

Research Article

Uncovering the neuroprotective powers of *Allium sativum*: exploring its potential to alleviate malathion- associated Parkinson's-like behavioral symptoms in a rat model

Bahareh Sadat Yousefsani^{1,2}, Ali Ghobadi^{1,2}, Kobra Shirani^{3,*}

¹ Institute for Studies in Medical History, Persian and Complementary Medicine, Iran University of Medical Sciences, Tehran, Iran

² Department of Traditional Medicine, School of Persian Medicine, Iran University of Medical Sciences, Tehran, Iran

³ Department of Toxicology, Faculty of Medical Sciences, Tarbiat Modares University, Tehran, Iran

ARTICLE INFO

Keywords:

Allium sativum
Parkinson 's disease
Malathion
Garlic
Oxidative stress
Inflammation

ABSTRACT

Background: Garlic, scientifically known as *Allium sativum*, has been a revered traditional medicine for millennia, rich in diverse phytochemicals with various medicinal properties, such as antioxidant, anticancer, anti-inflammatory, pain killer, hypoglycemic, antimicrobial, antiviral, and wound-healing properties. Its remarkable therapeutic potential makes garlic a beneficial natural treatment for many health issues. **Objective:** This research examined the neuroprotective effects of garlic against Malathion (Mal)-associated Parkinson's-like behavioral symptoms in rats. **Methods:** Animals were categorized into eight groups at random: (1) saline-treated group (control); (2) Mal group (100 mg kg⁻¹); (3) Mal + garlic (50 mg kg⁻¹); (4) Mal + garlic (100 mg kg⁻¹); (5) Mal + garlic (150 mg kg⁻¹); (6) Mal + L-DOPA (10 mg kg⁻¹); (7) garlic (150 mg kg⁻¹); and (8) polyethylene glycol (PEG) group (L-DOPA vehicle). Treatment lasted 28 days, followed by behavioral assessments and analyses of acetylcholinesterase (AChE) activity, malondialdehyde (MDA) level, antioxidants levels, and proinflammatory cytokines in the striatum. **Results:** Mal exposure caused neurobehavioral toxicity with increased MDA, tumor necrosis factor- α (TNF- α), and interleukin-6 (IL-6), alongside decreased GSH concentration and AChE activity. Garlic treatment successfully mitigated these effects. **Conclusion:** The findings suggest that garlic may have a favorable impact in treating Parkinson's disease by enhancing antioxidant levels and mitigating oxidative stress and inflammatory processes, countering the harmful effects of pesticides like Mal. Additional studies necessary to thoroughly explore garlic's protective benefits in this area.

Abbreviations: 6-OHDA, 6-hydroxydopamine; AChE, Acetylcholinesterase; AP-1, Activator protein 1; DTNB, 5,50-dithiobis 2- nitrobenzoic acid; GSH, Glutathione; IL-6, Interleukin-6; IP, Intraperitoneal injection; Mal, Malathion; MAPK, Mitogen-activated protein kinase; MDA, Malondialdehyde; MPTP, 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine; PEG, Polyethylene glycol; PUFAs, Polyunsaturated fatty acids; OFT, Open field test; ROS, Reactive oxygen species; SAC, S-allyl cysteine; SEC, S-ethyl cysteine; SN, Substantia nigra; SNpc, Substantia nigra pars compacta; SPC, S-propyl cysteine; TH, Tyrosine hydroxylase; TNF α , Tumor necrosis factor alpha; TPM, Traditional Persian medicine

*Corresponding author: k.shirani@modares.ac.ir

doi: [10.61186/jmp.23.89.68](https://doi.org/10.61186/jmp.23.89.68)

Received 29 May 2024; Received in revised form 24 August 2024; Accepted 26 August 2024

© 2023. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>)

1. Introduction

Parkinson was initially identified by James Parkinson as the "shaking palsy" and is now recognized as a major neurological disorder [1]. It is regarded as the one of the most widespread nervous system dysfunction, impacting more than 8.5 million individuals throughout the world, primarily the elderly population [2]. The main pathological manifestation of parkinson includes the death of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the aggregation of misconfigured α -synuclein into complexes called Lewy bodies. A notable reduction in the levels of tyrosine hydroxylase (TH), an enzyme crucial for dopamine production in the SNpc region serves as a significant marker for Parkinson's disease [3].

The primary signs of parkinson mainly encompass motor symptoms like rigidity, bradykinesia, and resting tremor, alongside nonmotor challenges including emotional distress, loss of cognitive abilities, hyposmia, anxiety, drowsiness, and gastrointestinal problems. Mutations in parkinson-related genes and contact with toxic substances like pesticides, welding emissions, and airborne particulate pollutants may possibly have linked with parkinson. Additional important pathological mechanisms of involve the regulation of α -synuclein proteostasis, dysfunctional mitochondria, increased reactive oxygen species (ROS) imbalance, disruption of calcium homeostasis, impaired axonal transport, and atypical neural inflammation responses [4, 5].

At present, FDA-approved methods for treating parkinson focus on enhancing dopamine production and inhibiting its breakdown. While these approaches effectively alleviate the motor symptoms by increasing dopamine levels, they do not stop the progression of the disease. L-DOPA is frequently utilized as a standard

treatment for parkinson, but it could trigger the development of dyskinesia, which further impairs the quality of life. Additionally, as the disease advances, both motor and nonmotor symptoms become more resistant to treatment. Hence, it is imperative to develop treatments that can modify the progression of the illness and delay disability in parkinson [6].

Interestingly, numerous nutraceuticals derived from plants offer significant advantages and have historically been utilized to address various disorders [7]. *Allium sativum* (Garlic) is a widely used vegetable in cooking to elevate the palatability and savor of culinary creations [8]. It has served as a valuable traditional remedy globally since ancient times, integrated into Ayurvedic medicine for 6000 years. It is considered among the most widely used phytomedicine, offering positive effects while causing minimal negative reactions [9]. The neuroprotective properties of garlic encompass antioxidant, anti-inflammatory, and anti-mitochondrial dysfunction attributes. The organosulfur compounds and polyphenols primarily account for their medicinal actions. Garlic supplements are accessible in various forms in the market [10]. Numerous studies propose that the administration of bioactive components or garlic as a treatment has demonstrated notable shielding effects in specific animal models of parkinson. It has been evidenced that these compounds can potentially reverse dopaminergic degeneration by targeting pathways linked to parkinson pathogenesis [11]. According to the texts of Traditional Persian medicine (TPM) books, it is mentioned under the name of SOOM, it has many properties. It is mentioned in the book "Makhz al-Adawieh" that this herb is a treatment for "paralysis, paralysis, tremors and most of the nervous diseases", which according to its properties, it seems that it can be

used for the treatment of parkinson [12]. The current investigation aimed to assess the antioxidative and anti-inflammatory properties of varying concentrations of garlic in a Malathion (Mal)-induced animal model of parkinson.

