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Toxicological Impact Of Clove Oil (Eugenol) On Channa Punctatus: A Dose-Dependent Analysis Of Histopathological, Histomorphometric, And Biochemical Alterations

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Abstract

This study investigates the toxicological impact of clove oil (eugenol) on *Channa punctatus*, focusing on dose-dependent histopathological, histomorphometric, and biochemical alterations. Fish were exposed to varying concentrations of clove oil (0 mg/L, 5 mg/L, 15 mg/L, and 25 mg/L) for different durations (1 hour, 6 hours, 12 hours, and 24 hours). Histopathological analysis revealed dose- and time-dependent neuronal degeneration in the brain, epithelial thickening and lamellar damage in the gills, and inflammation in the suprabranchial cavity. Histomorphometric measurements confirmed these changes, with significant reductions in neuronal cell density, gill epithelial thickness, and suprabranchial cavity volume at higher concentrations. Biochemical assays showed increased HSP70 expression, elevated SOD activity, and decreased total protein content, indicating oxidative stress and cellular damage. The results highlight the neurotoxic and respiratory impairments caused by clove oil exposure, emphasizing the importance of controlling concentration and exposure duration to prevent potential harm in aquaculture practices.

Keywords: *Clove oil, eugenol, Channa punctatus, toxicology, histopathology, histomorphometry, oxidative stress, SOD, HSP70, aquaculture.*

Introduction

The use of clove oil (eugenol) as an anesthetic in fish handling and aquaculture has gained widespread acceptance due to its efficiency and humane properties (Ali & Dhanapalan, 2020). It is commonly used for sedating fish during transport, surgery, and research (Fernando & Perera, 2020), with *Channa punctatus* being a commonly utilized model species for such studies (Kumar & Choudhury, 2020). Clove oil is derived from *Syzygium aromaticum*, and its active compound, eugenol, acts on the central nervous system, inducing sedation (Hossain & Islam, 2020). While clove oil is considered effective for anesthesia, concerns about its toxicity at higher concentrations and prolonged exposure periods are raised due to its potential to induce oxidative stress and biochemical alterations in fish (Das & Reddy, 2021). Several studies have documented the impact of clove oil on various fish species, focusing on its neurotoxic effects, including neuronal degeneration and vacuolization in brain tissues (Gauthier & Purcell, 2019; Kumar & Kaur, 2021). Histopathological analyses of

gill tissues have also revealed epithelial thickening, lamellar damage, and inflammation, which can lead to impaired respiratory function (Lee & Perera, 2021). The suprabranchial cavity, an important site for respiration and osmoregulation, has also shown alterations such as epithelial desquamation and constriction after exposure to clove oil (Jain & Mishra, 2021). These changes suggest that while clove oil is effective for sedation, its prolonged use can have detrimental effects on fish health, leading to reduced efficiency in respiration and overall stress. Additionally, biochemical markers such as heat shock proteins (HSP70) and superoxide dismutase (SOD) have been used to assess oxidative stress, revealing a dose-dependent increase in antioxidant activity following clove oil exposure (Shafique & Hussain, 2021; Tiwari & Sharma, 2020). Clove oil exposure has also been shown to reduce total protein content, indicating cellular damage and degradation (Kumar & Choudhury, 2020; Singh & Yadav, 2022). Studies have emphasized the need for further understanding of clove oil's effects at varying concentrations and exposure durations, particularly its dose-response relationship and the subsequent effects on fish physiology (Gupta & Pandey, 2022; Lethbridge & Lewis, 2022). Moreover, despite its widespread use, there is limited consensus on the safe concentrations and exposure durations, highlighting a need for comprehensive toxicological studies across different aquatic species (Rahman & Hossain, 2020). This study seeks to bridge this gap by examining the toxicological impact of clove oil on *Channa punctatus*, evaluating histopathological changes in the brain, gills, and suprabranchial cavity, histomorphometric alterations, and biochemical markers of stress (Li & Zhang, 2020). Through this investigation, we aim to provide a clearer understanding of the dose-dependent toxicity of clove oil and contribute valuable insights for its safer application in aquaculture and research (Wang & Li, 2021). Understanding the toxicological effects of clove oil is essential not only for optimizing its use in aquaculture but also for ensuring the welfare of aquatic species, thereby preventing potential long-term adverse effects on their health and survival in controlled environments (Fernando & Perera, 2020; Jha & Yadav, 2021). Furthermore, the outcomes of this study will aid in the formulation of safer guidelines for clove oil use, promoting better management practices and the sustainable use of anesthetics in the fish industry (Siddiqui & Khalid, 2022). By incorporating recent findings and expanding on the existing knowledge base, this research will offer a comprehensive analysis of the toxicological impacts of clove oil on *Channa punctatus*, providing an essential resource for both aquaculture professionals and toxicologists working in the field of aquatic animal welfare.

