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Effects of Clove Oil Exposure on Freshwater Fish: A Comprehensive Study on Neurological, Respiratory, and Biochemical Responses in *Channa punctatus*

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ABSTRACT: This study investigates the toxicological effects of clove oil (eugenol) on *Channa punctatus*, focusing on neurological, respiratory, and biochemical responses across different concentrations (0 mg/L, 5 mg/L, 15 mg/L, and 25 mg/L) and exposure durations (1 hour, 6 hours, 12 hours, and 24 hours). Histopathological analysis revealed dose- and time-dependent neuronal degeneration, gill epithelial thickening, and suprabranchial cavity inflammation. Histomorphometric measurements confirmed these observations, showing significant reductions in neuronal density, gill epithelial thickness, and suprabranchial cavity volume at higher concentrations. Biochemical assays indicated increased HSP70 expression and SOD activity, reflecting oxidative stress, while total protein content decreased, indicating cellular damage. The results underscore the importance of regulating clove oil concentrations and exposure durations to minimize adverse effects on fish health in aquaculture. These findings contribute to a broader understanding of clove oil's toxicological impact and provide a basis for establishing safer usage guidelines in fish handling and aquaculture practices.

Keywords: Clove oil, Eugenol, *Channa punctatus*, Neurological Effects, Respiratory Effects, Biochemical Responses, Oxidative Stress, HSP70, SOD, Aquaculture.

INTRODUCTION

Clove oil, derived from the dried flower buds of *Syzygium aromaticum*, is commonly used in aquaculture as a sedative and anesthetic for fish handling, transport, and research. Eugenol, the primary bioactive compound in clove oil, is known for its analgesic and anesthetic properties, making it a popular choice for humane euthanasia and sedation of aquatic species. However, despite its widespread use, the toxicological effects of clove oil on fish remain poorly understood, particularly with regard to its dose-dependent impact on neurological, respiratory, and biochemical processes. The freshwater fish species *Channa punctatus* (snakehead fish) is commonly used in toxicological studies due to its adaptability to various environmental conditions and its relevance in aquaculture (Ali & Dhanapalan 2020). This study aims to assess the effects of clove oil exposure on *Channa punctatus*, focusing on neurological, respiratory, and biochemical alterations, and to explore the potential risks associated with clove oil exposure at different concentrations and exposure durations. The use of clove oil in fish anesthesia has gained traction due to its relatively low cost, ease of use, and effectiveness in inducing sedation without significant harm when used within recommended dosage limits (Das & Reddy 2021). Studies have shown that at appropriate concentrations, clove oil can be a humane and effective method for anesthetizing fish, especially in cases of surgical procedures or transport, reducing stress and minimizing physical injury (Kumar & Kaur 2021). However, when used in excess or over prolonged periods, clove oil may cause harmful physiological and biochemical alterations in fish, leading to oxidative stress, tissue damage, and even mortality (Gauthier & Purcell 2019; Fernando & Perera 2020). These detrimental effects are of particular concern in aquaculture settings, where clove oil is frequently utilized for various management practices, such as breeding, handling, and harvesting (Hossain & Islam 2020). Previous studies on the toxicological effects of clove oil have demonstrated that exposure to high concentrations can lead to significant histopathological changes, including neuronal damage, gill epithelial thickening, and liver dysfunction (Jain & Mishra 2021; Gupta & Pandey 2022). Histopathological observations in fish exposed to clove oil often show neuronal degeneration and vacuolization, particularly in the brain, which could impair cognitive functions and behavior (Ali & Dhanapalan 2020). Similarly, gill tissues exhibit epithelial thickening and lamellar damage, which can disrupt respiration and limit oxygen intake, affecting the overall health of the fish (Jha &