2. Materials and methods

2.1. Treatment protocols

Thirty two adult male Wistar rats weighing between 250 and 300 g and aged 70 days were acclimated in polystyrene cages for a week before the commencement of the study. They were housed under 12-h light and 12-h dark (12:12 LD) conditions, at a room temperature ranges from 20°C to 25 °C, and 50 % relative humidity. Food and water were available ad libitum to the rats. The animals were given time to adjust to their surroundings prior to the initiation of the experimental procedures. The animal were allocated to eight groups at random, each comprising six animals. The groups were categorized as follows: (1) saline-treated group (control); (2) malathion (Mal)- group (100 mg kg⁻¹); (3) Mal + garlic (50 mg kg⁻¹) group; (4) Mal + garlic (100 mg kg⁻¹) group; (5) Mal + garlic (150 mg kg⁻¹) group; (6) Mal + L-DOPA (10 mg kg⁻¹) group; (7) garlic (150 mg kg⁻¹) group; and (8) polyethylene glycol (PEG) group (L-DOPA vehicle) [13]. Ethical approval number is [IR.IUMS.REC.1398.606](https://doi.org/10.61186/jmp.23.89.68).

2.2. Behavioral tests

2.2.1. The open field test (OFT)

The OFT test was done to analyze motor functioning as previously described. The parameters assessed during OFT included the total number of squares crossed (overall locomotion), the number of outer squares crossed (indicating peripheral locomotion), and the number of inner squares crossed (central

locomotion). The OFT was done on the initial day and after the final treatment [14].

2.2.2. Rotarod test

One day after the final treatment, an accelerating rotarod test was administered. Prior to the assessment, each rat underwent a training session on the morning of the test, where they were required to remain on the rotarod apparatus for 30 seconds, with the device revolving at a speed of 5 rpm. During the actual test, the rats were placed on a rotarod set at a speed of 10 rpm. The duration (in seconds) that each animal remained on the rod was meticulously recorded. A cutoff time of 180 seconds was established for the test [13].

2.2.3. Catalepsy

The catalepsy test, as outlined by Costall and Naylor (1974), evaluated the time it took for an animal to remove one or both of its forelimbs from a horizontal metal bar. During the assessment, the animal was positioned with its forelimbs resting on the bar, which was situated 9 cm above the base, allowing for the measurement of muscular rigidity [15].

2.2.4. Determination of acetylcholinesterase (AChE) activity

The animals were euthanized and their serum was collected for the subsequent measurement of plasma AChE activity. This assessment was conducted following the Ellman method. In short, 20 µl of the collected serum sample was combined with 100 µl of a 5 % (w/v) acetylthiocholine iodide solution and 3 ml of Ellman's reagent (5, 5'-dithiol-bis- [2-nitrobenzoic acid]). The absorbance of the resulting mixture was then recorded at 0.5-minute intervals for a total duration of 2 minutes. The calculation of enzyme inhibition percentage

was determined by the following formula: Inhibition (%) = $(\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}) / \text{Absorbance}_{\text{control}} \times 100$ [14].

2.3. Measurement of MDA in the striatum tissue

Following euthanasia by decapitation, the animals' brains were excised, and the striatum was immediately divided in an ice-cold environment. The isolated striatum samples were then homogenized for 2 minutes at 4 °C in a 1.15 % KCl solution. This procedure was undertaken to measure the levels of malondialdehyde (MDA), a byproduct formed from the degradation of arachidonic acid and other polyunsaturated fatty acids (PUFAs), as per the methodology established by Fernandez et al. (1997) described previously [16].

2.4. Assessment of GSH levels

The 1 ml striatum homogenate sample was subjected to precipitation by the addition of 1 ml of 10 % trichloroacetic acid (TCA). The resulting precipitate was then separated through centrifugation at 3,000 g for 5 minutes. Next, 0.5 ml of the supernatant was combined with 2 ml of DTNB (5,5'-dithiobis (2-nitrobenzoic acid)), and the total volume was adjusted to 3 ml using a 0.2 M phosphate buffer (pH 8.0). The absorbance of the obtained mixture was subsequently assessed at a wavelength of 412 nm. The glutathione (GSH) content of the tissues was reported in units of nmol/g of tissue [17].

2.5. Detection of tumor necrosis factor alpha (TNF α) and interleukin-6 (IL-6) in striatum tissue

The frozen striatum samples were homogenized and the total protein level was analyzed by a protein assay kit (Bio-Rad). Afterward, the levels of the inflammatory

cytokines TNF α and IL-6 were assessed by ELISA kits (Invitrogen, USA), following the related instructions [18].

2.6. Statistical analysis

The data were expressed as the mean \pm standard deviation (SD). Statistical evaluation was conducted using one-way ANOVA, followed by Tukey's multiple comparison test, facilitated by PRISM software (version 6.00, GraphPad Software Inc., San Diego, CA, USA). A p-value of under 0.05 was regarded as statistically significant.

3. Results

3.1. Open-field test

The assessment of locomotor activity was conducted through the open-field test on both the initial and concluding days of the experiment. On the first day, treatment with Mal alone or in plus garlic and L-DOPA did not have any significant effect on total locomotion (TL) (Figure 1a) (F for PL = 2.2, F for TL = 0.91 and F for CL = 0.33).

However, on the final day, Mal (100 mg kg⁻¹, IP) diminished the parameters of peripheral locomotion (PL), central locomotion (CL), and TL compared to the saline-treated group (P < 0.001). Interestingly, the addition of garlic (all doses) and L-DOPA to Mal treatment extremely enhanced PL, TL and CL (Figure 1b). Additionally, the administration of garlic at a dosage of 150 mg kg⁻¹ did not lead to any substantial changes in PL, TL, and CL when compared to both the Mal-exposed group and the saline-treated group at 28th day of experiment (P > 0.05). (F for PL = 128.19, F for TL = 77.69 and F for CL = 143.46).

3.2. Rotarod test

As shown in Figure 2, the rotarod test yielded clear results indicating a decrease in the time it took

for the Mal group to fall compared to the saline-treated group ($P < 0.001$). The Mal group exhibited a significant decline in muscle coordination when compared to the normal saline group. However, the administration of garlic (all doses) and L-DOPA in combination with Mal partially restored muscular coordination. Interestingly, no meaningful difference was observed between the group treated with garlic alone (150 mg kg⁻¹) and the control group ($F = 17.48$).

3.3. Catalepsy

As indicated in Figure 3, the bar test results indicated that subchronic exposure to Mal led to

a notable increase in cataleptic immobilization in contrast the control group ($P < 0.001$). Conversely, the co-treatment of garlic at all doses and L-DOPA with Mal substantially mitigated the cataleptic effects induced by Mal ($P < 0.001$). Additionally, no considerable variances were seen in the duration of time spent on the bar test among the groups treated with PEG or garlic alone and the control group. Furthermore, there were no notable differences in the cataleptic immobilization between the PEG or garlic alone treated animals and the control animals ($F=251.8$).

