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Original article

Berberine ameliorates malathion-triggered rat brain cholinergic dysfunction besides attenuation of oxidative stress, pyroptosis, and apoptosis

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Abstract

Objective: Exposure to pesticides such as malathion induces neurotoxic effects that may lead to brain injury. Berberine, a bioactive alkaloid isolated from *Coptis chinensis*, has demonstrated significant neuroprotective properties. This study aimed to evaluate the protective effects of berberine against malathion-induced brain injury in rats.

Materials and methods: Adult male rats were assigned to four groups: control, malathion, malathion + berberine 10 mg/kg, and malathion + berberine 50 mg/kg. Malathion (100 mg/kg/day, i.p.) was administered for two weeks to induce neuronal injury. Berberine was given orally daily for the same period. Oxidative stress parameters including malondialdehyde (MDA), reactive oxygen species (ROS), and glutathione (GSH) levels, and superoxide dismutase (SOD) and catalase (CAT) activities were assessed. Acetylcholinesterase (AChE) activity was measured as an index of cholinergic function. Moreover, caspase 1 and caspase 3 enzymatic activities were determined, and hippocampal CA1 pyramidal neuron density was evaluated histologically.

Results: Malathion exposure significantly impaired antioxidant activity and cholinergic function while increasing oxidative stress, pyroptosis, and apoptosis markers. Berberine treatment, particularly at 50 mg/kg, significantly reversed these malathion-induced alterations by restoring SOD, CAT, and AChE activities and reducing MDA and ROS levels, along with caspase 1 and caspase 3 activities. Although berberine improved GSH levels and CA1 neuronal density compared to the malathion group, these effects did not reach statistical significance.

Conclusion: Berberine exerts neuroprotective effects against malathion-induced brain injury by attenuating cholinergic dysfunction, oxidative stress, and pyroptotic and apoptotic pathways. These findings suggest that berberine may represent a promising therapeutic candidate for pesticide-induced neurotoxicity.

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Introduction

Malathion, a widely employed organophosphate insecticide, exerts its effects through irreversible acetylcholinesterase inhibition, resulting in acetylcholine accumulation at synaptic junctions and subsequent cholinergic neurotransmission disruption (Elmorsy et al. 2022). Recent investigations have provided insights into malathion neurotoxicity beyond cholinergic mechanisms, demonstrating its ability to provoke oxidative stress, mitochondrial impairment, neuroinflammation, and neuronal apoptosis. Research indicates that malathion triggers apoptotic cell death via enhanced lysosomal membrane permeabilization, thereby exacerbating progressive neurological pathologies (Massoud et al. 2022; Venkatesan et al. 2017; Yadav et al. 2025). For instance, Narasimhamurthy et al. showed that combined low-dose malathion exposure and radiation disrupts critical neural processes including synaptic transmission and plasticity, ultimately driving neurodegeneration in murine models. Together, these observations underscore malathion diverse neurodegenerative pathways, positioning it as a key environmental contributor to neurological disease risk (Narasimhamurthy et al. 2024).

Berberine, a naturally occurring isoquinoline alkaloid extracted primarily from the roots, stems, and bark of plants such as *Coptis chinensis*, has been extensively investigated for its broad pharmacological profile encompassing anti-inflammatory, antitumor, and metabolic regulatory activities (Cheng et al. 2025). Although rooted in traditional medicine, recent findings highlight berberine therapeutic promise in addressing chronic conditions including diabetes, cardiovascular disease, and neurodegeneration. These effects stem from its modulation of pivotal cellular processes governing energy homeostasis, oxidative balance, and inflammatory signaling, positioning it as a compelling candidate for clinical translation (Fan et al. 2025). Berberine profoundly influences cellular energy dynamics via AMP-activated protein kinase (AMPK) activation, thereby alleviating oxidative injury and neuroinflammatory responses to confer neuroprotection (Tian et al. 2023). Furthermore, it dampens neuroinflammation by suppressing proinflammatory cytokines and inhibiting nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) pathway activation—a cornerstone of neurodegenerative cascades. In addition to free radical scavenging and anti-inflammatory actions, berberine safeguards neurons by modulating apoptosis and pyroptosis through regulation of Bcl-2 family proteins and caspase activity, thereby promoting neuronal survival in the context of neurodegenerative insults (Begh et al. 2025; Zhang et al. 2025). It concurrently attenuates nicotinamide adenine dinucleotide phosphate (NADPH) oxidase function to curb reactive oxygen species (ROS) generation while bolstering endogenous antioxidants such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), thereby countering neural oxidative stress (Fan et al. 2019).

Therefore, this study aimed to evaluate berberine neuroprotective efficacy against malathion-induced cerebral impairments.

Materials and Methods

Animals

Male albino Wistar rats (10–12 weeks old, 200–240 g), obtained from the Pasteur Institute of Tehran (Iran), were acclimatized and housed in the animal facility under standard laboratory conditions (21–23°C, 30–40% humidity, 12-hr light/dark cycle). Animals had free access to standard commercial pellet diet and tap water *ad libitum*. All experimental procedures were conducted in accordance with ethical guidelines designed to minimize animal numbers and suffering.

Experimental groups

Neurotoxicity was induced by daily intraperitoneal injection of malathion (100 mg/kg/day) for 14 consecutive days. The experimental groups consisted of: (1) control (intraperitoneal malathion vehicle + oral Kolliphor EL); (2) malathion (intraperitoneal malathion + oral Kolliphor EL); (3) malathion + berberine 10 (intraperitoneal malathion + oral berberine 10 mg/kg); and (4) malathion + berberine 50 (intraperitoneal malathion + oral berberine 50 mg/kg). Berberine hydrochloride (Santa Cruz Biotechnology, USA) was administered daily throughout the experimental period according to the protocol described by Akbarizadeh-Mashkani et al. (Akbarizadeh-Mashkani et al. 2025).

Tissue homogenate preparation

Rats were deeply anesthetized via intraperitoneal administration of ketamine (150 mg/kg) and xylazine (10 mg/kg). Transcardial perfusion with ice-cold phosphate-buffered saline was conducted, followed by careful brain extraction and processing. Right hippocampus tissues were homogenized in ice-cold 150 mM Tris-HCl buffer (pH 7.4), while left hemispheres were immersion-fixed in 10% neutral buffered formalin for histological examination. Homogenates were centrifuged at 5000 rpm for 15 min at 4°C, and the resulting supernatants were collected for subsequent biochemical assays (Asgari et al. 2025).

Determination of hippocampal MDA concentration

Assessment of lipid peroxidation was carried out by measuring malondialdehyde (MDA) through the thiobarbituric acid reactive substance (TBARS) method. Briefly, supernatant aliquots were mixed with trichloroacetic acid solution, followed by the addition of TBARS reagent. The resulting mixtures were incubated in boiling water (100°C) for 80 min, cooled immediately on ice, and thereafter, centrifugation was carried out at 1000 × g for 10 min. The optical density of the clear supernatant was monitored at 532 nm using a spectrophotometer. Final results were calculated as MDA equivalents by comparison with a calibration curve generated from known concentrations of tetraethoxypropane (Sedaghat et al. 2014).