Literature review

A growing body of research has highlighted the toxicological effects of clove oil (eugenol) on fish species, particularly *Channa punctatus*, emphasizing dose- and time-dependent alterations in histopathological, histomorphometric, and biochemical parameters. Studies by Ali and Dhanapalan (2020) and Hossain and Islam (2020) demonstrate the effectiveness of clove oil as an anesthetic but caution against its potential toxicity at higher concentrations, leading to damage in gill and liver tissues (Gauthier & Purcell, 2019). Histopathological examinations consistently show neuronal degeneration, epithelial thickening, and lamellar damage, especially at concentrations above 15 mg/L (Jain & Mishra, 2021; Siddiqui & Khalid, 2022). Biochemical markers of oxidative stress, such as increased SOD activity and HSP70 expression, indicate significant cellular damage and stress responses (Kumar & Choudhury, 2020; Shafique & Hussain, 2021). Furthermore, reduced total protein content suggests protein degradation as a result of clove oil exposure (Rahman & Hossain, 2020). These findings underline the importance of optimizing clove oil use in aquaculture, ensuring safe concentrations to avoid adverse impacts on fish health (Wang & Li, 2021).

Author(s)	Year	Title	Focus of Study	Key Findings
Ali & Dhanapalan	2020	Effects of clove oil on the anesthetic and recovery responses in freshwater fish *Channa punctatus*	Anesthetic effects of clove oil on *Channa punctatus*	Clove oil effectively anesthetizes *Channa punctatus* but can cause recovery delays and moderate physiological changes, with higher concentrations leading to increased stress.
Alvarado et al.	2019	Histopathological analysis of gill and liver tissues of *Channa punctatus* exposed to clove oil under different concentrations	Histopathological impact of clove oil on gill and liver tissues	Significant histopathological changes, including epithelial thickening and cellular necrosis, were observed in gills and liver at higher concentrations.
Das & Reddy	2021	Clove oil as an anesthetic: A review of its toxicity and safety in aquatic organisms	Review of clove oil's safety and toxicity in fish	Clove oil is a common anesthetic in aquaculture, but its toxicity depends on concentration and exposure time, with adverse effects on gill and liver tissues observed at higher doses.
Fernando & Perera	2020	Biochemical changes in the liver of *Channa punctatus* during clove oil-induced anesthesia	Biochemical responses to clove oil in the liver	Liver enzymes and oxidative stress markers (SOD) increased in response to clove oil exposure, indicating potential liver damage.
Gauthier & Purcell	2019	Evaluation of histopathological and biochemical responses in *Channa punctatus* exposed to sub-lethal doses of clove oil	Histopathological and biochemical responses to clove oil	The study found dose-dependent changes in brain and gill histology, with increased oxidative stress in response to clove oil exposure.
Gupta & Pandey	2022	The toxicological impact of essential oils in fish: A comparative study of clove oil and other plant-derived oils	Comparative study of clove oil and other oils in fish	Clove oil was identified as less toxic compared to other plant oils, but prolonged exposure caused oxidative stress and damage to gills.
Hossain & Islam	2020	An assessment of clove oil as an anesthetic agent for freshwater fish: Biochemical and histopathological changes in *Channa punctatus*	Anesthetic and toxic effects of clove oil on *Channa punctatus*	Fish exposed to clove oil showed changes in oxidative stress markers, including elevated SOD activity and decreased total protein levels.
Jain & Mishra	2021	Assessment of clove oil's impact on oxidative stress and protein degradation in *Channa punctatus*	Oxidative stress and protein degradation in *Channa punctatus*	Significant oxidative stress was observed, with a decrease in total protein content and increased HSP70 expression indicating cellular damage.
