Original Article

Thymol and Brain Tissue Oxidative Stress Responses Caused by Mercury Metal Poisoning in Common Carp (*Cyprinus carpio*)

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ABSTRACT

Background: One of the most significant heavy metals that is not biodegradable is mercury. Fish exposed to mercury may experience adverse effects, including reduced brain tissue. Using herbal compounds or their active constituents is one of the best ways to lower the negative effects of heavy metals. The essential oils of plants like thyme contain thymol, a naturally occurring monoterpene.

Objectives: This study aimed to evaluate the protective effects of thymol on oxidative stress responses in the brain tissue of juvenile carp (*Cyprinus carpio*) following mercury exposure.

Methods: The 120 common carp (*C. carpio*) used in this study were randomly divided into four groups: Control, $HgCl_2$, thymol, and thymol+$HgCl_2$. Each group had 10 fish and was reproduced three times. The fish spent 56 days in captivity. For the control group, the fish were kept in water devoid of mercury(II) chloride and fed a simple diet. Thymol and thymol+$HgCl_2$ fish were fed food containing 100 mg/kg of thymol for 56 days. Fish in the thymol+$HgCl_2$ and $HgCl_2$ groups had 0.44 mg/L of mercury chloride in their tank water. A necropsy and removal of the fish's brain tissue followed their euthanasia after 56 days. Brain tissues were rinsed with phosphate-buffered saline (PBS) and homogenized in 0.9 M PBS (pH 7.4). The supernatant obtained after centrifugation was used to assess oxidative stress markers. Malondialdehyde (MDA), total antioxidant capacity (TAC), and catalase (CAT) activity in brain tissue were quantified using commercial colorimetric assays

Results: TAC and CAT activity significantly increased in the thymol group ($P<0.0001$), while both were markedly reduced in the mercury group ($P<0.0001$). Thymol treatment restored TAC and CAT levels in mercury-exposed fish to near-control values ($P<0.0001$). MDA levels decreased in the thymol and mercury+thymol groups, but the changes were not statistically significant. These results highlight thymol's antioxidant potential against mercury-induced stress.

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Conclusion: Thymol significantly enhanced antioxidant defenses in juvenile carp exposed to mercury by increasing TAC and CAT levels. It partially restored oxidative balance and reduced lipid peroxidation, though MDA levels did not differ significantly. These findings support thymol's potential as a natural protective agent against heavy metal-induced neurotoxicity.

Keywords: Heavy metals, Mercury chloride, Oxidative stress responses, Thymol, Common carp

Introduction

Large volumes of pollutants have been released into the environment over the past few decades due to industrial expansion. Inadequate adherence to environmental regulations has exacerbated the environmental impacts of these contaminants (Vutukuru, 2005). Aquatic life forms are seriously threatened by heavy metal contamination in aquatic environments when these levels exceed acceptable levels (Shahjahan et al., 2022). Heavy metal salts fall into the latter category of pollutants, which are often divided into two categories: Degradable and non-degradable (Güven et al., 1999).

Fish, other aquatic life, and aquatic plants are all negatively impacted by elevated amounts of these chemicals (Malik et al., 2010). Aquatic environments contain heavy metals that enter the food chain and eventually accumulate in fish tissues. As a result, these contaminants are exposed to people who eat contaminated fish (Farombi et al., 2007; El-Naga et al., 2005; Lakshmanan et al., 2009). Fish poisoning caused by heavy metal contamination is currently a major worldwide concern (Taslima et al., 2022). The following heavy metals are considered to be of special concern: Lead (Pb), mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), zinc (Zn), and copper (Cu) (Landis et al., 2003; Agbugui & Abe, 2023). The digestive system and permeable membranes, such as fish gills, are the two main ways that heavy metals typically enter living things and, consequently, the food chain (Yesaki, 1994). Fish species frequently collect heavy metals over time (Sharma et al., 2024).

Around the world, mercury is recognized as a widespread environmental contaminant. Water, sediments, soil, and the atmosphere are among the environmental compartments where this metal can be found. Natural occurrences and industrial processes, including coal burning, cement manufacture, the production of fuel and chemical waste, and gold mining, are important sources of mercury discharge into aquatic habitats. Furthermore, mercury resists decomposition (Li et al., 2009; Lidskog et al., 2018; Kimáková et al., 2019; Keerthana & Qureshi, 2020; Zulkipli et al., 2021). Mercury has been shown to have detrimental effects on fish growth rates, the immune and reproductive systems, the brain, muscles, and liver tissues. Additionally, it causes changes in energy metabolism, calcium homeostasis, oxidative stress, and cellular structural degeneration. (Gonza´lez-Estecha et al. 2014; Macirella et al. 2016).

