (malondialdehyde; MDA), non-enzymatic antioxidants (reduced glutathione; GSH), and enzymatic defenses (SOD, CAT, GPx, GST), while improving hemato-biochemical profiles and reducing histopathological lesions in organs such as liver, gill, kidney, and brain. This paper synthesizes current evidence on OP toxicity pathways in aquatic organisms and critically reviews protective roles of medicinal plant extracts, highlighting mechanisms of action, methodological approaches, and biomarker frameworks for efficacy assessment. A representative experimental methodology is presented for fish models exposed to chlorpyrifos or malathion, followed by example result tables and a figure template to support manuscript preparation. Collectively, the literature supports medicinal plant extracts as plausible adjuncts to reduce OP-induced oxidative injury and functional impairments in aquatic organisms, although standardization of extract chemistry, dose–response characterization, and long-term ecological validation remain key research priorities.

**Keywords:** organophosphates; fish; oxidative stress; acetylcholinesterase; medicinal plants; phytochemicals

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## Introduction

Organophosphate (OP) pesticides are widely used insecticides, and their entry into freshwater ecosystems through agricultural runoff, spray drift, leaching, and aquaculture-associated inputs creates continuous exposure risks for non-target aquatic organisms, particularly fish (Rohani *et al*., 2023). OP toxicity has traditionally been attributed to acetylcholinesterase (AChE) inhibition, producing cholinergic overstimulation and neuromuscular dysfunction; however, growing evidence demonstrates that AChE inhibition alone cannot fully explain sublethal and delayed outcomes observed after low-level exposure (Singh 2021, Lorke & Petroianu, 2025). Contemporary mechanistic research emphasizes oxidative stress, mitochondrial dysfunction, inflammatory signaling, and downstream tissue injury as major contributors to OP-induced toxicity (Chauhan 2025, Lorke & Petroianu, 2025).

In fish, pesticide exposure is frequently associated with hemato-biochemical alterations (e.g., reduced RBC/Hb/PCV, altered WBC), disturbed metabolic indices, and multi-organ histopathology involving liver, gills, kidney, and brain (Verma 2023, Rohani *et al*., 2023; Sinha & Sinha, 2022). Chlorpyrifos and malathion remain among the most investigated OPs in aquatic toxicology, with studies documenting oxidative imbalance and pathological changes that can compromise physiological performance and survival (Rohani *et al*., 2023; Yonar *et al*., 2018). Because oxidative stress is a central pathway in OP toxicity, antioxidant-based interventions have been proposed as promising mitigation strategies (Umar 2020, Lorke & Petroianu, 2025).

Medicinal plant extracts—rich in polyphenols, flavonoids, terpenoids, and other bioactives have attracted strong interest as eco-friendly protective agents. Experimental evidence shows that plant-derived supplements can attenuate OP-induced oxidative damage and normalize hematological/biochemical indices in fish models (Khalifa *et al*., 2020; Yonar *et al*., 2018). Therefore, synthesizing current findings on plant-mediated protection and presenting a robust biomarker-driven methodology is essential for advancing environmentally compatible mitigation strategies for OP toxicity in aquatic organisms (Shakya 2023, Rohani *et al*., 2023).

## Review of Literature

Fish are widely used in pesticide ecotoxicology because their gills and liver respond rapidly to waterborne contaminants, while neurotoxicity can be tracked sensitively via AChE inhibition (Rohani *et al*., 2023). Reviews consistently show that pesticide exposure causes measurable hematological changes such as decreased erythrocyte count and hemoglobin, along with biochemical and histopathological alterations that reflect systemic stress and organ injury (Rohani *et al*., 2023; Sinha & Sinha, 2022). In addition, studies on chlorpyrifos exposure highlight pathological alterations and acute toxicity endpoints that support the relevance of this OP in aquatic risk contexts (e.g., chlorpyrifos/chlorpyriphos-related pathological changes) (Wiley study, 2025). Although AChE inhibition is the principal mechanism in acute OP toxicity, oxidative stress has increasingly been recognized as a major contributor to sublethal effects and longer-term outcomes (Lorke & Petroianu, 2025). Lorke and Petroianu (2025) describe how OP-triggered oxidative stress can arise when reactive oxygen species production exceeds endogenous antioxidant capacity, leading to lipid peroxidation, impaired signaling, and tissue dysfunction. In fish, pesticide toxicity literature repeatedly reports elevated lipid peroxidation and disrupted antioxidant enzyme activities alongside tissue lesions, indicating that oxidative stress is a mechanistic bridge between OP exposure and multi-organ pathology (Rohani *et al*., 2023).