Determination of hippocampal ROS level

ROS levels were quantified using the non-fluorescent probe dichlorofluorescein diacetate (DCFDA), which is hydrolyzed by intracellular esterases and subsequently oxidized by ROS to yield the fluorescent product 2′,7′-dichlorofluorescein (DCF). Fluorescence intensity was measured with excitation at 488 nm and emission at 525 nm. Quantification was achieved using a calibration curve generated from DCF standards of known concentrations (Tayanloo-Beik et al. 2022).

Measurement of hippocampal SOD activity

Superoxide dismutase (SOD) activity in hippocampal homogenates was quantified using a commercially available assay kit (Kiazist, Iran) according to the manufacturer's instructions. Briefly, tissue homogenates were prepared and incubated with the provided reaction mixture containing xanthine oxidase and a chromogenic substrate at 37°C for 20–30 min. Enzymatic activity was determined by measuring the inhibition of superoxide radical formation, reflected by the reduction in absorbance at 450 nm using a microplate reader. SOD activity was subsequently calculated based on the degree of inhibition and is expressed as units per milligram of protein (Faryadras et al. 2025).

Measurement of hippocampal catalase activity

Catalase (CAT) enzymatic activity, which catalyzes the decomposition of hydrogen peroxide, was measured

according to the protocol of Claiborne. Hydrogen peroxide was mixed with potassium phosphate buffer and sample supernatant, and its decomposition rate was monitored by measuring the decrease in absorbance at 240 nm (Claiborne 1985).

Determination of hippocampal GSH concentration

The GSH concentration, representing a key non-enzymatic antioxidant, was evaluated according to previously described protocol (Sedlak and Lindsay 1968). Briefly, supernatant was subjected to a second centrifugation step in the presence of 5% trichloroacetic acid (TCA). The resulting clear supernatant was then mixed with phosphate buffer (pH 8.4), 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB), and distilled water. GSH levels were quantified by measuring absorbance spectrophotometrically at 412 nm.

Determination of acetylcholinesterase activity

Following two weeks of treatment, rats were euthanized and serum samples were collected. Acetylcholinesterase (AChE) activity was quantified using the Ellman assay. This assay measures the rate of acetylthiocholine iodide hydrolysis by monitoring thiol release at 405 nm, where thiols react with DTNB to produce a chromogenic product. Absorbance was recorded at 30-sec intervals over 2 min. Cholinesterase activity was calculated using the formula: Cholinesterase activity (mU/ml at 25°C) = Δabsorbance/30 sec × 23400 (Gorun et al. 1978).

Determination of caspase 1 activity

Caspase 1 activity was evaluated using a specific assay kit from Abcam following the manufacturer's instructions. In brief, 100 µl of reaction buffer containing 10 mM dithiothreitol (DTT) was prepared, and 25 µl of the sample supernatant was added and maintained on ice. Subsequently, 10 µl of 2 mM Tyr-Val-Ala-Asp-p-nitroanilide (YVAD-p-NA) substrate was introduced, and the mixture was incubated at 37°C for 1 hr in the dark. Finally, absorbance value was measured at 405 nm using a microplate reader (Tashakori-Miyanroudi et al. 2022).

Determination of caspase 3 activity

For the assessment of the activity of caspase 3, an apoptosis indicator, was measured with the Asp-Glu-Val-Asp-p-nitroanilide (DEVD-p-NA) substrate. Production of the chromophore p-nitroaniline was monitored and quantified by measuring absorbance at 405 nm, and results are presented as optical density (OD) values (Fahanik-Babaei et al. 2019).

Protein assay

The total protein content was assessed via the bicinchoninic acid (BCA) method, a colorimetric technique based on the reduction of copper ions by protein in alkaline conditions, resulting in a color change proportional to protein concentration. Absorbance was measured at 560 nm, and protein levels were determined by comparison to a standard curve generated from known protein standards (Lowry et al. 1951).

Histological studies

Hippocampal tissue blocks were processed, embedded in paraffin, and sectioned into 5-µm coronal slices using a rotary microtome (DidSabz, Urmia, Iran). Cresyl violet acetate staining was applied to alternate tissue sections. For each sample, pyramidal neurons specifically located in the CA1 subfield of the hippocampus were counted across multiple evenly spaced sections, with counts performed twice and averaged. Only cells exhibiting clearly defined boundaries and nucleoli were included. Quantification was performed using ImageJ software version 1.49 (NIH) (Baluchnejadmojarad and Roghani 2024).

Statistical analysis

Data were analyzed using GraphPad Prism version 10.4 software. Results are presented as mean ± standard error of the

mean (SEM). Statistical analysis of variables across groups was performed using one-way *ANOVA* followed by Tukey's *post hoc* test. Statistical significance was set at $p<0.05$.

Results

The effect of berberine on hippocampal indices of oxidative stress and antioxidants

In hippocampal tissue, malathion significantly elevated MDA levels compared to control ($p<0.001$). Both berberine treatment groups (10 and 50 mg/kg) showed significantly increased MDA levels relative to control ($p<0.001$ and $p<0.05$, respectively). Berberine at 50 mg/kg significantly reduced MDA levels compared to the malathion-only group ($p<0.05$) (Figure 1A).

Malathion administration markedly increased ROS levels in the hippocampus compared to the control group ($p<0.01$). Malathion-exposed rats treated with berberine at 10 mg/kg also showed increased hippocampal ROS accumulation compared to controls ($p<0.05$). Berberine at 50 mg/kg significantly lowered ROS levels in malathion-treated rats compared to the malathion group ($p<0.05$) (Figure 1B).

In malathion-treated rats, SOD enzymatic activity in hippocampal tissue was significantly reduced compared to controls ($p<0.001$). This decrease persisted following berberine treatment at 10 and 50 mg/kg ($p<0.001$, $p<0.05$, vs. control group, respectively). However, berberine at 50 mg/kg significantly increased SOD activity compared to the malathion-only group ($p<0.01$) (Figure 2A).

In malathion-intoxicated animals, CAT activity was significantly decreased compared to controls ($p<0.001$). Neither berberine dose (10 nor 50 mg/kg) restored hippocampal CAT activity in malathion-exposed rats ($p<0.001$ and $p<0.05$ vs. control, respectively). However, berberine at 50 mg/kg significantly increased CAT activity compared to malathion alone ($p<0.05$) (Figure 2B).

GSH levels were significantly reduced in rats receiving malathion alone compared to controls ($p<0.01$). Co-administration of berberine at 10 mg/kg also significantly decreased GSH levels ($p<0.05$ vs. control group), although this reduction did not differ significantly from that observed with malathion alone ($p>0.05$). Berberine at 50 mg/kg showed no significant effect on GSH content in malathion-injected rats ($p>0.05$ vs. malathion group) (Figure 2C).