Mercury is mostly found in aquatic environments as elemental mercury (Hg^0), inorganic compounds (such as HgS, Hg_2Cl_2, and $HgCl_2$), or organic molecules (such as $[CH_3]_2Hg$ and CH_3Hg). Aquatic systems are most frequently exposed to mineral mercury through industrial discharges. Fish tissues are more toxically affected by this form of mercury than by the other two (Zhang et al., 2016a; Zhang et al., 2016b; de Almeida Rodrigues et al., 2019; Zulkipli et al., 2021). Exposure to inorganic mercury in aquatic organisms can cause a variety of problems, such as long-term harm to kidney and liver tissues, neurological impairment, cardiac damage, immune system disruptions, problems with reproduction, and, most importantly, growth and development disorders (Chen et al., 2021; Huang et al., 2010; Zhang et al., 2016a; Zhou et al., 2020). For instance, studies on *Sander vitreus* fish in Canada showed that growth factors were significantly reduced with increased mercury accumulation in their muscles (Simoneau et al., 2005).

One practical way to reduce heavy metal pollution in fish is to avoid contaminating water and subsurface resources, but using compounds with chelating or antioxidant qualities is also a good option. Taleghani et al. (2019), for example, showed that extracts from *Rosa damascena* reduce the negative effects of zinc exposure on the liver of common carp (*Cyprinus carpio*). Another study found that oral administration of curcumin reduces the negative effects of lead on the immunological and antioxidant responses of common carp (Giri et al., 2021). Furthermore, the adverse effects of food poisoning with zinc oxide nanoparticles were successfully mitigated by oral administration of *Allium hirtifolium* extract (Mahboub et al., 2022). In the gill tissue of common carp, cinnamon aldehyde has also been found to be useful in minimizing damage caused by zinc oxide poisoning (Heidardokht et al., 2023). By either preventing heavy metals from being absorbed or reducing their effects on tissues of different species, including fish, these substances can lower pollutant levels in both human and animal populations. The need to address this issue is underscored by the persistence of non-biodegradable pollutants, such as heavy metals, already present in the environment. Recently, there has been a significant increase in the use of plants as chelators for various compounds and as sources of antioxidants in scientific research (Arzi et al., 2011).

Essential oils derived from plants, such as those from oregano (Zheng et al. 2009) and thyme (Hoseini & Yousefi 2019), naturally contain the monoterpene thymol (2-isopropyl-5-methylphenol). As a plant-based feed additive, thymol has been effectively added to fish diets to boost performance, improve the structure and function of the digestive tract, speed up metabolism, and reduce damage from free radicals (Ran et al., 2016; Ezzat Abd El-Hack et al., 2016; Anyu et al., 2018). Thymol is an efficient anesthetic and has anti-inflammatory qualities in a variety of fish species, including silver catfish (Bianchini et al., 2017) and common carp (Yousefi et al., 2018). In addition to promoting growth (Anyu et al. 2018), thymol exhibits antibacterial activity against *Aeromonas hydrophila* bacteria (da Cunha et al., 2019). The studies have shown that dietary thymol supplementation improves the growth performance of Nile tilapia (*Oreochromis niloticus*) (Aanyu et al., 2018; Amer et al., 2018) and common carp (*C. carpio*) (Rahmati-Holasso et al., 2025b). Additionally, the antioxidant properties of thymol have been verified (Amer et al., 2018).

This study aims to assess the protective effects of thymol against oxidative stress responses induced by waterborne mercury chloride toxicity, considering both thymol's antioxidant benefits in fish and the detrimental effects of mercury metal toxicity on fish oxidative stress responses.

Materials and Methods

Diet preparation

Commercial carp fish food (Beyza 21 Manufacturing Company, Fars, Iran) was utilized as the primary food source for the fish. The food must first be thoroughly and consistently pulverized before thymol may be added to the basic diet. Thymol (Merck, Germany) at a dose of 100 mg/kg was added to the base diet (Morselli et al., 2020). To create pellets of the right size, each ingredient was thoroughly mixed, pelletized, air-dried, and finally sieved. Every week, fresh feed was prepared and kept at 4 °C.