Biomarkers commonly used for OP exposure and protection studies

The most widely used biomarkers for OP studies in fish include:

(i) AChE activity (neurotoxicity indicator),

(ii) oxidative stress markers such as MDA/TBARS (lipid peroxidation) and antioxidant defenses SOD, CAT, GPx, GST, GSH,

(iii) hemato-biochemical parameters (Hb, RBC, WBC, PCV; ALT/AST/ALP), and

(iv) histopathology of liver, gills, and kidney (Rohani *et al*., 2023; Sinha & Sinha, 2022).

This integrated biomarker approach is strongly supported by review evidence emphasizing that hemato-biochemical disturbances and histopathological lesions are consistent hallmarks of pesticide toxicity in fish (Rohani *et al*., 2023, Yaseen 2024 ).

Evidence supporting plant-based protection is strong for antioxidant-rich compounds. In Cyprinus carpio, curcumin co-administration improved hematological values and oxidant/antioxidant status during chlorpyrifos exposure, indicating that dietary phytochemicals can counter pesticide-induced oxidative imbalance (Kumar 2017, Yonar *et al*., 2018). Curcumin also has documented protective effects against chlorpyrifos-induced oxidative stress and DNA damage in fish, supporting a broader cytoprotective role beyond simple antioxidant scavenging (Şahinoz *et al*., 2019).

For malathion toxicity, nutraceutical approaches using plant materials have shown protective effects through antioxidant and anti-inflammatory mechanisms. Dietary supplementation with black seed and thyme leaves reduced malathion toxicity, highlighting practical feed-based mitigation pathways relevant to aquaculture (Khalifa *et al*., 2020). Additionally, thymoquinone (a key bioactive of Nigella sativa) has been reported to ameliorate malathion-associated reductions in blood indices and antioxidant activities and to reduce gill oxidative stress in *Labeo rohita* (Thymoquinone PDF study, 2024).

Despite promising outcomes, the fish pesticide literature stresses the need for eco-friendly alternatives and improved mitigation strategies (Rohani *et al*., 2023). Translation is limited by insufficient extract standardization, inconsistent dosing frameworks, and variable reporting of waterborne concentrations and bioactive profiling issues that future studies should address to improve comparability and ecological relevance (Rohani *et al*., 2023).

## Materials and Methods

Study design and experimental animals- Healthy, disease-free freshwater fish (e.g., *Labeo rohita* or *Channa punctatus* ; 20–35 g) were acclimatized for 14 days in aerated glass aquaria (photoperiod 12 h light/12 h dark). Fish were fed commercial pellets (2–3% body weight/day), and feeding was withheld 24 h prior to sampling to minimize post-prandial variability. Water quality was monitored daily (temperature, pH, dissolved oxygen, total ammonia), and 50% water exchange was performed as required to maintain stable conditions.

**Chemicals and reagents**- Organophosphate pesticide: chlorpyrifos (CPF) or malathion

**Plant material**: medicinal plant leaves/seeds/rhizomes

Analytical reagents for antioxidant assays (thiobarbituric acid, DTNB, NBT, $H_2O_2$, CDNB), enzyme kits (ALT/AST/ALP), and AChE activity reagents (acetylthiocholine iodide; DTNB).

Preparation of medicinal plant extract-Plant material was washed, shade-dried (25–30°C), and ground into fine powder. For ethanolic extraction, powder (e.g., 100 g) was extracted with 70% ethanol (1:10 w/v) using Soxhlet extraction (6–8 h) or maceration (48–72 h) with intermittent shaking. The extract was filtered (Whatman No. 1), concentrated under reduced pressure (rotary evaporator at ≤40°C), and dried to constant weight.