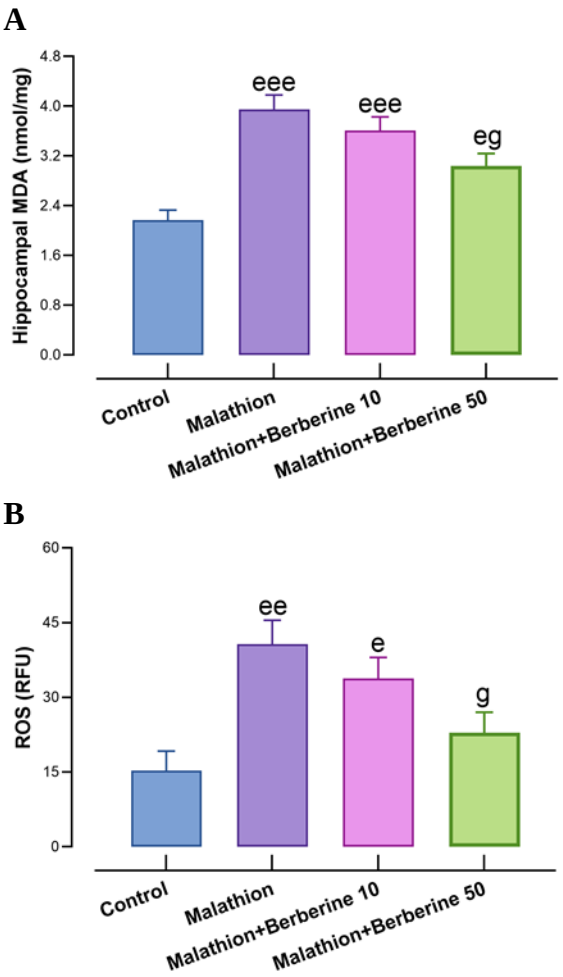


Figure 1. Data of oxidative stress factors comprising malondialdehyde (MDA) (pane A), and reactive oxygen species (ROS) (pane B). Berberine effectively reduced the oxidative stress caused by malathion, as demonstrated by one way *ANOVA* followed by Tukey's *post hoc* test. Statistical significance: e ($p<0.05$), ee ($p<0.01$), and eee ($p<0.001$) vs. control; g ($p<0.05$), vs. malathion (Means ± SEM, n = 7/group).

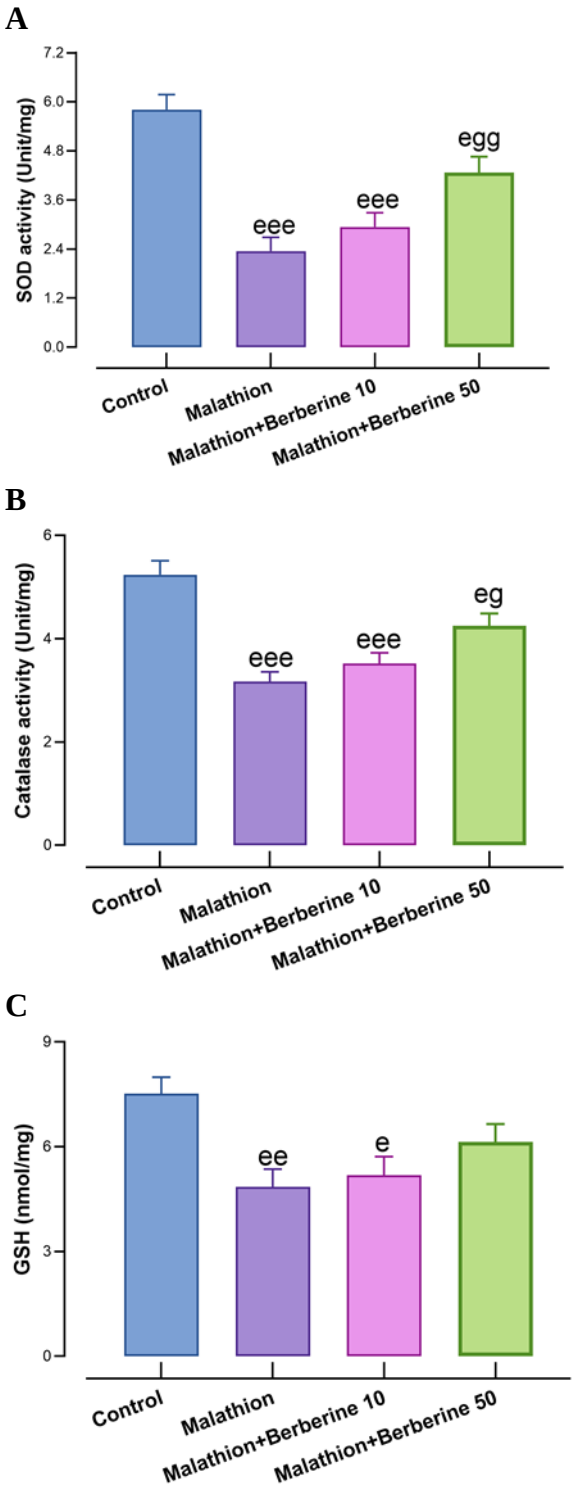


Figure 2. Data of antioxidant factors comprising superoxide dismutases (SOD) activity (pane A), catalase activity (pane B), and glutathione (GSH) level (pane C). Berberine effectively increased levels of antioxidants other than GSH, as demonstrated by one way *ANOVA* followed by Tukey's *post hoc* test. Statistical significance: e ($p<0.05$), ee ($p<0.01$), and eee ($p<0.001$) vs. control; g ($p<0.05$), gg ($p<0.01$), and ggg ($p<0.001$) vs. malathion (Means ± SEM, n = 7/group).

The effect of berberine on hippocampal indices of cholinergic activity

A significant decline in AChE enzymatic activity was observed in the malathion and malathion + berberine 10 groups compared to control animals ($p<0.01$ and $p<0.05$, respectively). In contrast, administration of the higher berberine dose (50 mg/kg) to malathion-treated rats significantly enhanced AChE activity relative to the malathion-only group ($p<0.05$), achieving levels comparable to controls ($p>0.05$) (Figure 3).

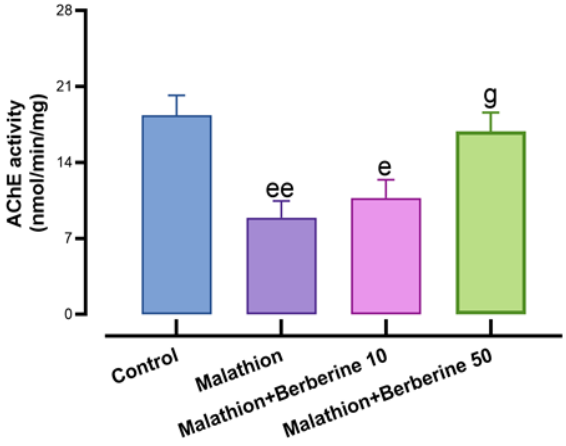


Figure 3. Data for hippocampal level of acetylcholinesterase (AChE) activity, as analyzed by one way *ANOVA* and Tukey tests. Berberine treatment at the higher dose modulates AChE activity in malathion-exposed group. Statistical significance: e ($p<0.05$), ee ($p<0.01$) vs. control; g ($p<0.05$), vs. malathion (Means ± SEM, n = 7/group).