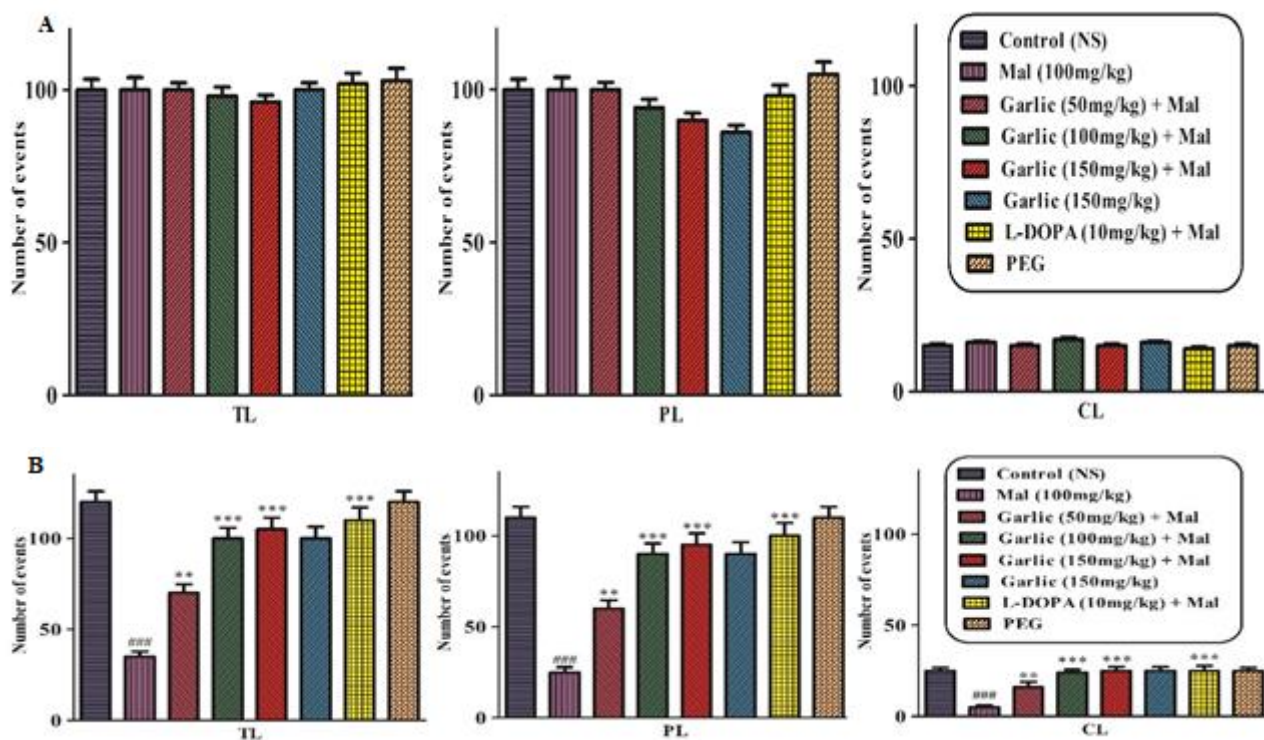


Fig. 1. Effects of garlic on locomotor activity in the open field test (OFT) on the starting day of the study (A) and final day of the study (B). CL (central locomotion), Malathion (Mal), PEG (polyethylene glycol), PL (peripheral locomotion), and TL (total locomotion).

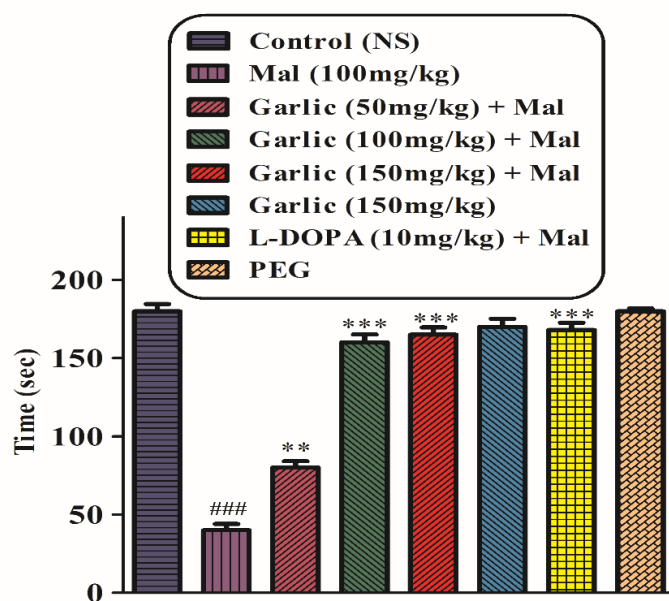


Fig. 2. Impacts of garlic on motor coordination in rotarod test. malathion (Mal), PEG (polyethylene glycol)

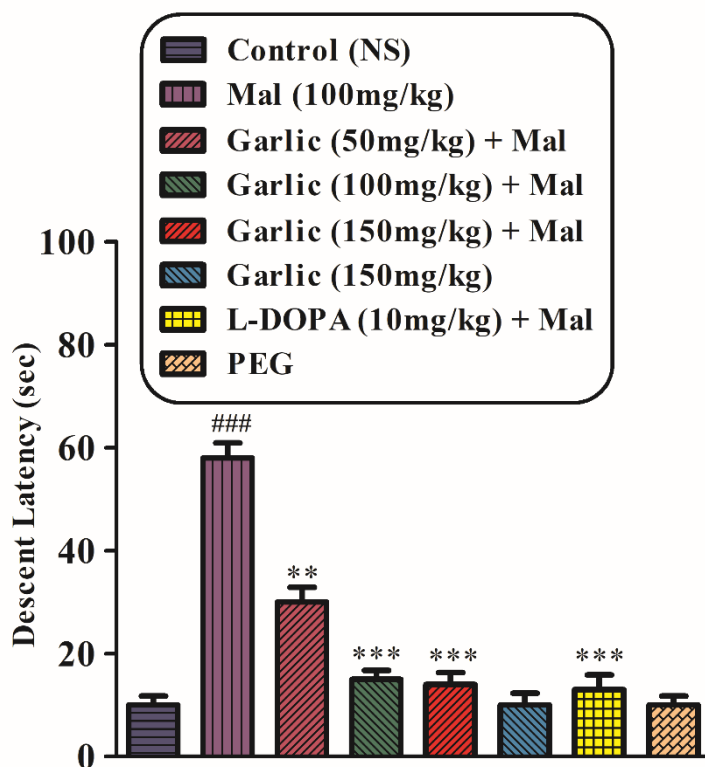


Fig. 3. Effects of garlic on Mal-induced catalepsy. Malathion (Mal) and PEG (polyethylene glycol)

3.4. Acetylcholinesterase activity

As illustrated in Figure 4, administration of Mal (100 mg kg⁻¹) to rats led to a notable reduction in plasma AChE activity after 28 days in comparison

to the control group (P < 0.001). Nevertheless, there were no considerable alterations in AChE activity between the groups treated with Mal plus garlic (50 mg kg⁻¹) and those treated solely with Mal (P >

0.05). Conversely, the groups receiving Mal plus garlic (100 and 150 mg kg⁻¹) as well as the L-DOPA group displayed a significant increase in AChE activity when compared to the Mal-only group ($P < 0.001$) ($F=1194.53$).