Jha & Yadav	2021	A study on the gill damage in *Channa punctatus* exposed to clove oil and its impact on respiratory function	Effect of clove oil on gill structure and respiratory function	Exposure to clove oil resulted in gill epithelial damage and decreased respiratory efficiency, especially at higher concentrations.
Kumar & Choudhury	2020	Effects of clove oil exposure on the physiological and biochemical indices of *Channa punctatus*	Physiological and biochemical responses to clove oil	Clove oil exposure led to altered biochemical markers of oxidative stress, indicating a disruption in normal physiological function.
Kumar & Kaur	2021	Clove oil as a humane anesthetic: Histopathological and biochemical analysis in *Channa punctatus*	Histopathological and biochemical effects of clove oil	The study demonstrated dose-dependent neuronal degeneration and oxidative stress in response to clove oil exposure, along with increased HSP70 levels.
Lee & Perera	2021	Toxicological effects of clove oil in fish: A focus on oxidative stress and liver damage in *Channa punctatus*	Toxic effects of clove oil on oxidative stress and liver function	Clove oil exposure increased oxidative stress markers and led to significant liver damage, confirming its toxicity at higher concentrations.
Lethbridge & Lewis	2022	Histomorphological and biochemical responses to clove oil in aquatic species: A review	Review of histomorphological and biochemical changes in fish due to clove oil	Clove oil caused significant changes in gill and liver histology and elevated oxidative stress markers, highlighting the risks of prolonged exposure.
Li & Zhang	2020	The influence of clove oil on oxidative stress markers in fish: Histopathological analysis in *Channa punctatus*	Oxidative stress and histopathological damage in fish exposed to clove oil	Significant oxidative damage was observed in both the brain and gills, accompanied by increased levels of SOD activity.
Rahman & Hossain	2020	The effects of clove oil on blood biochemical markers in *Channa punctatus* under sub-lethal concentrations	Biochemical alterations in blood following clove oil exposure	Increased blood levels of oxidative stress markers were detected, including elevated SOD and reduced total protein levels.
Reddy & Nageswara	2020	Analyzing the impact of clove oil on fish: A review of physiological alterations and histological changes	Review of physiological and histological effects of clove oil	Clove oil induces stress responses, including alterations in liver, gill, and brain tissues, leading to impaired organ function.
Shafique & Hussain	2021	Evaluation of clove oil exposure in freshwater fish: Its effect on gill histology and biochemical markers	Histological and biochemical impact of clove oil on gills	Significant epithelial thickening and oxidative stress were observed in gills, alongside increased SOD activity.

Siddiqui & Khalid	2022	Toxicological implications of clove oil on *Channa punctatus*: A dose-response study on gill and liver histology and oxidative stress	Dose-response effects of clove oil on gills and liver	Increased oxidative stress markers and significant gill and liver damage were observed at higher concentrations.
Singh & Yadav	2022	Clove oil as an anesthetic for fish: Impact on biochemical and histopathological changes in *Channa punctatus*	Biochemical and histopathological analysis of clove oil's impact on *Channa punctatus*	Exposure led to increased oxidative stress, significant gill damage, and decreased protein content, highlighting the risks of high-dose exposure.
Tiwari & Sharma	2020	Effects of clove oil on oxidative stress, histopathology, and reproductive health of fish: *Channa punctatus* as a model	Impact on oxidative stress, histology, and reproductive health	Oxidative stress markers were elevated, with damage to gill and brain tissues, and potential reproductive health risks observed.
Wang & Li	2021	Histological and biochemical analysis of *Channa punctatus* exposed to clove oil: Implications for fish welfare in aquaculture	Histological and biochemical analysis of clove oil in aquaculture	Significant histopathological damage to gills and brain, along with elevated biochemical markers of stress, was observed in *Channa punctatus*.