The effect of berberine on hippocampal indices of pyroptosis and apoptosis

For the detection of pyroptotic inflammatory mediated cellular death, we estimated caspase 1 activity. The malathion only and malathion given berberine at 10 mg/kg rats experienced a significantly higher levels of caspase 1 activity when compared to control group ($p<0.001$, $p<0.01$, respectively). In contrast, co-administration of malathion with berberine at 50 mg/kg resulted in significantly lower caspase 1 activities ($p<0.05$ vs. malathion group) (Figure 4A).

To assess apoptotic cell death, we measured caspase 3 activity. Compared to the control group, rats treated with

malathion alone and those received malathion followed by berberine at a dose of 10 mg/kg exhibited a significant increase in caspase 3 activity ($p<0.01$ and $p<0.05$, respectively). However, compared to the malathion group, rats receiving malathion and berberine at 50 mg/kg showed significantly decreased caspase 3 activity ($p<0.05$) (Figure 4B).

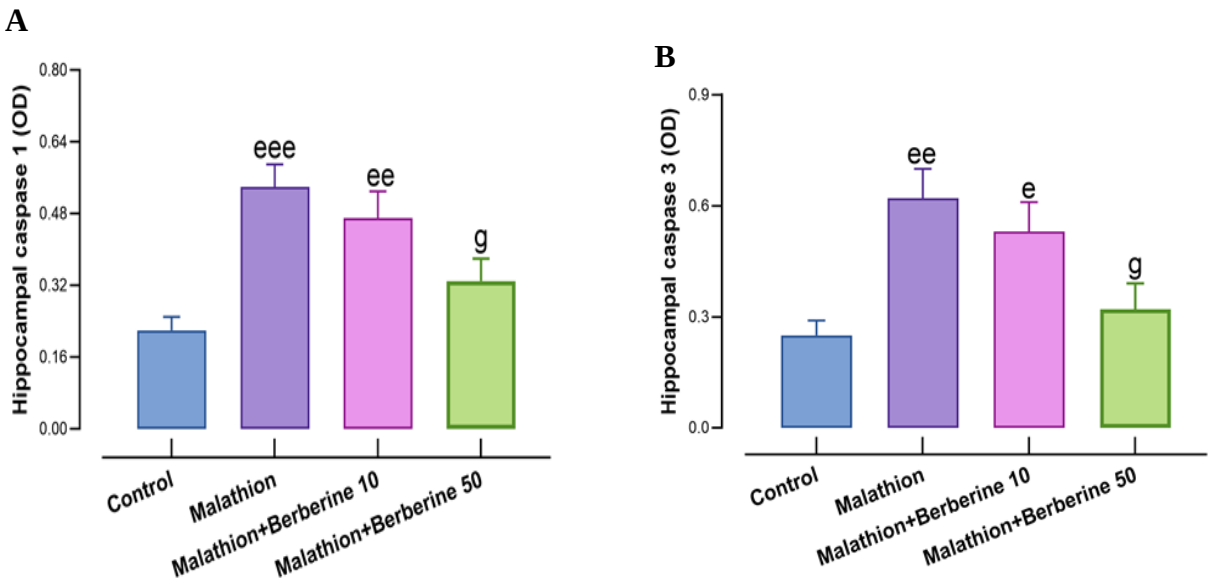
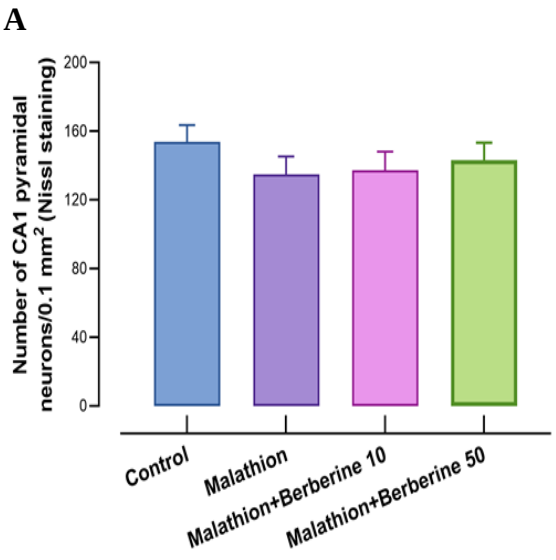


Figure 4. Data for hippocampal level of caspase 1 (pane A) and caspase 3 (pane B), as analyzed by one way *ANOVA* and Tukey tests. Berberine reversed malathion-induced changes in all factors. Statistical significance: e ($p<0.05$), ee ($p<0.01$), eee ($p<0.001$) vs. control; g ($p<0.05$), vs. malathion. (Means ± SEM, n = 7/group).

The effect of berberine on the number of CA1 pyramidal neurons

In the malathion group, microscopic examination revealed degenerative alterations characterized by pyramidal neuron damage within the CA1 hippocampal subfield, although these changes failed to reach statistical significance ($p>0.05$). Concurrent treatment with berberine at 10 and 50 mg/kg doses produced a modest, non-significant attenuation of these malathion-induced neurodegenerative effects compared to the malathion-only group ($p>0.05$) (Figure 5).



B

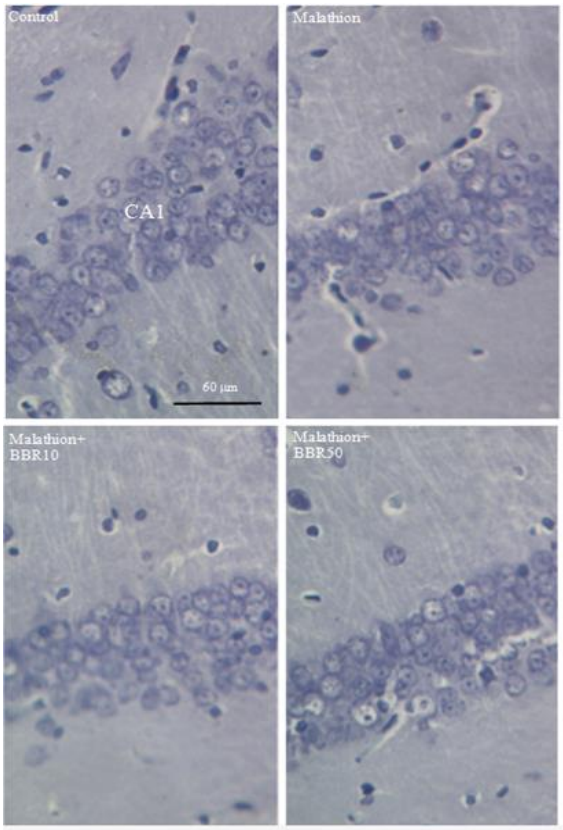


Figure 5. Quantification of CA1 pyramidal neuron density through Nissl staining, as analyzed by one way *ANOVA* and Tukey tests (pane A) and representative photomicrographs (pane B). No significant differences were observed among groups; neither malathion exposure nor berberine (BBR) treatment caused significant changes in the number of CA1 pyramidal neurons (Means ± SEM, n = 4/group).