Fish and experimental design

Fish were purchased from a breeding facility in Gilan Province, Iran. The Department of Aquatic Health, Aquatic Research Center, Faculty of Veterinary Medicine, University of Tehran, received 120 young common carp. After being moved to 1000-L aquariums, the fish had a 14-day adaptation period (Giri et al., 2021). The fish were harvested and inspected for illnesses after the adaptation period. Microscopical examinations revealed no internal or external parasites. The average weight of the fish was 17.4±1.08 g. After that, the fish were divided into groups of 1 to 4 and placed in 12 tanks, each holding 125 liters of water. Ten fish were considered for each of the three repetitions in each group. The water in group 1 (control) had no mercury chloride, and the fish were fed a simple diet. The fish in group 2 ($HgCl_2$) were given the standard diet, and mercury chloride (0.44 mg/L) was added to their water (Gül et al., 2004). Fish in group 3 (thymol) were fed thymol-containing food (100 mg/kg feed) without any mercury chloride in their water (Morselli et al., 2020). Fish in group 4 (thymol+$HgCl_2$) were fed thymol-containing food (100 mg/kg feed), and their water was supplemented with mercury (II) chloride (0.44 mg/L). After that, the fish were kept for 8 weeks (56 days). The fish were fed twice a day at a rate of 1% of their body weight.

Additionally, the fish's water was changed by 50% every day, and the amount of mercury extracted during each water change was determined and added back to the water to maintain the mercury content. Additionally, an electric heater and an air pump were used to maintain the water temperature for the fish. Throughout the study period, the water's average temperature was 24.02±0.8 °C, and its pH was 7.7±0.2.

Investigating oxidative stress responses in brain tissue

The fish were taken after the 56-day holding period, anesthetized, and then put down with a commercial fish anesthetic (PI-222, Pars Imen Daru, Iran) at twice the recommended dosage (4 mL in 10 L of water). Following a thorough euthanasia, the fish's skull bones were carefully removed, followed by the removal of all brain tissue and storage in a freezer set at -80 °C.

Brain samples were gently washed twice with phosphate-buffered saline (PBS) to remove residual blood and debris. The cleaned tissues were then homogenized at 10% (w/v) in 0.9 M PBS (pH 7.4) using a mechanical tissue homogenizer. The resulting homogenates were centrifuged to isolate the supernatant, which was subsequently used to analyze oxidative stress indices. The levels of malondialdehyde (MDA) and total antioxidant capacity (TAC), along with the catalase (CAT) enzymatic activity, were quantified using commercially available colorimetric assay kits (Kooshan Zist Azma, Iran) according to the manufacturer's protocols.

Statistical analysis

Data were analyzed using GraphPad Prism software, version 6.0. Results are expressed as Mean±SD. Statistical significance was assessed using one-way analysis of variance (ANOVA), followed by the Tukey post hoc test for multiple comparisons. A $P<0.05$ was considered statistically significant.

Results

The level of TAC significantly increased in the thymol group (507±93.46) compared to the control group (254.4±5.17) ($P<0.0001$). In contrast, TAC levels in the mercury group (176.6±16.97) decreased significantly compared with the control group ($P<0.001$). Furthermore, treatment of the mercury group with thymol (313.1±34.74) resulted in a significant increase in TAC levels compared to the mercury group ($P<0.0001$) (Figure 1a). CAT enzyme activity (nmol/min/mg protein) in the thymol group (26.09±2.95) showed a significant increase compared to the control group (11.27±0.38) ($P<0.0001$). In contrast, CAT activity in the mercury group (8.54±0.53) was significantly lower than in the control group ($P<0.0001$). Subsequently, treatment of the mercury group with thymol (12.15±1.14) led to a significant increase in CAT levels compared to the mercury group ($P<0.0001$), effectively restoring antioxidant capacity to near-control levels (Figure 1b).

The lipid peroxidation index, MDA, decreased in the thymol group (9.28±3.5) compared with the control group (11.74±9.93), although the difference was not statistically significant. Additionally, the MDA level in the thymol-treated mercury group (12.75±2.18) also showed a reduction compared to the mercury group, but the difference was not statistically significant (Figure 1c).

Discussion

In this study, 4 experimental groups (control, thymol, mercury, and mercury + thymol) were evaluated. The results demonstrated that thymol, as a phenolic plant-derived compound with strong antioxidant properties, could mitigate the negative effects of oxidative stress induced by chronic mercury intoxication in the brain tissue of common carp (*C. carpio*). This finding highlights the importance of utilizing natural compounds to improve the health of cultured fish.