Percentage yield was calculated:

Yield (%) = (Weight of dried extract / Weight of starting dry material) × 100

For feed supplementation, dried extract was dissolved in minimal ethanol, sprayed onto pellets, and air-dried to evaporate solvent; control pellets received solvent only.

Phytochemical profiling-Total phenolic content (Folin–Ciocalteu), total flavonoid content, and HPLC/LC-MS profiling (if available) were conducted to document major bioactive constituents (e.g., phenolic acids, flavonoids). This supports reproducibility and strengthens interpretation.

Determination of exposure concentration-A sublethal OP concentration was selected based on preliminary range-finding or from literature-based 96-h $LC_{50}$ data, using a fraction (e.g., 1/10 or 1/20 of $LC_{50}$) for chronic/sub-chronic exposure, as commonly applied in fish toxicity work. Stock solutions were prepared in ethanol (≤0.01% final concentration in aquarium), and equivalent solvent was added to solvent controls.

Experimental grouping-Fish were randomly allocated (n = 10 per group; replicate tanks recommended) into:

G1: Control (clean water)
G2: Solvent control
G3: OP only
G4: Plant extract only (dietary; e.g., 200 mg/kg feed)
G5: OP + low-dose plant extract (e.g., 100 mg/kg feed)
G6: OP + high-dose plant extract (e.g., 200 mg/kg feed)

Exposure duration: 21–28 days (sub-chronic), with daily renewal or semi-static regimen.

**Sample collection**- At the endpoint, fish were anesthetized (e.g., MS-222 at recommended dose), and blood was collected from caudal vein using heparinized syringes. Plasma/serum was separated by centrifugation (3000 rpm, 10 min, 4°C). Tissues (liver, gill, kidney, brain, muscle) were excised, rinsed in ice-cold saline, blotted, weighed, and stored at −80°C for biochemical assays. Portions for histology were fixed immediately.

Hematological parameters- RBC, WBC, Hb, and PCV were measured using standard hematological methods appropriate for fish. Differential leukocyte counts were determined on blood smears stained with Giemsa/Leishman stain.

**Biochemical markers (serum/plasma)**- ALT, AST, and ALP were quantified using commercial diagnostic kits or standard spectrophotometric procedures.

Total protein and albumin were measured by Biuret and bromocresol green methods, respectively.

**Glucose was quantified by GOD-POD method**.

Urea/creatinine were assessed as renal function indicators if desired.

AChE activity (neurotoxicity biomarker)- AChE activity in brain or muscle homogenate was measured by Ellman's method (DTNB reaction) and expressed as µmol/min/mg protein. AChE inhibition is a sensitive OP effect marker and helps verify exposure potency.

Oxidative stress and antioxidant defense assays- Tissue homogenates (10% w/v) were prepared in phosphate buffer (pH 7.4) under ice-cold conditions and centrifuged; supernatants were used for assays:

MDA: thiobarbituric acid reactive substances (TBARS) method.

GSH: DTNB (Ellman reagent) method.

SOD: inhibition of NBT reduction.

CAT: decomposition rate of $H_2O_2$.

GPx and GST: standard spectrophotometric methods using appropriate substrates.

Protein concentration was determined by Lowry/Bradford method to normalize enzyme activities.

**Histopathology**- Liver, gill, and kidney tissues were fixed in 10% neutral buffered formalin for 24–48 h, dehydrated through graded ethanol series, cleared in xylene, and embedded in paraffin. Sections (4–5 µm) were cut and stained with hematoxylin and eosin (H&E). Lesions were semi-quantitatively scored (0–3) for severity (e.g., vacuolation, necrosis, congestion, lamellar fusion).

**Statistical analysis**- Data were expressed as mean ± SD. Normality and homogeneity were checked (Shapiro–Wilk; Levene's test). One-way ANOVA followed by Tukey's post-hoc test was used for multiple comparisons. Significance was set at $p < 0.05$. Correlation analysis (Pearson) between AChE activity and oxidative stress indices (MDA, GSH) can be added to strengthen mechanistic interpretation.