Discussion

Pesticide intoxication has become a major public health concern in many developing nations. Widespread overuse and inappropriate application of these chemicals are responsible for severe poisoning cases and contribute to millions of fatalities (Nurulain et al. 2013). Malathion constitutes a globally prevalent organophosphate insecticide, extensively employed in agricultural and veterinary sectors to manage vector-borne diseases, particularly those propagated by mosquitoes (Organization 2016). Farmers and veterinary workers commonly encounter malathion toxicity through occupational exposure (Suratman et al. 2015).

Malathion and other organophosphates manifest neurotoxicity across both acute and chronic exposure paradigms. Prolonged organophosphate exposure correlates strongly with cognitive decline, manifesting as disruptions in mnemonic function, attentional capacity, visuospatial learning proficiency, and sensorimotor filtering mechanisms (Middlemore-Risher et al. 2010) The neurotoxic profile of organophosphates stems primarily from synergistic processes involving neuroinflammation, ROS generation, and consequent neuronal injury (Coban et al. 2015). Given the brain's pronounced lipid content and substantial oxygen demands, cerebral tissue exhibits heightened vulnerability to oxidative insults (Bodhinathan et al. 2010), which can precipitate cognitive deterioration and progression toward neurodegenerative pathologies (Kishida and Klann 2007).

Under normal physiological circumstances, the production of ROS is tightly controlled. Oxidative stress occurs when the equilibrium between ROS generation and the cell's antioxidant defense systems is disrupted (Mooli et al. 2022). Malathion exposure disrupts the pro-oxidant/antioxidant balance, leading to cellular damage through dual mechanisms: first, the oxidative conversion of malathion to its more toxic metabolite, malaoxon; and second, the consumption of GSH stores during phase II detoxification, thereby compromising the cellular antioxidant defense capacity. These processes enhance ROS formation and diminish antioxidant reserves, as reflected by elevated MDA levels and reduced GSH content (Althobaiti 2025). Malathion exposure in rats has been shown to promote apoptosis and pyroptosis, characterized by increased levels of caspase 3, Bax, NOD-like receptor protein 3 (NLRP3), and caspase 1, alongside a decrease in Bcl-2 protein levels (Saati 2024).

Increasing evidence indicates that organophosphate compounds can disrupt intracellular calcium homeostasis (Meijer et al. 2014), which may be partly linked to changes in mitochondrial respiratory chain activity (Hargreaves 2012). Redox imbalance constitutes a major driver of the toxic actions of malathion. The neurotoxic effects of this pesticide are strongly associated with oxidative damage. Upon acute exposure, malathion enhances cholinergic signaling by suppressing AChE activity, which is considered the main pathological pathway underlying its toxic effects. Beyond cholinergic pathways, malathion also contributes to neuronal loss through noncholinergic mechanisms observed in neurodegenerative conditions. By disrupting both enzymatic and nonenzymatic antioxidant defenses in the brain, malathion promotes mitochondrial dysfunction, triggers DNA damage, and interferes with normal apoptotic responses to oxidative damage mediated by free radical formation such as lipid peroxidation (Fortunato et al. 2006; Varol et al. 2015). Nevertheless, inhibition of AChEalone does not seem to fully account for the broad spectrum of toxic effects arising from malathion exposure.

Oxidative stress is regarded as the primary contributor to both the acute and chronic adverse effects of this pesticide. Because of its strong lipophilicity, malathion can easily associate with cell membrane phospholipids, promoting membrane instability and excessive free radical formation (Ozsoy et al. 2016). Experimental findings also indicate that malathion elevates MDA concentrations, increases oxidative stress burden, and weakens antioxidant defenses, as reflected by alterations in total antioxidant capacity (TAC) and SOD activities (Mohammadzadeh et al. 2020). In agreement with these reports, our results showed that intraperitoneal administration of malathion at 100 mg/kg enhanced lipid peroxidation (MDA), ROS production, and the activities of caspase 3 and caspase 1, while decreasing antioxidant enzymes.

Recent investigations have shown that, persistent oxidative stress induced by malathion can initiate autophagy, DNA fragmentation, and mitochondrial dysfunction, culminating in apoptotic cell death. In hippocampal tissue, malathion has been shown to elevate mitochondrial ROS generation and impair the activities of respiratory chain complexes I, II, and IV. This disruption compromises cellular bioenergetics and creates an imbalance between oxidant generation and antioxidant protection (Karami-Mohajeri et al. 2014). Earlier reports have indicated that malathion can impair the antioxidant defense system, manifested as lowered activity of critical enzymes like SOD, GSH, and GPx (Ullah et al. 2018). Such dysfunction is particularly critical in the brain due to its high metabolic demand and relatively weak antioxidant defense.

Oxidative damage to DNA and mitochondria can further drive apoptosis. Malathion exposure has been reported to lower the expression of anti-apoptotic proteins such as phosphorylated protein kinase B (AKT) and Bcl-2, while simultaneously increasing the Bax/Bcl-2 ratio and caspase 3 activity in brain tissue (Salama et al. 2019). Evidence showed that treatment of rats with malathion caused increased oxidative stress and inflammation, hyperphosphorylation of tau protein and apoptotic effects, as well as cognitive impairment (Mohammadzadeh et al. 2020). Dermal exposure to malathion, alone or with NN-diethyl-m-toluamide (DEET) and permethrin, significantly reduced neuronal density in the CA1 region of the hippocampus. Since CA1 is essential for learning and memory, this degeneration likely contributes to the observed behavioral deficits. These findings indicate that malathion toxicity may not be limited to cholinesterase inhibition but could also involve additional pathways including redox imbalance and neuroinflammation (Abdel-Rahman et al. 2004). Previous

studies have reported that exposure to malathion leads to neuronal loss in the granular layer and dislocation of the CA1 cellular layers in the hippocampus of rats (N'Go et al. 2021). The current investigation revealed no significant change in the population of CA1 pyramidal neurons following malathion exposure, which may be attributed to its tendency to activate programmed cell death pathways such as apoptosis and pyroptosis, as well as increased oxidative stress. These mechanisms might induce biochemical and cellular damage without leading to a substantial reduction in the total neuron count.

Berberine, the bioactive isoquinoline alkaloid derived from natural sources, continues to attract intensive investigation for its promising applications in neurodegenerative and CNS-related pathologies (Cheng et al. 2022). Through potent suppression of neuroinflammatory signaling, attenuation of oxidative damage, and modulation of endoplasmic reticulum stress pathways, it confers robust neuroprotection alongside antioxidant and anti-inflammatory benefits, thereby limiting neuronal degeneration and apoptotic cascades (Zhang et al. 2020). A growing body of research robustly affirms its efficacy in mitigating cognitive decline across varied experimental paradigms (Fang et al. 2020; Yao et al. 2023; Zhang et al. 2021). Recently, many investigations across various countries have explored the pharmacological effects and underlying mechanisms of berberine. These studies have demonstrated that berberine exerts distinctive pharmacological actions on several body systems, including the cardiovascular, nervous, and endocrine systems (Phogat et al. 2024).