Mercury is one of the most critical non-biodegradable pollutants in aquatic environments, directly causing cellular damage, particularly in sensitive tissues such as the brain (Macirella et al., 2016). These injuries are mainly triggered by the overproduction of reactive oxygen species (ROS) and disruption of the cellular antioxidant defense system (González-Estecha et al., 2014).

In the mercury group, antioxidant enzyme activity was markedly reduced, particularly CAT, which was strongly inhibited by mercury ions. This enzymatic disruption was accompanied by a pronounced decrease in total antioxidant capacity and an increase in MDA, indicating intensified lipid peroxidation and disturbance of cellular defense balance.

Conversely, the pure thymol group exhibited the highest CAT activity. In addition, TAC in this group was significantly elevated, reflecting a simultaneous enhancement of enzymatic and non-enzymatic defense pathways. This finding indicates that thymol not only activated the defense system but also elevated antioxidant capacity beyond basal levels. The phenolic structure of thymol and its ability to neutralize free radicals are the main drivers of these protective effects (Hoseini & Yousefi, 2019).

In the mercury + thymol group, CAT activity increased compared to the mercury group but remained lower than in the pure thymol group. TAC was also intermediate—neither as high as pure thymol nor as reduced as mercury alone. This pattern suggests that thymol partially counteracted mercury-induced damage, though its protective effect was limited in the presence of the pollutant. Specifically, reductions in MDA did not reach statistical significance, although biologically relevant trends were observed. The limited protective efficacy may be attributed to the intervention dose or duration being insufficient to induce significant changes in MDA, or to the small sample size.

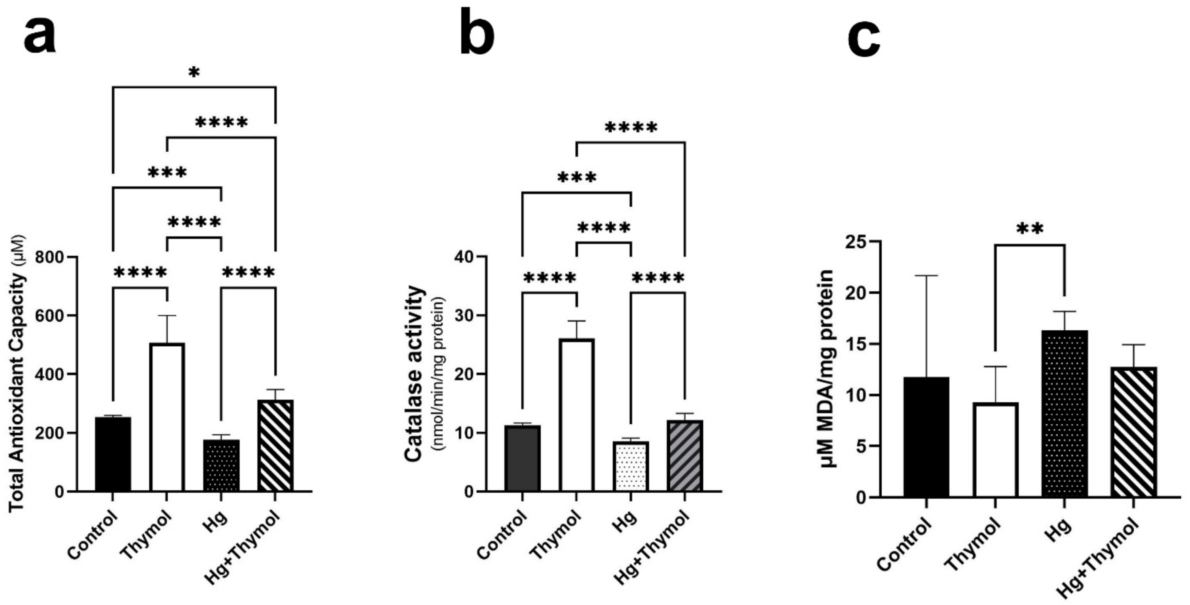

Figure 1. Protective effect of thymol on oxidative stress responses in the brain of common carp after chronic mercury exposure

MDA: Malondialdehyde; TAC: Total antioxidant capacity.

*P<0.05, **P<0.01, ***P<0.001, ****P<0.001.

Note: Control, untreated control group; Thymol, group treated with thymol; Hg, group exposed to mercury ($HgCl_2$); Hg + thymol, group exposed to mercury and treated with thymol. The results are presented as Mean±SD.