3.5. Oxidative Stress Markers

3.5.1. MDA

The MDA level in the striatum of rats was significantly increased after being exposed to Mal for a subchronic period, as compared to the saline-treated group ($P < 0.001$). Nevertheless, when garlic (at three different doses) and L-DOPA were co-administered with Mal, the MDA level in the rat striatum was effectively reduced in comparison to the Mal group ($P < 0.001$) (Figure. 5) ($F=63.35$).

3.5.2. GSH

Administration of both garlic at doses of 100 and 150 mg kg⁻¹, along with Mal, resulted in a notable

increase in the levels of GSH in the rat striatum when compared to the effects of Mal alone ($P < 0.001$). Furthermore, no meaningful differences were observed in the level of GSH between the groups that PEG or garlic alone treated group and the control group (Figure. 6) ($F = 39.73$).

3.5.3. Cytokine production

The levels of TNF- α and IL-6 in the striatum were significantly elevated in rats administered Mal, as depicted in Figure 7a and b. Conversely, the administration of garlic resulted in a notable decrease in TNF- α and IL-6 levels when compared to the Mal group. It is worth noting that the administration of garlic alone did not induce any notable alterations in TNF- α and IL-6 levels when compared to the saline-treated rats (F for TNF- α =52.2 and F for IL-6 =413.4).

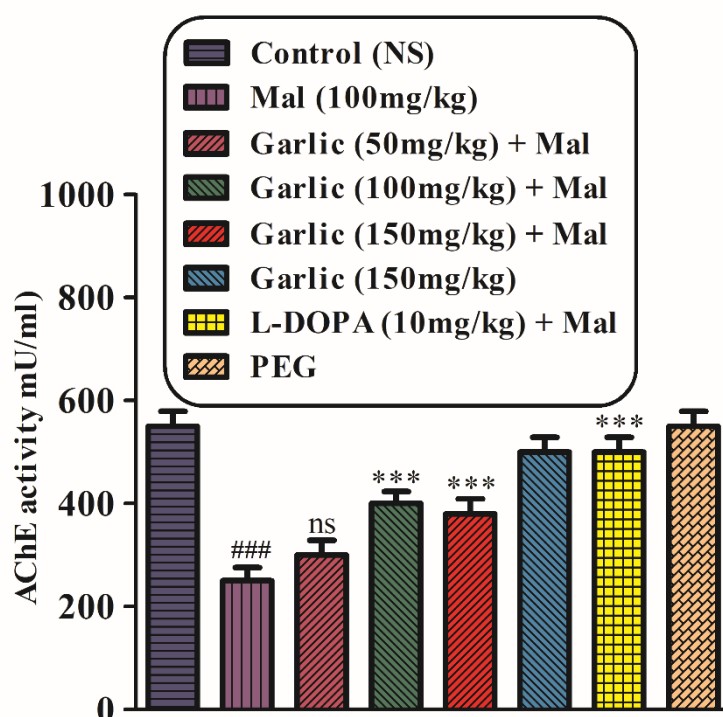


Fig. 4. Effects of garlic on AChE activity. AChE (Acetylcholinesterase), Malathion (Mal), and PEG (polyethylene glycol).

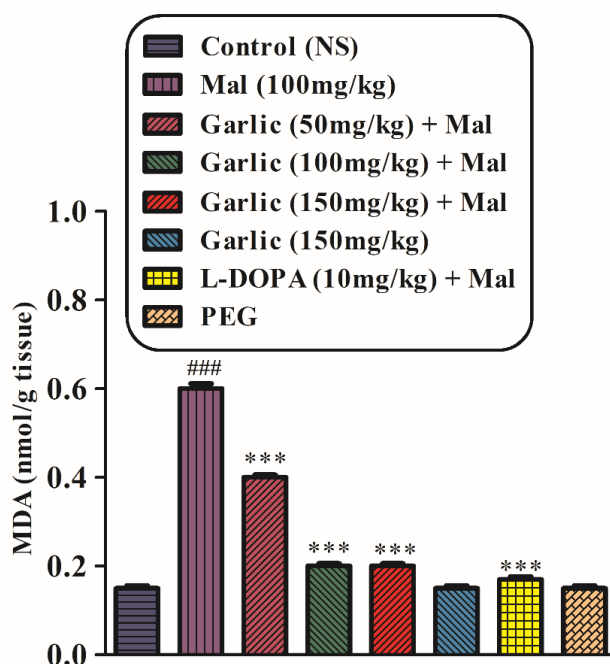


Fig. 5. Effect of garlic on Malathion induced lipid peroxidation in striatum of rat. Malathion (Mal), MDA (malondialdehyde), and PEG (polyethylene glycol)

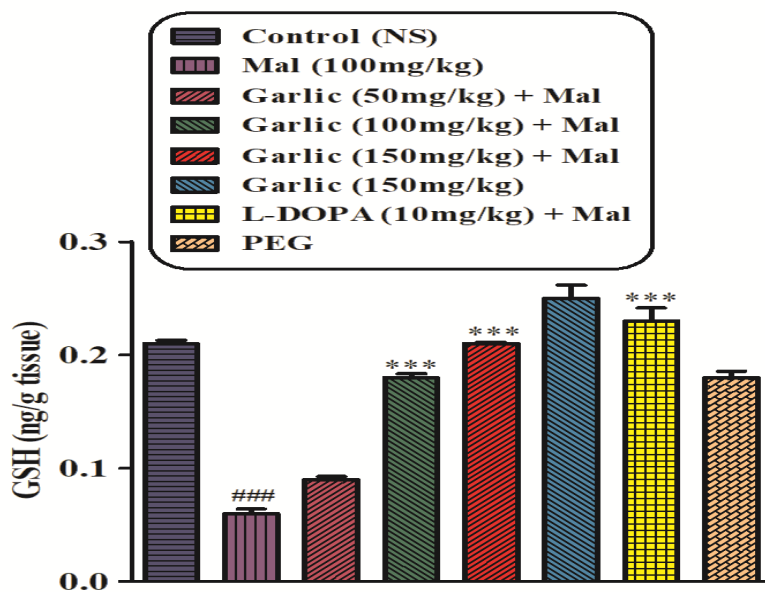


Fig. 6. Effect of garlic on GSH in rat striatum. Malathion (Mal), GSH (glutathione), PEG (polyethylene glycol).