Cha and colleagues have demonstrated that berberine significantly mitigates neurotoxic effects triggered by 6-hydroxydopamine (6-OHDA) through the suppression of oxidative damage, enhancement of mitochondrial performance, and normalization of autophagic processes in both *in vitro* and *in vivo* Parkinson's disease models (Cha et al. 2025). A separate investigation evaluated berberine protective role against cardiomyopathy induced by doxorubicin (DOX). The findings revealed that berberine markedly inhibited the development of cardiac diastolic impairment and fibrotic remodeling, leading to a decrease in cardiac MDA levels and ROS production, and an increase in antioxidant SOD activity in DOX-treated rats (Wang et al. 2023). Yardim et al. studied the possible protective effects of berberine on bortezomib-induced peripheral neuropathy. Investigations on the sciatic nerve and spinal cord revealed that bortezomib-induced oxidative stress elevated MDA concentrations as well as mRNA expression levels of nuclear factor erythroid 2–related factor 2 (Nrf2), heme oxygenase 1 (HO-1), NAD(P)H:quinone oxidoreductase 1 (NQO1), glutamate-cysteine ligase catalytic subunit (GCLC), and glutamate-cysteine ligase modulatory subunit (GCLM). Subsequent administration of berberine effectively lowered these oxidative stress markers. Additionally, antioxidant defenses including SOD, CAT, GPx, and GSH were significantly enhanced following berberine treatment. Bortezomib was also found to provoke inflammatory responses by upregulating pro-inflammatory cytokines such as NF-κB, tumor necrosis factor alpha (TNF-α), interleukin-1-beta (IL-1β), and interleukin-6 (IL-6), whereas berberine therapy successfully suppressed these cytokines, leading to reduced inflammation (Yardim et al. 2022). Studies have shown that berberine treatment improves learning and memory, and reduces hyperglycemia, oxidative stress, and acetylcholinesterase activity in diabetic rats (Bhutada et al. 2011).

In the present study, oral administration of berberine at 50 mg/kg body weight to the malathion-exposed group significantly diminished markers of oxidative damage (MDA and ROS) while enhancing the

activity of antioxidant enzymes (CAT and SOD) and acetylcholinesterase relative to the malathion-exposed group.

Li et al. demonstrated that berberine suppressed ROS generation, blocked programmed cell death, and preserved mitochondrial function in their cellular model. These observations indicate berberine capacity to mitigate cardiac aging via antioxidant and anti-apoptotic mechanisms, potentially mediated through modulation of the Klotho/SIRT1 signaling axis (Li et al. 2022). Research has shown that berberine significantly counteracts the elevation of TNF-α, IL-6, caspase 3, and Bax along with the rise in plasma cTn-T and serum MDA levels; furthermore, berberine significantly attenuates cardiac injury by significantly increasing cardiac IL-10 and serum GSH. Consequently, berberine mitigates cardiac ischemia/reperfusion damage in male rats through modulation of inflammation and suppression of programmed cell death (Abdulredha et al. 2021). Berberine can reduce oxidative stress damage and counteract the damage caused by inflammation in diabetic nephropathy by regulating antioxidant Nrf2 and subsequently regulating NLRP3-Caspase 1-GSDMD signaling to inhibit pyroptosis and reduce caspase 1 (Ding et al. 2021). Consistent with the above studies, we found that berberine treatment at a dose of 50 mg/kg reduced apoptosis (caspase 3) and pyroptosis (caspase 1) compared to the malathion group.

In a study, Nissl staining results demonstrated that berberine treatment in APP/PS1 mice reduced neuronal damage in the hippocampal CA1 region (Yang and Wang 2022). Additionally, findings from a study showed the potential of berberine to protect neurons in the CA1 region and the blood-brain barrier, increase antioxidant activity, and reduce memory impairments caused by global cerebral ischemia (Mehboodi et al. 2024). In our study, berberine treatment did not produce a significant effect on the number of neurons in the CA1 region following malathion-induced brain injury. This contrasts with previous reports indicating a neuroprotective effect of berberine on hippocampal neurons, which may be attributed to differences in experimental conditions such as injury models, dosage, treatment duration, or timing of assessment. Further investigations are needed to clarify these discrepancies.

Berberine treatment at 50 mg/kg significantly attenuated malathion-induced neurotoxicity, as demonstrated by enhanced antioxidant capacity, diminished apoptosis, and reduced pyroptosis. These findings position berberine as a promising neuroprotective agent against malathion toxicity. The present study comprehensively evaluated oxidative stress markers, antioxidant enzyme activities, apoptotic signaling, and pyroptotic pathways within hippocampal tissue. The present study limitations include the absence of detailed molecular pathway analyses, necessitating targeted future investigations. Furthermore, the examination was confined to the hippocampus, precluding evaluation of berberine potential effects across other cerebral territories or cellular populations susceptible to malathion toxicity. More comprehensive brain-wide assessments in future studies will provide deeper mechanistic insights.

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

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Ethical Considerations

All issues were reviewed by the review board and ethics committee of Shahed University of medical sciences, and the ethics code is IR.SHAHED.REC.1401.075.

Code of Ethics

IR.SHAHED.REC.1401.075.

Authors' Contributions

Saeid Iranzadeh: Investigation, Funding, Acquisition of data, Statistical analysis, Interpretation of data, Drafting of the manuscript and revision. Reza Sedaghat: Study concept and design, Methodology, Supervision, Acquisition of data, Statistical analysis, Interpretation of data, Drafting of the manuscript and revision. Mahdieh Taheri: Statistical analysis, Interpretation of data, Drafting of the manuscript and critical revision. Mehrdad Roghani: Study concept and design, Methodology, Supervision, Acquisition of data, Statistical analysis, Interpretation of data, Drafting of the manuscript and revision.

Data availability

The datasets can be obtained from the corresponding author if requested with a valid reason.

References

Abdel-Rahman A, Dechkovskaia AM, Goldstein LB, et al. (2004) Neurological deficits induced by malathion, DEET, and permethrin, alone or in combination in adult rats. J Toxicol Environ Health A 67(4):331-56 doi:10.1080/15287390490273569

Abdulredha A, Abosaooda M, Al-Amran F, Hadi NR (2021) Berberine Protests the Heart from Ischemic Reperfusion Injury via Interference with Oxidative and Inflammatory Pathways. Med Arch 75(3):174-179 doi:10.5455/medarh.2021.75.174-179

Akbarizadeh-Mashkani MH, Afshinmajd S, Iranzadeh S, Roghani M (2025) Involvement of synaptophysin and microtubule-associated protein 2 in the neuroprotective effect of berberine in an amyloid β-induced rat model of Alzheimer's disease. Avicenna J Phytomed 15(6):1741-1754 doi:10.22038/ajp.2025.26026

Althobaiti SA (2025) MitoQ alleviates malathion-induced hepatorenal toxicity via oxidative stress and inflammation modulation. Mol Med Rep 32(5):1-16 doi:10.3892/mmr.2025.13661

Asgari Z, Iranzadeh S, Roghani M (2025) Myricetin alleviates learning and memory deficits in trimethyltin Alzheimer's phenotype via attenuating hippocampal endoplasmic reticulum stress and regulating inflammation and oxidative stress. Brain Res Bull 227:111382 doi:10.1016/j.brainresbull.2025.111382