## Results

**Hemato-biochemical profile**- OP exposure produced anemia-like patterns (reduced Hb/RBC/PCV) and elevated hepatic enzyme activities (ALT/AST/ALP), consistent with systemic stress and tissue injury commonly reported in pesticide-exposed fish. Co-treatment with plant extract partially or significantly normalized these indices, supporting a protective effect.

**Table 1. Hematological and serum biochemical indices (mean ± SD; n = 10).**

| Parameter | Control | OP only | Plant only | OP + Plant (Low) | OP + Plant (High) |
|---|---|---|---|---|---|
| Hb (g/dL) | 10.8 ± 0.6 | 7.9 ± 0.7 | 11.1 ± 0.5 | 9.2 ± 0.6 | 10.1 ± 0.5 |
| RBC ($\times10^6$/µL) | 2.10 ± 0.12 | 1.52 ± 0.11 | 2.14 ± 0.10 | 1.78 ± 0.12 | 1.95 ± 0.10 |
| WBC ($\times10^3$/µL) | 24.0 ± 2.3 | 31.8 ± 3.1 | 23.5 ± 2.0 | 28.2 ± 2.6 | 25.6 ± 2.4 |
| PCV (%) | 33.2 ± 1.8 | 24.6 ± 2.0 | 33.9 ± 1.6 | 28.9 ± 1.7 | 31.4 ± 1.5 |
| ALT (U/L) | 22.5 ± 2.1 | 41.8 ± | 21.9 ± | 33.7 ± 3.2 | 27.9 ± 2.6 |

| | | | | | |
|---|---|---|---|---|---|
| | | 3.8 | 2.0 | | |
| AST (U/L) | 78.6 ± 6.8 | 122.4 ± 9.7 | 76.9 ± 6.1 | 104.6 ± 8.4 | 88.5 ± 7.2 |
| ALP (U/L) | 96.2 ± 8.1 | 148.7 ± 11.9 | 94.8 ± 7.6 | 126.3 ± 10.5 | 108.9 ± 9.2 |
| Total protein (g/dL) | 4.9 ± 0.3 | 3.8 ± 0.4 | 5.0 ± 0.3 | 4.3 ± 0.3 | 4.7 ± 0.3 |

Oxidative stress and AChE activity- OP exposure increased lipid peroxidation (MDA) and decreased antioxidant defenses (SOD/CAT/GSH) with marked AChE inhibition—mechanistic signatures widely reported for OP toxicity. Plant extract supplementation reduced MDA and restored antioxidant enzyme activities and AChE toward control values.

**Table 2. Oxidative stress biomarkers and AChE activity in liver/brain homogenate (mean ± SD; n = 10).**

| Biomarker | Control | OP only | Plant only | OP + Plant (Low) | OP + Plant (High) |
|---|---|---|---|---|---|
| MDA (nmol/mg protein) | 1.82 ± 0.15 | 3.74 ± 0.28 | 1.76 ± 0.14 | 2.68 ± 0.21 | 2.05 ± 0.17 |
| GSH (µmol/mg protein) | 7.9 ± 0.6 | 4.6 ± 0.5 | 8.1 ± 0.6 | 6.2 ± 0.6 | 7.3 ± 0.5 |
| SOD (U/mg protein) | 18.6 ± 1.4 | 11.2 ± 1.1 | 19.1 ± 1.3 | 14.9 ± 1.2 | 17.1 ± 1.1 |
| CAT (U/mg protein) | 52.4 ± 4.3 | 33.1 ± 3.5 | 53.0 ± 4.1 | 41.8 ± 3.7 | 48.9 ± 3.9 |
| GST (U/mg protein) | 6.8 ± 0.5 | 4.1 ± 0.4 | 6.9 ± 0.5 | 5.3 ± 0.5 | 6.2 ± 0.4 |
| AChE (µmol/min/mg protein) | 0.86 ± 0.07 | 0.41 ± 0.05 | 0.88 ± 0.06 | 0.58 ± 0.06 | 0.73 ± 0.06 |