Methodology:

1. Research Design

A quantitative experimental research design was adopted to assess the toxicological effects of clove oil (eugenol) on *Channa punctatus* (snakehead fish). The study utilized a randomized controlled design (RCT), with exposure to different concentrations of clove oil (0 mg/L, 5 mg/L, 15 mg/L, and 25 mg/L) for time periods of 1 hour, 6 hours, 12 hours, and 24 hours.

2. Sample Selection and Acclimatization

- **Fish Selection**: A total of 120 healthy adult *Channa punctatus* were selected, weighing between 15–25 grams and measuring 6–8 cm in length.
- **Acclimatization**: Fish were acclimatized in 500 L tanks for 7 days under controlled conditions: temperature at 25°C, pH at 7.2, and dissolved oxygen at 5 mg/L.

3. Clove Oil Solution Preparation

- **Stock Solution**: Clove oil (eugenol) was dissolved in ethanol to prepare a stock solution with a concentration of 100 mg/mL.
- **Dilution**: The required volumes of stock solution were diluted in water to achieve target concentrations of 5 mg/L, 15 mg/L, and 25 mg/L.

4. Experimental Exposure Procedure

- **Tank Setup**: Fish were randomly assigned to 15 experimental tanks (5 tanks per concentration group), each containing 50 L of water.
- Exposure: Fish were exposed to clove oil concentrations for the designated periods. Water quality (pH, dissolved oxygen, temperature) was continuously monitored to ensure consistency.

5. Sample Collection and Tissue Preparation

- Tissue Collection: After exposure, the fish were euthanized using an overdose of clove oil. The brain, gills, and suprabranchial cavity were dissected and preserved in 10% formalin for histopathological analysis. For biochemical analysis, small portions of the tissues were frozen at -80°C.
- Histopathological Analysis: Tissue sections were stained with Hematoxylin and Eosin (H&E) and observed under a light microscope.
- Histomorphometric Measurements: Quantitative measurements of neuronal cell density, gill epithelial thickness, and suprabranchial cavity volume were made using ImageJ software.

6. Biochemical Analysis

- **Biochemical Assays**:
  - HSP70 Expression was measured using Western blotting.
  - SOD Activity was assessed using the NBT reduction method.
  - Total Protein Content was quantified using the Bradford assay.

7. Statistical Analysis
Data were analyzed using One-Way ANOVA followed by Tukey's HSD post-hoc test to compare the means across different groups. Pearson's correlation analysis was used to evaluate relationships between variables. A p-value of <0.05 was considered statistically significant.

Results

The results of this study explore the toxicological impact of clove oil (eugenol) on *Channa punctatus*, assessing histopathological, histomorphometric, and biochemical alterations in response to different concentrations of clove oil exposure. Four concentrations of clove oil were tested: control (0 mg/L), 5 mg/L, 15 mg/L, and 25 mg/L, with exposure durations of 1 hour, 6 hours, 12 hours, and 24 hours. The primary objectives were to assess the dose-dependent changes induced by clove oil and to explore the time-dependent toxicity. The results are presented in a structured manner, with data from histopathological analysis, histomorphometric measurements, and biochemical assays, along with statistical analyses that highlight the significance of the findings.

4.1 Histopathological Observations
Histopathological analysis of the brain, gills, and suprabranchial cavity provided clear evidence of the toxic effects of clove oil exposure. The extent of damage was observed to increase with both higher concentrations of clove oil and longer exposure durations.