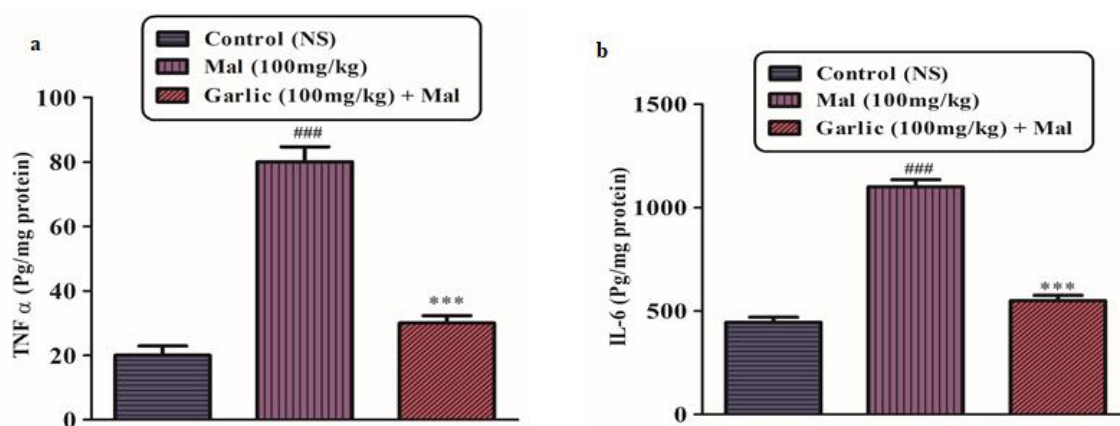


Fig. 7. Effects of garlic on TNF- α (a) and IL-6 (b) levels in rat striatum. Malathion (Mal), IL-6 (interleukin-6), and TNF α (tumor necrosis factor alpha)

4. Discussion

The goal of this research was to examine the neuroprotection ability of garlic against Mal-induced Parkinson's-like behavioral manifestations in rats through the implementation of comprehensive behavioral assessments. Mal is a consumable organophosphate pesticide that cause oxidative stress by via ROS formation and antioxidant defence mechanisms [19]. The findings of our investigation showed that exposure to Mal led to a considerable reduction in locomotor activity and reduced the time spent on the rotarod device, along with enhanced catalepsy. Notably, garlic administration was found to effectively ameliorate these Mal-induced detrimental effects. To comprehensively evaluate motor perturbation, we employed the rotarod test, which takes into account various factors, including lack of coordination, slow movement, and muscular rigidity. This well-established test has been extensively utilized to assess motor abilities in rodent models of parkinson [20, 21]. The results of the present study indicated that the administration of Mal for a duration of 28 days led to a notable decrease in the time spent on the

rotarod apparatus in comparison to the saline-treated group. These outcomes implies that Mal exposure compromised muscular coordination in the treated animals. Interestingly, subjects that received a combination of garlic and Mal exhibited a marked enhancement in muscle coordination and grip strength. Furthermore, by the end of the treatment period, the Mal group displayed a remarkable increase in catalepsy and postural instability compared to the saline-treated group. Importantly, these Mal-induced alterations in cataleptic behavior and postural stability were substantially ameliorated in the garlic group when compared to the Mal-only treatment group. Catalepsy, a hallmark feature of parkinson, is frequently utilized as an indicator to assess the degree of nigrostriatal neurodegeneration. The standard bar test method, involving placing a mice on a horizontal bar and evaluation the time taken for the mice to withdraw its forepaws, is widely employed to induce and evaluate catalepsy in animal models [22]. Neurotoxic substances, such as 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP) and 6-hydroxydopamine (6-OHDA), as well as organophosphorus pesticides, have been

demonstrated to induce catalepsy in lab animal. These experimental models are invaluable for investigating the molecular mechanisms of parkinson and for assessing possible therapeutic interventions [23]. Indeed, the cataleptic behavior observed in animals due to exposure to the neurotoxin Mal in our investigation is associated with degeneration of the dopaminergic nigrostriatal pathway. Importantly, the open-field test revealed that Mal exposure led to a decrease in overall locomotor activity. Remarkably, the administration of garlic played a significant role in safeguarding against these Mal-induced detrimental effects on locomotion [13, 24]. Taken together, the findings of the research indicated that the negative effects on behavior in rats induced by Mal, resembling those seen in parkinson, were improved by the use of garlic. This suggests that garlic has the potential to be a beneficial neuroprotective treatment for parkinson. The outcomes of this study align with the traditional medicinal uses of garlic in Traditional Persian Medicine [25]. Previous research has demonstrated that the administration of L-DOPA (10 mg kg⁻¹) effectively ameliorates the behavioral impairments induced by Mal. This finding aligns with earlier studies that have shown the potential of L-DOPA treatment in reversing deficits in behavior associated with the degeneration of the nigrostriatal dopamine system [26].

Acetylcholine, a vital neurotransmitter, plays a pivotal role in the normal functioning of brain and body, including muscle contraction, memory, and attention. The enzyme AChE is accountable for the degradation of acetylcholine, thus controlling its concentration. The suppression of AChE function may cause an increase in acetylcholine levels, leading to overstimulation of cholinergic receptors and consequent neurotoxic effects [27].

Consequently, inhibition of AChE activity serves as a reliable indicator for organophosphorus pesticides neurotoxicity in both acute and chronic exposures. Organophosphorus pesticides, such as Mal, mainly produce their pharmacological and toxicological impacts through the inhibition of AChE. Studies have demonstrated that administering subacute doses of Mal (50 and 150 mg kg⁻¹, intraperitoneal) notably decrease AChE activity in rats. In accordance with earlier investigations, our results exhibited a significant decline in plasma AChE activity in rats exposed to Mal. Intriguingly, our findings also suggest that garlic could potentially increase AChE activity and mitigate the neurobehavioral disturbances induced by Mal exposure. These results indicate that the modulation of cholinergic signaling may related to the protective properties of garlic against the neurobehavioral toxicity caused by Mal. The regulation of acetylcholine levels, a critical neurotransmitter involved in various physiological processes appears to be a key mechanism underlying the neuroprotective properties of garlic [28, 29].

Oxidative stress is a critical mechanism underlying dopaminergic neurodegeneration in parkinson. The neurotoxins are commonly used in preclinical models to induce parkinson-like pathology by selectively targeting and injuring dopaminergic neurons in the SNpc, leading to elevated ROS and cell death [30, 31]. The findings of this study indicate that the neural abnormalities observed in Mal-induced parkinson behavior can be attributed to the generation of free radicals and oxidative damage. Exposure to Mal leads to oxidative stress by upsetting the balance between ROS and antioxidants, resulting in elevated levels of MDA. The lipophilic nature of Mal enables it to interplay with brain membranes and impair

antioxidant defenses, rendering the central nervous system susceptible to oxidative damage. Mal has been found to influence the activity of antioxidant enzymes and decrease GSH levels, while raising GSSG levels, thereby reducing the GSH/GSSG ratio. This decrease in GSH concentration mirrors the observations made in parkinson, underscoring the neurotoxicity of Mal through a pro-oxidative mechanism [28, 29]. To investigate the potential anti-parkinson impact of garlic, its antioxidant properties against Mal-induced oxidative stress were examined. Figure 6 and 5 demonstrate that treatment with garlic incomparably mitigated the production of MDA induced by Mal and increased the concentration of GSH. These findings suggest that garlic has the ability to ameliorate oxidative imbalances and contribute to the maintenance of redox homeostasis. Allicin, a compound found in garlic extract, has demonstrated neuroprotective effects in 6-OHDA-induced cell toxicity in PC12 cells. Allicin pretreatment substantially declined intracellular ROS generation, lipid peroxidation, and protein oxidative damage in these cells. Additionally, allicin helped maintain the activity of endogenous antioxidant enzymes, which were impaired by 6-OHDA exposure [32]. Another garlic-derived compound, S-allyl cysteine (SAC), has also shown neuroprotective effects in animal models of parkinson. SAC was able to decrease lipid peroxidation, preserve Cu-Zn-SOD activity, and improve locomotor malfunction and dopamine levels in the striatum of mice treated with the neurotoxin MPP⁺. The proposed mechanisms involve SAC's free radical scavenging properties and its ability to restore antioxidant enzyme activity (33). Furthermore, pretreatment with other garlic-derived sulfur compounds like S-ethyl cysteine (SEC), has also been shown to decrease oxidative damage and

enhance antioxidant defenses in an MPTP-induced mouse model of parkinson [34].