Yadav 2021). Moreover, the suprabranchial cavity, an important site for respiration and osmoregulation, has been found to exhibit inflammation and epithelial thickening, which may impair the fish's ability to regulate gas exchange and maintain homeostasis under stressful conditions (Kumar & Choudhury 2020). Such tissue alterations are particularly concerning for fish in aquaculture, where optimal health and performance are crucial. The biochemical effects of clove oil exposure are also of significant interest, as it is known to induce oxidative stress in fish. Oxidative stress occurs when the production of reactive oxygen species (ROS) exceeds the capacity of the antioxidant defense system, leading to cellular damage (Hossain & Islam 2020). This is particularly evident through increased activity of superoxide dismutase (SOD), a key antioxidant enzyme that scavenges ROS to protect cells from damage (Shafique & Hussain 2021). The induction of oxidative stress by clove oil is associated with increased expression of heat shock proteins (HSP70), which play a role in cellular protection by aiding protein folding and preventing protein aggregation under stress conditions (Siddiqui & Khalid 2022). However, prolonged exposure to clove oil can lead to cellular damage, as reflected by a decrease in total protein content, indicating protein degradation and impaired cellular function (Tiwari & Sharma 2020). Understanding the biochemical responses to clove oil is essential for assessing its safety and establishing appropriate usage guidelines in aquaculture. Studies on the neurotoxic effects of clove oil have demonstrated its impact on brain function, leading to neurodegeneration and behavioral changes in aquatic organisms. Clove oil has been shown to affect the central nervous system (CNS) of fish by altering neurotransmitter levels and inducing neuronal vacuolization (Lee & Perera 2021). These alterations may affect the fish's ability to respond to environmental stimuli, impair learning and memory, and reduce overall fitness, which is particularly problematic in both wild and cultured populations. The extent of these effects is dose-dependent, with higher concentrations of clove oil leading to more severe neurotoxic effects (Kumar & Kaur 2021). The concentration and exposure time are critical factors that influence the severity of these neurotoxic effects, making it essential to establish safe dosage limits for clove oil use in fish anesthesia.

LITERATURE REVIEW

Clove oil, specifically its active compound eugenol, has been widely used as an anesthetic in aquaculture for the sedation and euthanasia of fish species. Its effectiveness, coupled with its low cost and easy application, has made it a preferred choice for fish handling and transportation (Ali & Dhanapalan 2020). Eugenol acts on the central nervous system of fish, inducing a state of anesthesia that is generally considered humane when used correctly. However, the potential toxicological effects of clove oil exposure at higher concentrations and prolonged durations have raised concerns regarding its safety, particularly in sensitive species like *Channa punctatus*. While studies have reported that clove oil is effective at sedating fish without causing severe harm at low concentrations, its toxicological impact has been less well-documented (Das & Reddy 2021). Histopathological studies have demonstrated that exposure to clove oil can lead to significant tissue damage in various fish species, particularly in the brain, gills, and liver. Gauthier and Purcell (2019) found that prolonged exposure to sub-lethal doses of clove oil resulted in neuronal vacuolization, degeneration, and other signs of neurotoxicity in the brain of *Channa punctatus*. Similar findings were reported by Fernando and Perera (2020), who observed liver damage and oxidative stress in fish exposed to clove oil. In addition, Jha and Yadav (2021) reported significant damage to gill tissues, including epithelial thickening and lamellar damage, which could impair respiratory function and reduce oxygen uptake. These findings are consistent with those of Kumar and Choudhury (2020), who found similar gill alterations and concluded that clove oil exposure could result in reduced respiratory efficiency and increased susceptibility to environmental stressors. In terms of biochemical responses, clove oil has been shown to induce oxidative stress, as evidenced by increased activity of antioxidant enzymes such as **superoxide dismutase (SOD)** and **catalase**, along with increased expression of **heat shock protein (HSP70)** (Kumar & Kaur 2021). These biochemical markers are indicative of cellular stress and damage due to the accumulation of reactive oxygen species (ROS). Several studies, including those by Shafique and Hussain (2021); Li and Zhang (2020), have highlighted that clove oil exposure leads to elevated oxidative stress markers, suggesting that the fish are undergoing cellular damage as part of a stress response. Furthermore, decreased total protein content has been observed in fish exposed to clove oil, reflecting protein degradation and cellular damage (Siddiqui & Khalid 2022). Despite its widespread use, there is still a significant gap in understanding the full extent of the toxicological effects of clove oil in fish, especially at different concentrations and exposure times. Some studies, like those by Gupta and Pandey (2022), argue that while clove oil is less toxic compared to other anesthetics, prolonged exposure at higher concentrations may still result in substantial damage, particularly to vital organs like the liver and gills. Furthermore, while clove oil has been shown to be effective for short-term anesthesia, its long-term effects on fish health are not well understood. This study seeks to bridge this gap by focusing on *Channa punctatus*, a commonly used model organism in toxicological studies, to explore the dose-dependent effects of clove oil exposure on neurological, respiratory, and biochemical responses. In conclusion, while clove oil is widely used as an anesthetic for fish, its potential toxicological effects cannot be overlooked, particularly when used at higher concentrations or over extended periods. Previous studies have provided valuable insights into the histopathological and biochemical alterations induced by clove oil, but further research is needed to establish safe exposure limits and optimize its use in aquaculture. This study will contribute to the growing body of knowledge on the toxicological impacts of clove oil on *Channa punctatus* and will help

inform guidelines for its safe application in the aquaculture industry.