Previous studies corroborate this pattern. Yousefi et al. (2024) demonstrated that thymol increased TAC and reduced MDA in rainbow trout under thermal stress. Firmino et al. (2021) reported that thymol, combined with carvacrol and garlic, enhanced mucosal barrier function and increased the secretion of immune molecules in sea bream. Hafsan and Ghafari-Farsani (2022) also demonstrated that thymol improved growth indices, body composition, and antioxidant status in trout and common carp. Giannenas et al. (2012) reported that thymol, in rainbow trout, not only exerted antioxidant effects but also improved flesh quality and reduced lipid oxidation.

A study by Kong et al. (2021) in *Channa argus* revealed that thymol shifted immune regulation from a pro-inflammatory state toward balance by downregulating pro-inflammatory genes (*TNF-α*, *IL-1β*) and upregulating anti-inflammatory genes (*IL-10*, *TGF-β*). Abou-Zeid et al. (2023) in tilapia demonstrated that thymol reduced MDA and restored antioxidant enzyme activity to normal levels when exposed to ZnO nanoparticles. Similarly, Li et al. (2022) found that thymol activated the Nrf2/HO-1 pathway while inhibiting NF-κB and p53, thereby enhancing antioxidant gene expression and reducing cellular apoptosis.

Compared with typical antioxidants, Mohiseni et al. (2017) showed that thymol, in common carp, functions similarly to vitamin E in alleviating cadmium toxicity. Antache et al. (2014) further confirmed synergistic effects when thymol was combined with vitamin E in tilapia, significantly enhancing antioxidant indices and growth performance.

Studies by Abdel-Latif et al. (2021) also demonstrate that thymol, when combined with probiotics, improved fish growth, immunity, and resistance to bacterial diseases. These synergies were achieved through reduced microbial load, strengthened intestinal barriers, and upregulated immune gene expression.

Finally, Ran et al. (2016) reported that thymol modulated gut microbiota, thereby influencing the gut–brain axis and indirectly reducing oxidative stress in the brain. Other phenolic compounds, including cinnamaldehyde (Heidardokht et al., 2023), wild garlic extract (Mahboub et al., 2022), and *R. damascena* extract (Taleghani et al., 2019), have also been shown to exert similar effects in reducing lipid peroxidation and enhancing TAC.

Heavy metal pollution, particularly mercury, represents a major environmental challenge threatening not only aquatic ecosystems but also human health. Due to its bioaccumulation, mercury accumulates in fish tissues, inducing severe oxidative stress. Findings from this study indicate that even concentrations below the LC_{50} of $HgCl_2$ can cause significant brain damage in common carp. Thus, the application of natural compounds such as thymol may serve as an effective strategy to mitigate pollutant effects and support the sustainability of aquatic ecosystems.

Conclusion

These findings are further supported by the significant increase in TAC and CAT activity observed in thymol-treated fish, indicating enhanced antioxidant defense and partial restoration of oxidative balance under mercury-induced stress. Although MDA levels showed a downward trend, the lack of statistical significance suggests that thymol's protective effects may depend on dosage, duration, or severity of exposure. The ability of thymol to counteract mercury toxicity and improve biochemical indices highlights its therapeutic potential in neuroprotection. Taken together, the results reinforce thymol's role not only in mitigating oxidative damage but also in promoting resilience against environmental pollutants. Its integration into aquafeed formulations could offer a practical and eco-friendly approach to safeguarding fish health in contaminated aquatic systems.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the Research Ethics Committee of University of Tehran (Code: IR.UT.VETMED.REC.1404.014), Tehran, Iran. All methods were carried out in accordance with the University of Tehran Veterinary Ethical Review Committee's relevant guidelines and regulations.

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Authors' contributions

Conceptualization and study design: HosseinAli Ebrahimzadeh Mousavi, Seyedeh Mohadeseh PourMortazavi Bahambari, and Akram Vatannejad; Methodology, investigation and formal analysis: Seyedeh Mohadeseh PourMortazavi Bahambari, HosseinAli Ebrahimzadeh Mousavi, and Aghil Sharifzadeh; Writing the original draft: Seyedeh Mohadeseh PourMortazavi Bahambari; Review and editing: HosseinAli Ebrahimzadeh Mousavi and Akram Vatannejad; Final approval: All authors.

Conflict of interest

The authors declared no conflict of interest.

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