Neuroinflammation is a crucial pathological mechanism implicated in various neurodegenerative disorders, including parkinson. Neuroinflammation is typically mediated by the activation of microglia and astrocytes, leading to the to an increase of pro-inflammatory cytokines such as TNF- α and IL-6. The activation of specific signaling pathways, particularly the p38 Mitogen-activated protein kinase (MAPK) pathway, plays a central role in regulating neuroinflammation and contributing to neuronal damage [35]. TNF- α , a pivotal cytokine implicated in inflammation, immune response, and cellular arrangement, assumes a vital function in the inflammatory reaction (18). IL-6 is associated with a wide range of humoral and cellular immune responses in inflammation and tissue injury [36]. The findings of the research indicated that Mal treatment resulted in elevated levels of proinflammatory cytokines like IL-6 and TNF- α in striatal tissue, suggesting that inflammation could contribute to the development of Mal-induced neurotoxicity. Intriguingly, the garlic treatment notably decreased the levels of both IL-6 and TNF- α .

Garlic-derived sulfur compounds, such as thiacremonone, have demonstrated anti-inflammatory properties in both in vitro and in vivo models of parkinson. Thiacremonone reduced MPTP-induced behavioral disturbances, dopamine evacuation, microglia and astrocyte activation, and neuroinflammation. These neuroprotective effects were mediated by the inhibition of the p38 MAPK and activator protein 1 (AP-1) signaling pathways. Additionally, other garlic-derived compounds have been found to decline the concentration of proinflammatory cytokines (TNF- α , IL-1 β , and IL-6) in an MPTP-model of parkinson [37, 38]. This protective

effect is thought to be related to these compounds ability to inhibit ROS production.

A limitation of the this study design is that we did not assess the effects of the various garlic doses in the absence of MAL exposure. Assessing the independent effects of garlic at these doses would have provided valuable information to better differentiate the neuroprotective properties of garlic from any potential symptomatic effects. Additionally, some compounds can exhibit dose-dependent relationships, and including the full range of garlic-only groups would have enabled us to explore any non-linear effects. It is suggested in future research, to incorporate the complete set of garlic dose groups, both in the presence and absence of MAL, to provide a more comprehensive evaluation of garlic's neuroprotective potential and any dose-dependent mechanisms of action.

5. Conclusions

The findings of this study indicated that garlic consumption was effective in mitigating the symptoms resembling parkinson that were triggered by exposure to the pesticide Mal. This therapeutic effect was directly linked to garlic's ability to decrease the elevated levels of oxidative stress and pro-inflammatory cytokines

References

1. Yousefsani BS, Ghobadi A, Dadmehr M and Shirani K. Castor Oil. A natural remedy with promising potential for Parkinson 's disease prevention. *Jundishapur. J. Nat. Pharm. Prod.* 2024; 19(1): e143882. doi: 10.5812/jjnpp-143882.
2. Santiago JA and Potashkin JA. Physical activity and lifestyle modifications in the treatment of neurodegenerative diseases. *Front. Aging. Neurosci.* 2023; 15: 1185671. doi: 10.3389/fnagi.2023.1185671.

observed in the Mal subjects. These findings show that garlic may offer a promising and feasible natural approach to combating parkinson -like manifestations resulting from pesticide exposure. The widespread use of garlic in traditional and modern medicine underscores its potential as an effective and accessible treatment option. However, the study notes that further comprehensive investigation is necessary to fully understand the detailed action mechanisms and the full therapeutic capabilities of garlic in the context of parkinson and pesticide-induced neurological damage. Nonetheless, this study supplied valuable insights into the neuroprotective properties of this versatile plant and its potential to serve as a natural intervention against parkinson -like disorders.

Conflict of Interest

All authors declared that there are no conflicts of interest.

Author Contributions

Research conception and design: KSH and AGH. Data collection: KSH, BYS. Analysis and interpretation of data: BYS. Drafting of the manuscript: KSH, AGH and BYS. Review and editing: KSH. All authors contributed to the article and approved the submitted version.

3. Xu L and Pu J. Alpha-Synuclein in Parkinson 's disease: from Pathogenetic dysfunction to potential clinical Application. *Parkinsons. Dis.* 2016; 2016: 1720621. doi: 10.1155/2016/1720621.
4. Váradi C. Clinical features of Parkinson 's disease. The evolution of critical symptoms. *Biology (Basel).* 2020; 9(5): 103. doi: 10.3390/biology9050103.
5. Radad K, Moldzio R, Krewenka C, Kranner B and Rausch W-D. Pathophysiology of non-