METHODOLOGY

1. Research Design

A quantitative experimental design was used to assess the effects of clove oil (eugenol) on *Channa punctatus*. The study involved a randomized controlled design (RCT) with four clove oil concentrations (0 mg/L, 5 mg/L, 15 mg/L, and 25 mg/L) and exposure durations (1 hour, 6 hours, 12 hours, and 24 hours).

2. Sample Selection and Acclimatization

- **Fish Selection**: A total of 120 healthy *Channa punctatus* (weight: 15–25 g, length: 6–8 cm) were selected.
- Acclimatization: Fish were acclimatized for 7 days in 500 L tanks under controlled environmental conditions (temperature: 25°C, pH: 7.2, dissolved oxygen: 5 mg/L).

3. Clove Oil Solution Preparation

- **Stock Solution**: Clove oil (eugenol) was dissolved in ethanol to prepare a 100 mg/mL stock solution.
- **Dilution**: Target concentrations of 5 mg/L, 15 mg/L, and 25 mg/L were prepared by diluting the stock solution in water.

4. Experimental Exposure

- **Tank Setup**: Fish were randomly assigned to 15 experimental tanks (5 tanks per concentration group).
- Exposure: Fish were exposed to clove oil concentrations for the designated time periods. Water quality (pH, dissolved oxygen, temperature) was monitored continuously.

5. Tissue Collection and Analysis

- Tissue Collection: After exposure, fish were euthanized using an overdose of clove oil. Brain, gills, and suprabranchial cavity were dissected and preserved in 10% formalin for histopathological analysis.
- Biochemical Sample Collection: Small portions of the tissues were frozen at -80°C for biochemical assays.

6. Histopathological and Histomorphometric Analysis

- Histopathology: Tissues were sectioned and stained with Hematoxylin and Eosin (H&E) for microscopic examination.
- Histomorphometry: Neuronal cell density, epithelial thickness, and suprabranchial cavity volume were quantified using ImageJ software.

7. Biochemical Assays

- HSP70 Expression: Measured using Western blotting.
- SOD Activity: Assessed using the NBT reduction method.
- Total Protein Content: Quantified using the Bradford assay.

8. Statistical Analysis

Data were analyzed using One-Way ANOVA followed by Tukey's HSD post-hoc test for comparisons between groups. A p-value of <0.05 was considered statistically significant.

RESULTS

The results of this study aim to evaluate the effects of clove oil (eugenol) exposure on *Channa punctatus*, focusing on neurological, respiratory, and biochemical responses. A comprehensive examination was performed across different clove oil concentrations (0 mg/L, 5 mg/L, 15 mg/L, and 25 mg/L) and exposure durations (1 hour, 6 hours, 12 hours, and 24 hours). Data from histopathological, histomorphometric, and biochemical analyses were analyzed to assess the dose-dependent and time-dependent toxicological impact of clove oil on this freshwater fish species. This chapter presents these findings with the help of tables and descriptive results based on the observed histological damage, histomorphometric measurements, and biochemical alterations.

A. Histopathological Findings

Histopathological analysis of brain, gills, and suprabranchial cavity tissues revealed significant damage associated with clove oil exposure. The extent of damage was found to be dose-dependent, with higher concentrations and prolonged exposure leading to more severe tissue alterations.

(i) Brain Tissue

- **Control Group**: No histopathological damage was observed, and the brain exhibited normal neuronal morphology with no signs of degeneration or vacuolization.
- **5 mg/L Group**: Mild vacuolization was observed in the brain after 1 hour, with moderate vacuolization and mild neuronal degeneration after 6 hours. By 12 and 24 hours, neuronal degeneration and vacuolization were evident in most brain sections.
- **15 mg/L Group**: Moderate vacuolization and mild neuronal degeneration were visible even after 1 hour of exposure, becoming severe by 12 hours, with extensive neuronal necrosis observed at 24 hours.
- **25 mg/L Group**: Extensive vacuolization and neuronal degeneration were visible after 1 hour of exposure. By 6 hours, neuronal necrosis was widespread, and by 12 and 24 hours, significant neuronal necrosis and tissue loss were observed.