Baluchnejadmojarad T, Roghani M (2024) Exploring the molecular mechanisms underlying neuroprotective effect of ellagic acid in okadaic acid-induced Alzheimer's phenotype. Metab Brain Dis 39(7):1417-1432 doi:10.1007/s11011-024-01405-9

Begh MZA, Amin MA, Shatu MM, et al. (2025) Unraveling Berberine's Molecular Mechanisms in Neuroprotection Against Neurodegeneration. Chem Biodivers 22(8):e202500170 doi:10.1002/cbdv.202500170

Bhutada P, Mundhada Y, Bansod K, et al. (2011) Protection of cholinergic and antioxidant system contributes to the effect of berberine ameliorating memory dysfunction in rat model of streptozotocin-induced diabetes. Behav Brain Res 220(1):30-41 doi:10.1016/j.bbr.2011.01.022

Bodhinathan K, Kumar A, Foster TC (2010) Redox sensitive calcium stores underlie enhanced after hyperpolarization of aged neurons: role for ryanodine receptor mediated calcium signaling. J Neurophysiol 104(5):2586-93 doi:10.1152/jn.00577.2010

Cha HR, Kim JS, Ryu JH, Cho HJ (2025) Investigating Neuroprotective Effects of Berberine on Mitochondrial Dysfunction and Autophagy Impairment in Parkinson's Disease. Int J Mol Sci 26(15):7342 doi:10.3390/ijms26157342

Cheng H, Li X, Wang Y, et al. (2025) Berberine pharmacological properties and therapeutic potential across cancer, digestive, metabolic, cardiovascular, and neurological diseases: an update review. EXCLI Journal 24:1225-1261 doi:10.17179/excli2025-8771

Cheng Z, Kang C, Che S, et al. (2022) Berberine: A Promising Treatment for Neurodegenerative Diseases. Front

Pharmacol 13:845591 doi:10.3389/fphar.2022.845591

Coban FK, Ince S, Kucukkurt I, Demirel HH, Hazman O (2015) Boron attenuates malathion-induced oxidative stress and acetylcholinesterase inhibition in rats. Drug Chem Toxicol 38(4):391-9 doi:10.3109/01480545.2014.974109

Ding B, Geng S, Hou X, et al. (2021) Berberine Reduces Renal Cell Pyroptosis in Golden Hamsters with Diabetic Nephropathy through the Nrf2-NLRP3-Caspase-1-GSDMD Pathway. Evid Based Complement Alternat Med 2021(1):5545193 doi:10.1155/2021/5545193

Elmorsy E, Al-Ghafari A, Al Doghaither H, Salama M, Carter WG (2022) An Investigation of the Neurotoxic Effects of Malathion, Chlorpyrifos, and Paraquat to Different Brain Regions. Brain Sci 12(8) doi:10.3390/brainsci12080975

Fahanik-Babaei J, Baluchnejadmojarad T, Nikbakht F, Roghani M (2019) Trigonelline protects hippocampus against intracerebral Abeta(1-40) as a model of Alzheimer's disease in the rat: insights into underlying mechanisms. Metab Brain Dis 34(1):191-201 doi:10.1007/s11011-018-0338-8

Fan D, Liu L, Wu Z, Cao M (2019) Combating Neurodegenerative Diseases with the Plant Alkaloid Berberine: Molecular Mechanisms and Therapeutic Potential. Curr Neuropharmacol 17(6):563-579 doi:10.2174/1570159X16666180419141613

Fan KQ, Zhang L, Song F, et al. (2025) Pharmacological properties and therapeutic potential of berberine: a comprehensive review. Front Pharmacol 16:1604071 doi:10.3389/fphar.2025.1604071

Fang Z, Tang Y, Ying J, Tang C, Wang Q (2020) Traditional Chinese medicine for anti-Alzheimer's disease: berberine and evodiamine from Evodia rutaecarpa. Chin Med 15:82 doi:10.1186/s13020-020-00359-1

Faryadras K, Golchoobian R, Iranzadeh S, Roghani M (2025) Promising neuroprotective potential of naringenin against trimethyltin-induced cognitive deficits and hippocampal neurodegeneration in rats. Front Neurosci 19:1567236 doi:10.3389/fnins.2025.1567236

Fortunato JJ, Feier G, Vitali AM, Petronilho FC, Dal-Pizzol F, Quevedo J (2006) Malathion-induced oxidative stress in rat brain regions. Neurochem Res 31(5):671-8 doi:10.1007/s11064-006-9065-3

Gorun V, Proinov I, Baltescu V, Balaban G, Barzu O (1978) Modified Ellman procedure for assay of cholinesterases in crude enzymatic preparations. Anal Biochem 86(1):324-6 doi:10.1016/0003-2697(78)90350-0

Hargreaves AJ (2012) Neurodegenerations induced by organophosphorous compounds. Adv Exp Med Biol 724:189-204 doi:10.1007/978-1-4614-0653-2_15

Karami-Mohajeri S, Hadian MR, Fouladdel S, et al. (2014) Mechanisms of muscular electrophysiological and mitochondrial dysfunction following exposure to malathion, an organophosphorus pesticide. Hum Exp Toxicol 33(3):251-63 doi:10.1177/0960327113493300

Kishida KT, Klann E (2007) Sources and targets of reactive oxygen species in synaptic plasticity and memory. Antioxid Redox Signal 9(2):233-44 doi:10.1089/ars.2007.9.ft-8

Li C, Jiang S, Wang H, Wang Y, Han Y, Jiang J (2022) Berberine exerts protective effects on cardiac senescence by regulating the Klotho/SIRT1 signaling pathway. Biomed Pharmacother 151:113097 doi:10.1016/j.biopha.2022.113097

Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193(1):265-75

Massoud A, SaadAllah M, Dahran NA, et al. (2022) Toxicological effects of Malathion at low dose on Wister male rats with respect to Biochemical and Histopathological alterations. Frontiers in Environmental Science 10:860359 doi:10.3389/fenvs.2022.860359

Mehboodi D, Shahedi A, Namavar MR, Yadegari M, Vakili M (2024) Effect of berberine on the hippocampal structure, biochemical factors, memory, and blood-brain barrier in rat model of transient global cerebral ischemia. Phytother Res 38(8):4230-4239 doi:10.1002/ptr.8234

Meijer M, Hamers T, Westerink RHS (2014) Acute disturbance of calcium homeostasis

in PC12 cells as a novel mechanism of action for (sub)micromolar concentrations of organophosphate insecticides. Neurotoxicology 43:110-116 doi:10.1016/j.neuro.2014.01.008

Middlemore-Risher ML, Buccafusco JJ, Terry AV, Jr. (2010) Repeated exposures to low-level chlorpyrifos results in impairments in sustained attention and increased impulsivity in rats. Neurotoxicol Teratol 32(4):415-24 doi:10.1016/j.ntt.2010.03.008

Mohammadzadeh L, Abnous K, Razavi BM, Hosseinzadeh H (2020) Crocin-protected malathion-induced spatial memory deficits by inhibiting TAU protein hyperphosphorylation and antiapoptotic effects. Nutr Neurosci 23(3):221-236 doi:10.1080/1028415X.2018.1492772