4.1.1 Brain Tissue
- **Control Group**: No visible damage was observed in the brain tissues of the control group, with normal neuronal morphology and no signs of vacuolization or degeneration.
- **5 mg/L Group**: Mild vacuolization was observed in the brain after 1 hour of exposure, which progressed to moderate vacuolization and mild neuronal degeneration at the 6-hour time point. By 12 and 24 hours, significant neuronal degeneration and vacuolization were observed.
- **15 mg/L Group**: After just 1 hour of exposure, moderate vacuolization and mild neuronal degeneration were evident. These changes became more severe at the 6-hour mark, with extensive neuronal degeneration and vacuolization becoming prominent by 12 hours. At 24 hours, significant neuronal necrosis was observed.
- **25 mg/L Group**: Extensive vacuolization and neuronal degeneration were visible even after 1 hour of exposure. By 6 hours, neuronal necrosis was widespread, and by 12 and 24 hours, extensive neuronal necrosis, coupled with significant tissue loss, was observed.

Table 4.1: Brain Histopathological Changes

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

Conclusion: The histopathological examination of the brain indicated a clear dose- and time-dependent relationship, with significant neuronal degeneration, vacuolization, and necrosis observed at higher concentrations and longer exposure durations.

4.1.2 Gills
The gills of *Channa punctatus* showed varying degrees of epithelial thickening, lamellar damage, and inflammation as the concentration of clove oil and exposure time increased.
- **Control Group**: The gills exhibited normal morphology, with no signs of epithelial thickening or damage.
- **5 mg/L Group**: Mild epithelial thickening was noted after 1 hour, progressing to moderate epithelial hyperplasia and mild lamellar damage by 6 hours. By 12 hours, the gills exhibited significant epithelial hyperplasia and lamellar damage, which became severe by 24 hours.

- **15 mg/L Group**: After just 1 hour of exposure, moderate epithelial thickening and mild lamellar damage were observed. These changes intensified with time, and by 12 and 24 hours, the gills exhibited significant epithelial desquamation and severe lamellar loss.
- **25 mg/L Group**: Severe epithelial thickening and lamellar damage were visible after 1 hour of exposure. By 6 hours, the gills showed severe epithelial hyperplasia and lamellar loss. At 12 and 24 hours, the gills exhibited extensive epithelial desquamation and complete loss of lamellae.

Table 4.2: Gills Histopathological Changes

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

The histopathological changes in the gills were also dose- and time-dependent. Clove oil exposure caused epithelial thickening, hyperplasia, and lamellar damage, leading to impaired respiratory function at higher concentrations.

4.1.3 Suprabranchial Cavity

Changes in the suprabranchial cavity were characterized by epithelial thickening, inflammation, and a reduction in cavity volume, indicating a disruption in the fish's respiratory and osmoregulatory functions.

- **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 of exposure. These changes became more pronounced by 6 hours, with moderate epithelial thickening and mild inflammation. After 12 and 24 hours, the cavity exhibited moderate inflammation and constriction.
- **15 mg/L Group**: After 1 hour, mild epithelial thickening and moderate inflammation were observed. These changes became severe by 6 hours, and by 12 and 24 hours, there was significant inflammation, epithelial desquamation, and constriction of the cavity.
- **25 mg/L Group**: Severe epithelial thickening and inflammation were observed even after 1 hour of exposure. After 6 hours, the suprabranchial cavity showed severe epithelial desquamation and constriction. By 12 and 24 hours, there was extensive inflammation and a significant reduction in cavity volume.

Table 4.3: Suprabranchial Cavity Histopathological Changes

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

The suprabranchial cavity also exhibited dose- and time-dependent changes, with higher concentrations of clove oil leading to significant inflammation, epithelial desquamation, and reduction in cavity volume. This could severely impair the fish's ability to regulate gas exchange and osmoregulation.

4.2 Histomorphometric Measurements

Histomorphometric measurements of the brain, gills, and suprabranchial cavity provided quantitative data on the changes observed in tissue structure. The following measurements were taken for each tissue type:

4.2.1 Brain Cell Density

- **Control Group**: The brain cell density was consistent with the baseline level, with no significant changes in neuronal density.
- **5 mg/L Group**: A slight decrease in neuronal cell density was observed after 6 hours (5%) and progressively worsened by 12 and 24 hours (15% and 30%, respectively).
- **15 mg/L Group**: A moderate decrease in neuronal cell density was observed after 6 hours (10%) and became more significant by 12 and 24 hours (20% and 30%, respectively).
- **25 mg/L Group**: A severe decrease in neuronal cell density was observed from the 1-hour exposure (20%), worsening by 6 hours (25%) and reaching a maximum loss of 40% by 24 hours.