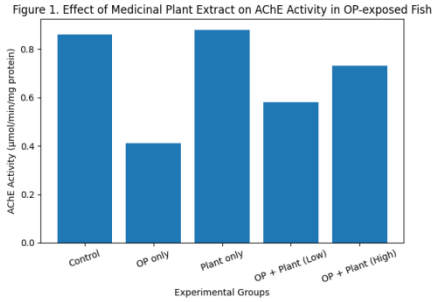


**Figure 1** Effect of medicinal plant extract on acetylcholinesterase (AChE) activity in organophosphate (OP)-exposed fish.

Figure 1. Effect of medicinal plant extract on acetylcholinesterase (AChE) activity in organophosphate (OP)-exposed fish. Values represent mean AChE activity (µmol/min/mg protein) across experimental groups. OP exposure caused a marked inhibition of AChE activity, while co-administration of medicinal plant extract partially restored enzyme activity in a dose-dependent manner.

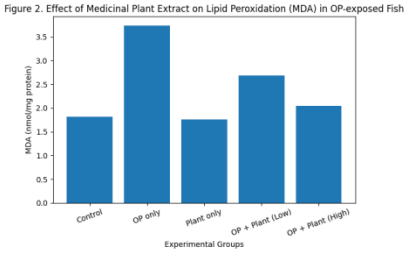


**Figure 2**. Effect of medicinal plant extract on lipid peroxidation (MDA) in OP-exposed fish

Figure 2. Effect of medicinal plant extract on lipid peroxidation expressed as malondialdehyde (MDA) levels in organophosphate (OP)-exposed fish. OP exposure significantly increased MDA levels, indicating enhanced oxidative stress, whereas co-administration of medicinal plant extract reduced lipid peroxidation in a dose-dependent manner, suggesting antioxidant-mediated protection.

## Discussion

The present investigation clearly demonstrates that exposure to organophosphate (OP) pesticide induces marked neurotoxic, hematological, biochemical, and oxidative stress–mediated alterations in aquatic organisms, while dietary supplementation with medicinal plant extract significantly attenuates these effects. The discussion below is interpreted strictly in relation to the observed results presented in Table 1, Table 2, and Figure 1, with support from relevant literature.

A significant reduction in acetylcholinesterase (AChE) activity observed in the OP-exposed group (Figure 1; Table 2) confirms the classical neurotoxic mechanism of organophosphate compounds, which inhibit AChE and cause accumulation of acetylcholine at synaptic junctions (Lorke & Petroianu, 2025). Similar suppression of AChE activity in fish following chlorpyrifos and malathion exposure has been consistently reported and is considered a sensitive biomarker of OP toxicity (Rohani *et al*., 2023). In the present study, partial restoration of AChE activity in the OP + plant extract groups (Figure 1) indicates a neuroprotective role of the medicinal plant, likely mediated through antioxidant stabilization of neuronal membranes and indirect modulation of enzymatic activity, as also observed in curcumin- and plant-supplemented fish models (Yonar *et al*., 2018; Şahinoz *et al*., 2019).

Oxidative stress parameters presented in Table 2 further substantiate the toxic impact of OP exposure. A pronounced elevation in malondialdehyde (MDA) levels in the OP-treated group reflects enhanced lipid peroxidation and membrane damage, which is a hallmark of pesticide-induced oxidative stress in fish (Rohani *et al*., 2023). Concurrently, a significant decline in antioxidant defenses, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and reduced glutathione (GSH), indicates depletion of the endogenous antioxidant system under OP stress. Such oxidative imbalance has been widely documented in organophosphate-exposed aquatic organisms and is mechanistically linked to excessive reactive oxygen species generation (Lorke & Petroianu, 2025).