Table 1: Show Brain Histopathological Changes.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	Normal	Mild vacuolization	Moderate vacuolization	Extensive vacuolization
6 hr	Normal	Moderate vacuolization, mild degeneration	Significant vacuolization, mild degeneration	Neuronal necrosis, degeneration
12 hr	Normal	Severe vacuolization, neuronal degeneration	Extensive neuronal degeneration	Extensive necrosis, tissue loss
24 hr	Normal	Neuronal degeneration, mild necrosis	Extensive necrosis	Complete neuronal necrosis, severe damage

(ii) Gills. Gills were the primary site of respiratory damage, and the severity of damage was strongly linked to the concentration and duration of clove oil exposure.

- **Control Group**: No histopathological changes were observed in the gills.
- **5 mg/L Group**: Mild epithelial thickening was observed after 1 hour of exposure, with moderate epithelial hyperplasia and mild lamellar damage by 6 hours. By 12 hours, gills exhibited significant epithelial hyperplasia and lamellar damage, which worsened by 24 hours.
- **15 mg/L Group**: Moderate epithelial thickening and mild lamellar damage were observed after 1 hour of exposure. By 12 and 24 hours, significant epithelial desquamation and lamellar loss were evident.
- **25 mg/L Group**: Severe epithelial thickening and lamellar damage were observed after 1 hour of exposure, progressing to severe epithelial hyperplasia, lamellar loss, and gill destruction by 12 and 24 hours.

Table 2: Show Gills Histopathological Changes.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	Normal	Mild thickening	Moderate thickening, mild lamellar damage	Severe thickening, early damage
6 hr	Normal	Moderate thickening, hyperplasia	Significant thickening, lamellar damage	Severe epithelial hyperplasia, lamellar loss
12 hr	Normal	Severe thickening, hyperplasia	Significant epithelial desquamation, lamellar damage	Severe epithelial desquamation, lamellar loss
24 hr	Normal	Severe epithelial hyperplasia, lamellar loss	Extensive epithelial damage, lamellar loss	Complete epithelial loss, severe damage

(iii) Suprabranchial Cavity. Changes in the suprabranchial cavity were observed at higher concentrations of clove oil. These changes were characterized by inflammation and epithelial thickening.

- **Control Group**: The suprabranchial cavity exhibited normal epithelial structure with no signs of inflammation.
- **5 mg/L Group**: Mild epithelial thickening and slight inflammation were observed after 1 hour, which became moderate by 6 hours. After 12 and 24 hours, there was moderate inflammation and constriction of the cavity.
- **15 mg/L Group**: Mild epithelial thickening and moderate inflammation were visible after 1 hour, with severe inflammation and constriction by 12 hours. By 24 hours, epithelial desquamation was observed, accompanied by significant cavity constriction.
- **25 mg/L Group**: Severe epithelial thickening and inflammation were observed after 1 hour of exposure, with extensive epithelial desquamation and cavity constriction by 6 hours. By 12 and 24 hours, the cavity showed significant reduction in volume and extensive tissue damage.

Table 3: Show Suprabranchial Cavity Histopathological Changes.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	Normal	Mild thickening, slight inflammation	Mild thickening, moderate inflammation	Severe thickening, mild inflammation
6 hr	Normal	Moderate thickening, mild inflammation	Significant thickening, inflammation	Severe epithelial hyperplasia, lamellar loss
12 hr	Normal	Mild inflammation, mild constriction	Severe inflammation, constriction	Extensive inflammation, cavity constriction
24 hr	Normal	Moderate inflammation, constriction	Severe inflammation, epithelial desquamation	Severe inflammation, severe constriction

B. Histomorphometric Measurements

Histomorphometric analysis provided quantitative data to support the histopathological observations, showing significant changes in neuronal cell density, epithelial thickness, and suprabranchial cavity volume.

(i) Brain Cell Density

- **Control Group**: No significant change in neuronal cell density was observed.
- **5 mg/L Group**: A slight decrease (5%) in neuronal density was observed after 6 hours, worsening to 15% at 24 hours.
- **15 mg/L Group**: A 10% decrease in neuronal density was seen after 6 hours, reaching 30% by 24 hours.
- **25 mg/L Group**: A 20% decrease in neuronal cell density was observed at 1 hour, with a 40% reduction by 24 hours.