Table 4.4: Brain Cell Density Measurements

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

The histomorphometric measurements of brain cell density confirmed the histopathological observations of neuronal degeneration, with more significant cell loss observed in the 15 mg/L and 25 mg/L groups.

4.2.2 Gills Epithelial Thickness

- **Control Group**: The gills exhibited normal epithelial thickness with no significant changes.
- **5 mg/L Group**: Mild thickening of the gill epithelium was observed after 6 hours (5%) and became more pronounced by 12 and 24 hours (10% and 25%, respectively).
- **15 mg/L Group**: Epithelial thickness increased significantly by 6 hours (15%) and continued to thicken by 12 and 24 hours (20% and 30%, respectively).
- **25 mg/L Group**: The greatest increase in epithelial thickness was observed, starting at 25% after 6 hours and reaching 50% by 24 hours.

Table 4.5: Gills Epithelial Thickness

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

The gills showed progressive epithelial thickening with increasing concentrations of clove oil, suggesting impaired gill function due to cellular hypertrophy.

4.2.3 Suprabranchial Cavity Volume

- **Control Group**: The suprabranchial cavity volume remained consistent with baseline measurements.
- **5 mg/L Group**: A slight decrease in cavity volume was observed (5%) after 6 hours, worsening with time (10% at 12 hours and 20% at 24 hours).
- **15 mg/L Group**: A more significant reduction in cavity volume was observed (10%) after 6 hours, reaching 25% by 24 hours.
- **25 mg/L Group**: The most significant reduction in cavity volume was observed, with a 25% reduction at 6 hours and a 40% reduction by 24 hours.

Table 4.6: 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%

The reduction in suprabranchial cavity volume correlates with increased inflammation and epithelial desquamation, particularly at higher concentrations of clove oil.

4.3 Biochemical Results

Biochemical analysis was performed to assess the effects of clove oil on oxidative stress and protein damage. The following markers were evaluated: **HSP70 expression**, **SOD activity**, and **total protein content**.

4.3.1 HSP70 Expression

- **Control Group**: No significant change in HSP70 expression was observed, maintaining baseline levels.
- **5 mg/L Group**: Mild increase in HSP70 expression was observed (10%) after 6 hours, progressing to 20% by 12 and 24 hours.
- **15 mg/L Group**: HSP70 expression increased significantly (15%) after 6 hours and reached 30% by 24 hours.
- **25 mg/L Group**: The highest increase in HSP70 expression was observed, with a 20% increase at 6 hours, reaching 40% by 24 hours.

Table 4.7: HSP70 Expression

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

The increase in HSP70 expression reflects the stress response to clove oil exposure, with higher concentrations leading to stronger activation of stress pathways.

4.3.2 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 12 and 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 4.8: SOD Activity

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

- **Conclusion**: The increase in SOD activity indicates heightened oxidative stress due to clove oil exposure, with higher concentrations causing greater increases in antioxidant activity.

4.3.3 Total Protein Content

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

Table 4.9: Total Protein Content

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

The reduction in total protein content reflects protein degradation and cellular damage, with the most significant effects observed at the highest concentration.

Conclusion

The results from this study provide strong evidence of the dose- and time-dependent toxic effects of clove oil on *Channa punctatus*. Histopathological changes, such as neuronal degeneration in the brain, epithelial thickening in the gills, and inflammation in the suprabranchial cavity, were observed across all exposure groups, with the most severe effects seen at higher concentrations and longer exposure periods. Histomorphometric measurements confirmed these changes, showing a significant reduction in neuronal density, gill epithelial thickness, and suprabranchial cavity volume at higher clove oil concentrations. Biochemical assays further demonstrated increased oxidative stress, with elevated HSP70 expression and SOD activity, as well as decreased total protein content. These findings highlight the potential risks of clove oil exposure, especially at higher concentrations, and emphasize the need for careful monitoring of its use in fish handling and transport.

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