Mooli RGR, Mukhi D, Ramakrishnan SK (2022) Oxidative Stress and Redox Signaling in the Pathophysiology of Liver Diseases. Compr Physiol 12(2):3167-3192 doi:10.1002/cphy.c200021

N'Go PK, Diboh E, Sylla I, et al. (2021) Comparison of the effects of argan and nigella oils on malathion-induced cognitive-behavioral alterations and brain histopathology in male Wistar rats. Toxicology 17

Narasimhamurthy RK, Venkidesh BS, Nayak S, et al. (2024) Low-dose exposure to malathion and radiation results in the dysregulation of multiple neuronal processes, inducing neurotoxicity and neurodegeneration in mouse. Environ Sci Pollut Res Int 31(1):1403-1418 doi:10.1007/s11356-023-31085-4

Nurulain SM, Szegi P, Tekes K, Naqvi SN (2013) Antioxidants in organophosphorus compounds poisoning. Arh Hig Rada Toksikol 64(1):169-77 doi:10.2478/10004-1254-64-2013-2294

Organization WH (2016) Use of malathion for vector control: report of a WHO meeting, Geneva, 16-17 May 2016. World Health Organization

Ozsoy AZ, Nursal AF, Karsli MF, et al. (2016) Protective effect of intravenous lipid emulsion treatment on malathion-induced ovarian toxicity in female rats. Eur Rev Med Pharmacol Sci 20(11):2425-34

Phogat A, Singh J, Malik V (2024) Pharmacological effects of Berberine – A Chinese medicine, against xenobiotics toxicity. Pharmacological Research - Modern Chinese Medicine 13:100507 doi:10.1016/j.prmcm.2024.100507

RA CAG (1985) Catalase activity. CRC Handbook of Methods for Oxygen Radical Research. Boca Raton: CRC Press

Saati AA (2024) Hesperidin Alleviates Malathion-Induced nephrotoxicity in Rats: Impact on Oxidative Damage, Inflammation, Pyroptosis and Apoptosis. Bulletin of Egyptian Society for Physiological Sciences 0(0):95-108 doi:10.21608/besps.2024.333609.1187

Salama OA, Attia MM, Abdelrazek MAS (2019) Modulatory effects of swimming exercise against malathion induced neurotoxicity in male and female rats. Pestic Biochem Physiol 157:13-18 doi:10.1016/j.pestbp.2019.01.014

Sedaghat R, Roghani M, Khalili M (2014) Neuroprotective effect of thymoquinone, the nigella sativa bioactive compound, in 6-hydroxydopamine-induced hemi-parkinsonian rat model. Iran J Pharm Res 13(1):227-34

Sedlak J, Lindsay RH (1968) Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal Biochem 25(1):192-205 doi:10.1016/0003-2697(68)90092-4

Suratman S, Edwards JW, Babina K (2015) Organophosphate pesticides exposure among farmworkers: pathways and risk of adverse health effects. Rev Environ Health 30(1):65-79 doi:10.1515/reveh-2014-0072

Tashakori-Miyanroudi M, Ramazi S, Hashemi P, Nazari-Serenjeh M, Baluchnejadmojarad T, Roghani M (2022) Acetyl-L-Carnitine Exerts Neuroprotective and Anticonvulsant Effect in Kainate Murine Model of Temporal Lobe Epilepsy. J Mol Neurosci 72(6):1224-1233 doi:10.1007/s12031-022-01999-8

Tayanloo-Beik A, Kiasalari Z, Roghani M (2022) Paeonol ameliorates cognitive deficits in streptozotocin murine model of sporadic Alzheimer's disease via attenuation of oxidative stress, inflammation, and mitochondrial dysfunction. Journal of Molecular Neuroscience 72(2):336-348

Tian E, Sharma G, Dai C (2023) Neuroprotective Properties of Berberine: Molecular Mechanisms and Clinical

Implications. Antioxidants (Basel) 12(10) doi:10.3390/antiox12101883

Ullah S, Li Z, Hasan Z, Khan SU, Fahad S (2018) Malathion induced oxidative stress leads to histopathological and biochemical toxicity in the liver of rohu (Labeo rohita, Hamilton) at acute concentration. Ecotoxicol Environ Saf 161:270-280 doi:10.1016/j.ecoenv.2018.06.002

Varol S, Başarslan S, Fırat U, et al. (2015) Detection of borderline dosage of malathion intoxication in a rat's brain. Eur Rev Med Pharmacol Sci 19(12):2318-2323

Venkatesan R, Park YU, Ji E, Yeo EJ, Kim SY (2017) Malathion increases apoptotic cell death by inducing lysosomal membrane permeabilization in N2a neuroblastoma cells: a model for neurodegeneration in Alzheimer's disease. Cell Death Discov 3(1):17007 doi:10.1038/cddiscovery.2017.7

Wang Y, Liao J, Luo Y, et al. (2023) Berberine Alleviates Doxorubicin-Induced Myocardial Injury and Fibrosis by Eliminating Oxidative Stress and Mitochondrial Damage via Promoting Nrf-2 Pathway Activation. Int J Mol Sci 24(4):3257 doi:10.3390/ijms24043257

Yadav P, Nair A, Chawla R, et al. (2025) From cell to organ: Exploring the toxicological correlation of organophosphorus compounds in living system. Toxicology 511:154049 doi:10.1016/j.tox.2025.154049

Yang M, Wang J (2022) Berberine Ameliorates Cognitive Disorder via GSK3beta/PGC-1alpha Signaling in APP/PS1 Mice. J Nutr Sci Vitaminol (Tokyo) 68(3):228-235 doi:10.3177/jnsv.68.228

Yao J, Wei W, Wen J, Cao Y, Li H (2023) The efficacy and mechanism of berberine in improving aging-related cognitive dysfunction: A study based on network pharmacology. Front Neurosci 17:1093180 doi:10.3389/fnins.2023.1093180

Yardim A, Gur C, Comakli S, et al. (2022) Investigation of the effects of berberine on bortezomib-induced sciatic nerve and spinal cord damage in rats through pathways involved in oxidative stress and neuro-inflammation. Neurotoxicology 89:127-139 doi:10.1016/j.neuro.2022.01.011

Zhang JH, Zhang JF, Song J, et al. (2021) Effects of Berberine on Diabetes and Cognitive Impairment in an Animal Model: The Mechanisms of Action. Am J Chin Med 49(6):1399-1415 doi:10.1142/s0192415x21500658

Zhang N, Gao Y, Yu S, Sun X, Shen K (2020) Berberine attenuates Aβ42-induced neuronal damage through regulating circHDAC9/miR-142-5p axis in human neuronal cells. Life Sci 252:117637 doi:10.1016/j.lfs.2020.117637

Zhang Y, Lv S, Huang P, Xiao L, Lin N, Huang E (2025) Network pharmacology study on the mechanism of berberine in Alzheimer's disease model. NPJ Sci Food 9(1):16 doi:10.1038/s41538-025-00378-y