Table 4: Show Brain Cell Density Measurements.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	100%	95%	90%	80%
6	100%	90%	85%	75%
12	100%	85%	80%	60%
24	100%	70%	70%	60%

(ii) Gills Epithelial Thickness

- **Control Group**: No significant change in epithelial thickness was observed.
- **5 mg/L Group**: Mild thickening (5%) was observed after 6 hours, progressing to 25% by 24 hours.
- **15 mg/L Group**: A 15% increase in epithelial thickness was observed at 6 hours, with a 30% increase at 24 hours.
- **25 mg/L Group**: The highest increase was observed, with a 25% increase after 6 hours and a 50% increase by 24 hours.

Table 5: Show Gills Epithelial Thickness.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	100%	105%	110%	125%
6 hr	100%	110%	115%	130%
12 hr	100%	115%	120%	145%
24 hr	100%	125%	130%	150%

(iii) Suprabranchial Cavity Volume

- **Control Group**: No significant reduction in cavity volume was observed.
- **5 mg/L Group**: A 5% reduction in cavity volume was observed after 6 hours, with 20% reduction at 24 hours.
- **15 mg/L Group**: A 10% decrease was observed after 6 hours, reaching 25% by 24 hours.
- **25 mg/L Group**: The greatest reduction was observed, with a 25% decrease at 6 hours and a 40% reduction by 24 hours.

Table 6: Show Suprabranchial Cavity Volume Measurements.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1	100%	100%	95%	90%
6	100%	95%	90%	75%
12	100%	90%	85%	60%
24	100%	80%	75%	60%

C. Biochemical Results

Biochemical analyses were conducted to assess oxidative stress and protein degradation in response to clove oil exposure. **HSP70 expression**, **SOD activity**, and **total protein content** were measured.

(i) HSP70 Expression

- **Control Group**: No significant change in HSP70 expression was observed.
- **5 mg/L Group**: A 10% increase in HSP70 expression was seen after 6 hours, increasing to 20% by 24 hours.
- **15 mg/L Group**: A 15% increase was seen after 6 hours, reaching 30% by 24 hours.
- **25 mg/L Group**: The highest increase in HSP70 expression was observed, with a 20% increase at 6 hours and a 40% increase by 24 hours.

Table 7: Show HSP70 Expression.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	100%	110%	115%	120%
6 hr	100%	120%	130%	140%
12 hr	100%	130%	140%	150%
24 hr	100%	140%	150%	160%

(ii) SOD Activity

- **Control Group**: No significant change in SOD activity was observed.
- **5 mg/L Group**: A mild increase in SOD activity (10%) was observed after 6 hours, progressing to 20% by 24 hours.
- **15 mg/L Group**: SOD activity increased significantly (15%) at 6 hours, reaching 25% by 24 hours.
- **25 mg/L Group**: The highest increase in SOD activity was observed, with a 20% increase at 6 hours, reaching 35% by 24 hours.

Table 8: Show SOD Activity.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	100%	110%	115%	120%
6 hr	100%	120%	130%	140%
12 hr	100%	130%	140%	150%
24 hr	100%	140%	150%	160%

(iii) Total Protein Content

- **Control Group**: No significant change in total protein content was observed.
- **5 mg/L Group**: A 5% decrease in total protein content was observed after 6 hours, with a 10% reduction by 24 hours.
- **15 mg/L Group**: A 10% reduction was observed after 6 hours, with a 20% decrease at 24 hours.
- **25 mg/L Group**: A 15% reduction was observed after 6 hours, reaching 25% by 24 hours.

Table 9: Show Total Protein Content.

Time (Hours)	Control	5 mg/L	15 mg/L	25 mg/L
1 hr	100%	95%	90%	85%
6 hr	100%	90%	85%	75%
12 hr	100%	85%	80%	70%
24 hr	100%	80%	70%	60%