- motor signs in Parkinson 's disease: some recent uparkinson ating with brief presentation. *Explor. Neuroprot. Ther.* 2023; 3: 24-46. doi: 10.37349/ent.2023.00036.
- 6.** Gouda NA, Elkamhawy A and Cho J. Emerging Therapeutic strategies for Parkinson 's disease and future prospects: A 2021 update. *Biomedicines* 2022; 10(2): 371. doi: 10.3390/biomedicines10020371.
- 7.** Shirani K, Hassani FV, Azar-Khiavi KR, Samie Moghaddam Z and Karimi GhR. Determination of methanol in Iranian herbal distillates. *J. Complement. Integr. Med.* 2016; 13(2): 123-7. doi: 10.1515/jcim-2015-0041.
- 8.** Shang A, Cao SY, Xu XY, Gan R-Y, Tang G-Y, Corke H, Mavumengwana V and Li H-B. Bioactive compounds and biological functions of Garlic (*Allium sativum* L.). *Foods* 2019; 8(7): 246. doi: 10.3390/foods8070246.
- 9.** Petrovska BB and Cekovska S. Extracts from the history and medical properties of Garlic. *Pharmacogn. Rev.* 2010; 4(7): 106-10. doi: 10.4103/0973-7847.65321.
- 10.** Verma T, Aggarwal A, Dey P, Chauhan AK, Rashid S, Chen K-T and Sharma R. Medicinal and therapeutic properties of garlic, garlic essential oil, and garlic-based snack food: An updated review. *Front. Nutr.* 2023; 10. doi: 10.3389/fnut.2023.1120377.
- 11.** Bigham M, Mohammadipour A, Hosseini M, Malvandi AM, Ebrahimzadeh-Bideskan A. Neuroprotective effects of garlic extract on dopaminergic neurons of substantia nigra in a rat model of Parkinson 's disease: motor and non-motor outcomes. *Metab. Brain. Dis.* 2021; 36(5): 927-937. doi: 10.1007/s11011-021-00705-8.
- 12.** Alavi Shirazi A, Mohammad Hadi SMH and Esfahani MM. Makhz al-Adawieh. Tehran, Iran: Iran University of Medical Sciences. Institute of Medical History Studies. Islamic and complementary medicine. 1387, P. 288. [Persian].
- 13.** Mohammadzadeh L, Hosseinzadeh H, Abnous K and Razavi BM. Neuroprotective potential of crocin against Mal-induced motor deficit and neurochemical alterations in rats. *Environ. Sci. Pollut. Res. Int.* 2018; 25(5): 4904-4914. doi: 10.1007/s11356-017-0842-0.
- 14.** Kraeuter A-K, Guest PC and Sarnyai Z. The open field test for measuring locomotor activity and anxiety-like behavior. *Methods Mol. Biol.* 2019; 1916: 99-103. doi: 10.1007/978-1-4939-8994-2_9.
- 15.** Costall B and Naylor RJ. On catalepsy and catatonia and the predictability of the catalepsy test for neuroleptic activity. *Psychopharmacologia.* 1974; 34(3): 233-41. doi: 10.1007/BF00421964.
- 16.** Yousefsani BS, Bahrami B, Qobadi A, et al. The selective cytotoxicity of the hydroalcoholic extract of Santalum Album Linn wood on A375 and SK-MEL-3 human malignant Melanoma cells: *Iran. J. Pharmaceutical. Sci.* 2022; 18(2). doi: 10.22037/ijps.v18.42139.
- 17.** Yousefsani BS, Salimi A, Imani F, Ramezani M, Shirani K, Seydi E and Pourahmad J. Risperidone toxicity on human blood lymphocytes in nano molar concentrations. *Drug. Res.* 2022; 72(6): 343-349. doi: 10.1055/a-1830-8701.
- 18.** Shirani K, Iranshahi M, Askari VR, Gholizadeh Z, Attaran Zadeh A, Zeinali M and Vahdati Hassani F. Comparative evaluation of the protective effects of oral administration of auraptene and umbelliprenin against CFA-induced chronic inflammation with polyarthritis in rats. *Biomed. Pharmacother.* 2021; 139: 111635. doi: 10.1016/j.biopha.2021.111635.
- 19.** Nikkhah E, Shirani K, Rezaee R and Karimi GhR. Protective effects of taurine against hepatotoxicity induced by pharmaceuticals and

- environmental chemicals. *Toxicol. Environ. Chem.* 2021; 103(1): 56-84. doi: 10.1080/02772248.2021.1892113.
- 20.** Recchia A, Rota D, Debetto P, Peroni D, Guidolin D, Negro A, Skaper SD and Giusti P. Generation of a α -synuclein-based rat model of Parkinson's disease. *Neurobiol. Dis.* 2008; 30(1): 8-18. doi: 10.1016/j.nbd.2007.11.002.
- 21.** Sedelis M, Schwarting RK and Huston JP. Behavioral phenotyping of the MPTP mouse model of Parkinson's disease. *Behav. Brain. Res.* 2001; 125(1-2): 109-25. doi: 10.1016/s0166-4328(01)00309-6.
- 22.** Prasad EM and Hung S-Y. Behavioral tests in Neurotoxin-Induced animal models of Parkinson's disease. *Antioxidants (Basel)*. 2020; 16; 9(10): 1007. doi: 10.3390/antiox9101007.
- 23.** Duty S and Jenner P. Animal models of Parkinson's disease: a source of novel treatments and clues to the cause of the disease. *Br. J. Pharmacol.* 2011; 164(4): 1357-91. doi: 10.1111/j.1476-5381.2011.01426.x.
- 24.** Tartaglione AM, Venerosi A and Calamandrei G. Early-life toxic insults and onset of sporadic neurodegenerative diseases—an overview of experimental studies. *Curr. Top. Behav. Neurosci.* 2016; 29: 231-264. doi: 10.1007/7854_2015_416.
- 25.** Jamshidi AH, Eghbalian F, Mahroozade S, Mohammadi Kenari H, Ghobadi A and Yousefsani BS. Recommended natural products in Alzheimer's disease based on traditional Persian medicine. *J. Med. Plants* 2020; 19(75): 17-29. doi: 10.29252/jmp.19.75.17.
- 26.** Nagatsu T and Sawada M. L-dopa therapy for Parkinson's disease: past, present, and future. *Parkinsonism. Relat. Disord.* 2009; 15(Supp. 1): S3-8. doi: 10.1016/S1353-8020(09)70004-5.
- 27.** Kamranfar F, Jaktaji RP, Shirani K, Jamshidi AH, Samiei F, Arjmand A, Khoramjouy M, Faizi M, Pourahmad J. Protective effect of a standardized *Allium jesdianum* extract in an Alzheimer's disease induced rat model. *Neurosci. Lett.* 2023; 815: 137491. doi: 10.1016/j.neulet.2023.137491.
- 28.** Colović MB, Krstić DZ, Lazarević-Pašti TD, Bondžić AM and Vasić VM. Acetylcholinesterase inhibitors: pharmacology and toxicology. *Curr. Neuropharmacol.* 2013; 11(3): 315-35. doi: 10.2174/1570159X11311030006.
- 29.** Nakamagoe K, Watanabe M, Takeda T, Mizutani T and Tamaoka A. Parkinsonism with organophosphate poisoning. *BMJ Case Rep.* 2009; 2009: bcr0420091766. doi: 10.1136/bcr.04.2009.1766.
- 30.** Dias V, Junn E and Mouradian MM. The role of oxidative stress in Parkinson's disease. *J. Parkinsons. Dis.* 2013; 3(4): 461-91. doi: 10.3233/JPD-130230.
- 31.** Chakraborty S, Bornhorst J, Nguyen TT and Aschner M. Oxidative stress mechanisms underlying Parkinson's disease-associated neurodegeneration in *C. elegans*. *Int. J. Mol. Sci.* 2013; 14(11): 23103-28. doi: 10.3390/ijms141123103.
- 32.** Liu H, Mao P, Wang J, Wang T, Xie C-H. Allicin protects PC12 cells against 6-OHDA-induced oxidative stress and Mitochondrial dysfunction via regulating Mitochondrial dynamics. *Cell. Physiol. Biochem.* 2015; 36: 966-979. doi: 10.1159/000430271.
- 33.** Rojas P, Serrano-García N, Medina-Campos ON, Pedraza-Chaverri J, Maldonado PD and Ruiz-Sánchez E. S-Allylcysteine, a garlic compound, protects against oxidative stress in 1-methyl-4-phenylpyridinium-induced parkinsonism in mice. *J. Nutr. Biochem.* 2011; 22(10): 937-944. doi: 10.1016/j.jnutbio.2010.08.005.
- 34.** Khovarnagh N and Seyedalipour B. Antioxidant, histopathological and biochemical outcomes of short-term exposure to acetamiprid

in liver and brain of rat: The protective role of N-acetylcysteine and S-methylcysteine. *Saudi Pharm. J.* 2021; 29(3): 280-289. doi: 10.1016/j.jsps.2021.02.004.