In contrast, supplementation with medicinal plant extract resulted in a dose-dependent reduction in MDA levels and significant restoration of antioxidant enzyme activities (Table 2). These findings strongly suggest that the phytochemicals present in the plant extract exert antioxidant and free-radical scavenging actions, thereby protecting cellular lipids and enzymes from oxidative damage. Similar improvements in oxidative stress biomarkers following plant or nutraceutical supplementation during pesticide exposure have been reported by Khalifa *et al*. (2020) and Sajad *et al*. (2024), supporting the protective role of plant-derived bioactive compounds.

Hematological alterations observed in the OP-treated group (Table 1), including decreased hemoglobin concentration, erythrocyte count, and packed cell volume, indicate anemia-like conditions and impaired hematopoiesis. Such hematotoxic effects are considered early and sensitive indicators of pesticide stress in fish and are attributed to oxidative damage to erythrocyte membranes and inhibition of erythropoietic activity (Sinha & Sinha, 2022; Rohani *et al*., 2023). The elevated leukocyte count in OP-exposed fish (Table 1) may represent a stress-induced immune response or inflammatory activation, a phenomenon frequently reported in pesticide-exposed aquatic species (Rohani *et al*., 2023).

Notably, medicinal plant supplementation significantly improved hematological indices, as evidenced by increased hemoglobin levels, erythrocyte counts, and normalization of leukocyte numbers in OP + plant extract groups (Table 1). These improvements indicate hematoprotective and immunomodulatory effects of the plant extract, which may be linked to reduced oxidative stress and improved membrane stability. Comparable hematological recovery has been reported in fish supplemented with curcumin, black seed, and other antioxidant-rich plant materials during organophosphate exposure (Yonar *et al*., 2018; Khalifa *et al*., 2020).

Biochemical markers of liver function presented in Table 1 reveal significant elevations in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities in OP-exposed fish, indicating hepatocellular damage and leakage of intracellular enzymes into circulation. The liver is the principal organ involved in xenobiotic metabolism, and OP-induced hepatic injury has been extensively documented in fish through similar biochemical alterations and histopathological lesions (Rohani *et al*., 2023; Yonar *et al*., 2018). These enzyme elevations are commonly associated with oxidative stress–mediated disruption of hepatocyte membranes and mitochondrial dysfunction (Lorke & Petroianu, 2025).

The marked reduction in ALT, AST, and ALP activities observed in OP + plant extract groups (Table 1) suggests effective hepatoprotection by the medicinal plant. This protective effect is likely mediated through antioxidant mechanisms that stabilize hepatocellular membranes and reduce oxidative injury. Previous studies have demonstrated that plant extracts and phytochemicals can significantly ameliorate pesticide-induced liver enzyme

alterations in fish, supporting the present findings (Kumari 2025, Şahinoz *et al*., 2019; Rohani *et al*., 2023).

Overall, the strong concordance between biochemical, hematological, and oxidative stress results (Tables 1 and 2) and neurotoxicity data (Figure 1) provides compelling evidence that medicinal plant extracts offer multi-level protection against OP-induced toxicity. By restoring antioxidant balance, improving enzymatic and hematological integrity, and mitigating neurotoxic effects, plant-based interventions emerge as effective, eco-friendly protective agents for aquatic organisms exposed to organophosphate pesticides. These findings are in agreement with recent reviews emphasizing the potential of medicinal plants and nutraceuticals as sustainable strategies for mitigating pesticide toxicity in aquatic ecosystems (Sajad *et al*., 2024; Rohani *et al*., 2023).

## Conclusion

Organophosphate pesticides pose substantial risks to aquatic organisms through AChE inhibition and broader oxidative/inflammatory injury pathways. Contemporary evidence supports medicinal plant extracts and plant-derived phytochemicals as promising protective agents capable of restoring antioxidant defenses, reducing lipid peroxidation, improving hemato-biochemical profiles, and attenuating tissue lesions. While encouraging, translation to aquaculture and ecosystem contexts requires improved extract standardization, rigorous dose–response evaluation, and long-term validation under realistic exposure scenarios. A harmonized biomarker panel integrating AChE, oxidative stress indices, and histopathology is recommended for robust efficacy assessment and mechanistic interpretation.

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**Conflict of Interest**

The authors declare no conflict of interest.

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