DISCUSSION

The results of this study clearly demonstrate the toxic effects of clove oil (eugenol) on *Channa punctatus*, with significant dose-dependent and time-dependent alterations in histopathological, histomorphometric, and biochemical parameters. Histological changes, including neuronal degeneration, gill epithelial thickening, and suprabranchial cavity inflammation, were more pronounced at higher concentrations and longer exposure periods. Histomorphometric analysis confirmed these findings, showing a decrease in neuronal cell density, increased gill epithelial thickness, and reduced suprabranchial cavity volume with increased clove oil exposure. Additionally, biochemical assays indicated significant oxidative stress, with increased SOD activity and HSP70 expression, along with decreased total protein content, reflecting cellular damage. These findings underscore the need for careful management of clove oil concentrations and exposure times in aquaculture to minimize harm to aquatic species. The results of this study clearly demonstrate that clove oil (eugenol) exposure significantly affects the neurological, respiratory, and biochemical responses of *Channa punctatus* in a dose- and time-dependent manner. Histopathological analysis revealed that higher concentrations of clove oil (15 mg/L and 25 mg/L) and prolonged exposure durations (12 and 24 hours) led to marked neuronal degeneration, vacuolization, and necrosis in the brain, indicating neurotoxic effects. These findings are consistent with previous studies that reported similar neuronal damage in other fish species exposed to clove oil (Ali & Dhanapalan 2020; Gupta & Pandey 2022). The observed neuronal vacuolization and degeneration suggest that clove oil interferes with normal brain function, potentially affecting sensory processing and motor coordination. In addition to neurological damage, significant respiratory impairments were observed in the gills, where epithelial thickening, hyperplasia, and lamellar damage were more pronounced at higher concentrations and longer exposure times. This aligns with the findings of Gauthier and Purcell (2019); Jha and Yadav (2021), who noted similar gill damage in other fish species exposed to clove oil. The gills, being the primary site for gas exchange, are highly sensitive to environmental stressors. The damage to gill morphology could lead to impaired oxygen uptake, potentially compromising the fish's overall health and survival. The suprabranchial cavity, another critical structure for respiration and osmoregulation, also showed signs of epithelial thickening and inflammation, particularly at higher concentrations (15 mg/L and 25 mg/L) and prolonged exposure durations (12 and 24 hours). The reduction in cavity volume and the presence of inflammation could indicate functional impairments, further supporting the idea that clove oil exposure can disrupt normal respiratory and osmoregulatory processes (Kumar & Kau, 2021; Li & Zhang 2020). From a biochemical perspective, this study demonstrated a dose-dependent increase in HSP70 expression and SOD activity, markers of cellular stress and oxidative damage, respectively. These findings are consistent with those of Hossain and Islam (2020); Tiwari and Sharma (2020), who observed similar increases in oxidative stress markers following clove oil exposure. The elevated SOD activity suggests that the fish were attempting to counteract the oxidative stress induced by clove oil, although this response may not be sufficient to prevent cellular damage at higher concentrations and longer exposure times. Moreover, the reduction in total protein content, particularly at the highest concentrations of clove oil, further supports the idea that protein degradation and cellular damage occur as a result of oxidative stress (Jain & Mishra 2021; Shafique & Hussain 2021).

Overall, the results of this study highlight the importance of regulating clove oil concentrations and exposure times in aquaculture and fish handling. While clove oil can be an effective anesthetic, its prolonged use or exposure at higher concentrations poses significant risks to fish health. The neurological, respiratory, and biochemical alterations observed in *Channa punctatus* emphasize the need for further research to establish safe exposure limits and to explore alternative, less toxic anesthetics for use in aquaculture. These findings also contribute to a broader understanding of the toxicological effects of clove oil on aquatic organisms, underscoring the need for more stringent management practices in the aquaculture industry to ensure the welfare of fish.

CONCLUSIONS

This study provides significant insights into the toxicological effects of clove oil (eugenol) on *Channa punctatus*, highlighting the dose- and time-dependent neurological, respiratory, and biochemical alterations induced by its exposure. The histopathological changes observed in the brain, gills, and suprabranchial cavity indicate that clove oil, particularly at higher concentrations and with prolonged exposure, causes substantial cellular damage, including neuronal degeneration, gill epithelial thickening, and inflammation in the suprabranchial cavity. Biochemically, the increase in HSP70 expression and SOD activity suggests an oxidative stress response, while the reduction in total protein content points to significant cellular damage. These findings underscore the need for caution in the use of clove oil in aquaculture, as prolonged exposure or high

concentrations can impair respiratory and neurological functions, ultimately threatening fish health. This research emphasizes the necessity for establishing safe exposure guidelines for clove oil in fish handling and aquaculture practices to prevent adverse impacts on fish welfare and ensure sustainable aquaculture operations. Further studies are needed to refine these guidelines and explore safer alternatives for fish anesthesia that minimize toxicity while maintaining effective sedation.

FUTURE SCOPE

Future research on clove oil exposure in *Channa punctatus* can further explore its long-term effects on fish populations, particularly in terms of reproductive health and environmental sustainability. Studies could focus on developing guidelines for safe clove oil use in aquaculture and conservation, assessing its potential for mitigating stress in fish during handling. Additionally, investigating genetic variations in response to clove oil and its broader ecological impact could provide valuable insights into preserving biodiversity and minimizing human impacts on aquatic ecosystems.

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