35. Shao F, Wang X, Wu H, Wu Q and Zhang J. Microglia and neuroinflammation: Crucial pathological mechanisms in traumatic brain Injury-Induced neurodegeneration. *Front. Aging Neurosci.* 2022; 14: 825086. doi: 10.3389/fnagi.2022.825086.

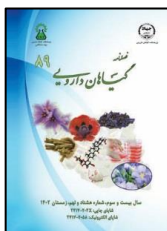
36. Hirano T. IL-6 in inflammation, autoimmunity and cancer. *Int. Immunol.* 2021; 33(3): 127-148. doi: 10.1093/intimm/dxaa078.

37. Ban JO, Oh JH, Kim TM, Kim Dj, Jeong H-S, Han SB and Hong JT. Anti-inflammatory and arthritic effects of thiacremonone, a novel sulfur compound isolated from garlic via inhibition of NF-kappaB. *Arthritis. Res. Ther.* 2009; 11(5):

R145. doi: 10.1186/ar2819.

38. Lin G, Lee Y-J, Choi D-Y, Han SB, Jung JK, Hwang BY, Moon DC, Kim Y, Lee MK, Oh K-W, Jeong HS, Leem JY, Shin HK, Lee JH and Hong JT. Anti-amyloidogenic effect of thiacremonone through anti-inflammation *in vitro* and *in vivo* models. *J. Alzheimers. Dis.* 2012; 29(3): 659-676. doi: 10.3233/JAD-2012-111709.

How to cite this article: Yousefsani BS, Ghobadi A, Shirani K. Uncovering the neuroprotective powers of *Allium sativum*: exploring its potential to alleviate malathion-associated Parkinson's-like behavioral symptoms in a rat model. *Journal of Medicinal Plants* 2024; 23(89): 68-82. doi: 10.61186/jmp.23.89.68



فصلنامه گیاهان دارویی

Journal homepage: www.jmp.irپژوهشکده گیاهان دارویی
جهاد دانشگاهی

مقاله تحقیقاتی

کشف قدرت محافظت عصبی *Allium sativum*: بررسی پتانسیل آن برای کاهش علائم مشابه پارکینسون ناشی از مالاتیون در مدل موش صحرایی بهاره‌سادات یوسف ثانی^{۱،۲}، علی قبادی^{۱،۳}، کبری شیرانی^{۳*}

^۱ موسسه مطالعات تاریخ پزشکی، طب فارسی و مکمل، دانشگاه علوم پزشکی ایران، تهران، ایران
^۲ گروه طب سنتی، دانشکده طب فارسی، دانشگاه علوم پزشکی ایران، تهران، ایران
^۳ گروه سم‌شناسی، دانشکده علوم پزشکی، دانشگاه تربیت مدرس، تهران، ایران

اطلاعات مقاله	چکیده
گل‌واژگان: <i>Allium sativum</i> بیماری پارکینسون مالاتیون سیر استرس اکسیداتیو التهاب	<p>مقدمه: سیر، با نام علمی <i>Allium sativum</i>، یک گیاه دارویی معروف است که از هزاران سال پیش در سراسر جهان مورد استفاده قرار گرفته است. این گیاه، با داشتن ترکیبات گیاهی غنی، خواص دارویی متنوعی از جمله آنتی‌اکسیدان، ضد سرطان، ضد التهاب، و فعالیت‌های ترمیم زخم را دارد. پتانسیل درمانی آن سیر را به درمان طبیعی مفیدی برای بیماری‌های مختلف تبدیل کرده است. هدف: پژوهش حاضر به بررسی خواص حفاظتی سیر در کاهش تظاهرات رفتاری شبیه پارکینسون ناشی از مالاتیون (Mal) در موش‌های صحرایی پرداخته است. روش بررسی: موش‌ها به هشت گروه تقسیم شدند: (۱) کنترل (نرمال سالین)، (۲) مالاتیون (۱۰۰ میلی‌گرم در کیلوگرم)، (۳) Mal + سیر (۵۰ میلی‌گرم در کیلوگرم)، (۴) Mal + سیر (۱۰۰ میلی‌گرم در کیلوگرم)، (۵) Mal + سیر (۱۵۰ میلی‌گرم در کیلوگرم)، (۶) Mal + L-DOPA (۱۰ میلی‌گرم در کیلوگرم)، (۷) سیر (۱۵۰ میلی‌گرم در کیلوگرم)، و (۸) پلی‌اتیلن‌گلیکول (PEG) به عنوان حلال L-DOPA. پس از ۲۸ روز درمان، ارزیابی‌های عصبی رفتاری و آنالیز فعالیت استیل کولین استراز (AChE)، سطوح پراکسیداسیون لیپیدی و گلوتاتیون (GSH) در جسم مخطط انجام شد. نتایج: سمیت عصبی-رفتاری ناشی از Mal به افزایش مالون دی‌آلدئید (MDA)، فاکتور نکروز تومور-α (TNF-α)، و اینترلوکین-۶ (IL-6) و کاهش GSH و فعالیت AChE منجر شد که با درمان سیر کاهش یافت. نتیجه‌گیری: این نتایج نشان می‌دهند که سیر ممکن است با افزایش سطوح آنتی‌اکسیدانی و کاهش استرس اکسیداتیو و التهاب، در درمان پارکینسون مؤثر باشد. نیاز به تحقیقات بیشتری برای درک جامع خواص حفاظتی سیر در این زمینه وجود دارد.</p>

مخفف‌ها: OHDA-۶، ۶-هیدروکسی دوپامین؛ AChE، استیل کولین استراز؛ AP-1، پروتئین فعال‌کننده ۱؛ DTNB، ۵،۵-دیتیوبیس (۲-نیتروبنزویک اسید)؛ GSH، گلوتاتیون؛ IL-6، اینترلوکین-۶؛ IP، تزریق داخل صفاقی؛ MAL، مالاتیون؛ MAPK، پروتئین کیناز فعال شده با میتوزن؛ MDA، مالون دی‌آلدئید؛ MPTP، ۱-متیل-۴-فیل-۱،۲،۳-تتراهیدروپیریدین؛ PEG، پلی‌اتیلن‌گلیکول؛ PUFAS، اسیدهای چرب غیراشباع چندانگانه؛ ROS، گونه‌های اکسیژن فعال؛ SAC، اس آلبل سیستمین؛ SEC، اس اتیل سیستمین؛ SN، توده سیاه؛ SPC، اس پروپیل سیستمین؛ TH، تیروزین هیدروکسیلاز؛ TNF α ، فاکتور نکروز تومور آلفا؛ TPM، طب سنتی ایرانی

* نویسنده مسؤول: k.shirani@modares.ac.ir

تاریخ دریافت: ۹ خرداد ۱۴۰۳؛ تاریخ دریافت اصلاحات: ۳ شهریور ۱۴۰۳؛ تاریخ پذیرش: ۵ شهریور ۱۴۰۳

doi: [10.61186/jmp.23.89.68](https://doi.org/10.61186/jmp.23.89.68)© 2023. Open access. This article is distributed under the terms of the Creative Commons Attribution-NonCommercial 4.0 International License (<https://creativecommons.org/licenses/by-nc/4.0/>)