

Protective Role of Aloe vera Against Ethanol-Induced Behavioural and Respiratory Stress in *Channa marulius*

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ABSTRACT

Aquatic pollution caused by anthropogenic activities has become a major environmental concern due to its harmful impact on aquatic organisms. The present study was conducted to evaluate the effect of ethanol exposure on the behavioral and respiratory responses of *Channa marulius* under controlled laboratory conditions and to investigate the protective role of Aloe vera powder against ethanol-induced stress. Healthy fishes were acclimatized and divided into control and treatment groups. Ethanol-exposed fishes exhibited significant physiological and behavioral alterations compared to the control group. Increased opercular movement, erratic swimming, restlessness, surface gulping, reduced feeding activity, sluggish movement, and loss of equilibrium were among the major stress responses observed during exposure. The severity of these responses increased with higher ethanol concentration and prolonged exposure duration, indicating respiratory and neurobehavioral disturbances in the fish. Opercular movement proved to be a sensitive biomarker for evaluating ethanol-induced respiratory stress. Treatment with Aloe vera powder showed noticeable improvement in fish behavior and respiration. The opercular movement gradually returned toward normal levels, and fishes regained stable swimming behavior with reduced stress symptoms. The recovery-enhancing effect of Aloe vera may be associated with its antioxidant and protective bioactive compounds. The findings of the present study suggest that ethanol significantly affects the normal physiological functioning of *Channa marulius*, while Aloe vera possesses potential protective properties against ethanol-induced toxicity. The study also highlights the usefulness of *Channa marulius* as a potential bioindicator species for freshwater pollution monitoring and ecotoxicological studies.

Keywords: *Channa marulius*, Ethanol toxicity, Opercular movement, Behavioural stress, Aloe vera.

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INTRODUCTION

Aquatic ecosystems are continuously exposed to various pollutants generated through industrialization, agricultural activities, domestic sewage, and laboratory discharges. The release of these contaminants into freshwater systems significantly affects water quality and threatens the survival and normal functioning of aquatic organisms. Fish are considered one of the most reliable bioindicators of aquatic pollution because they respond rapidly to environmental stress through

physiological, biochemical, and behavioural alterations (Mousaviyon et al., 2025; Chukwuka et al., 2022). Changes in swimming behaviour, respiratory activity, feeding response, and stress-related movements are commonly used as sensitive indicators of toxic exposure in ecotoxicological studies. Among emerging aquatic contaminants, ethanol has gained attention due to its widespread use in pharmaceutical industries, chemical laboratories, beverages, and industrial processing units. Accidental release of ethanol-containing waste into

aquatic environments can disturb fish metabolism and neurological functioning. Previous studies have shown that ethanol and other solvent-based toxicants can induce abnormal swimming patterns, respiratory distress, excessive mucus secretion, lethargy, and loss of equilibrium in freshwater fish species (Nash et al., 2005; Adegun & Sahaf, 2022). Opercular movement rate is considered an important physiological biomarker because it reflects respiratory stress and oxygen demand in fishes exposed to toxic substances (Chukwuka et al., 2022). Increased opercular activity is generally associated with impaired gill function and physiological stress caused by environmental pollutants. *Channa marulius* (giant snakehead) is an ecologically and commercially important freshwater fish species widely distributed in South Asian aquatic ecosystems. Due to its hardy nature, air-breathing capability, and tolerance to fluctuating environmental conditions, this species is considered suitable for aquatic toxicology and bioindicator studies. However, limited scientific information is available regarding the effect of ethanol exposure on the behavioural and respiratory physiology of *Channa marulius*. Most previous studies have focused on other freshwater fish species, leaving a significant research gap in understanding toxicological responses in this species. Recently, herbal and plant-based products have gained considerable importance for their protective and recovery-enhancing effects against toxic stress in aquatic organisms. Aloe vera is a medicinal plant known for its antioxidant, antimicrobial, anti-inflammatory, and healing properties. Several studies have suggested that Aloe vera and its bioactive compounds may reduce oxidative stress and improve physiological recovery in fish exposed to toxic substances (Nirmala et al., 2022; Yang et al., 2022). Herbal supplementation has also been reported to enhance immunity, antioxidant defense systems, and stress tolerance in aquatic animals. Therefore, the present study was designed to evaluate the effect of ethanol exposure on behavioural responses and opercular movement in *Channa marulius* under controlled laboratory conditions and to investigate the possible protective role of Aloe vera powder against ethanol-induced stress. The findings of this research may contribute to aquatic toxicology, fish physiology, and freshwater pollution monitoring by establishing *Channa marulius* as a potential bioindicator species.

MATERIALS AND METHODS

Experimental Fish

Healthy freshwater fish, *Channa marulius* (giant snakehead), were selected for the present study due to their ecological importance and sensitivity to environmental stress. Fish of nearly uniform size and

weight were procured from a local fish market and transported carefully to the laboratory in aerated containers. Before the experiment, fishes were acclimatized under laboratory conditions for 10–14 days in glass aquaria containing dechlorinated tap water. During the acclimatization period, fishes were fed with commercial pellet feed twice daily, and water was renewed regularly to maintain proper hygienic conditions. Dead or unhealthy fishes, if any, were removed immediately. Water quality parameters such as temperature, pH, and dissolved oxygen were maintained within suitable ranges for freshwater fish survival following standard aquatic toxicology procedures (APHA, 2017).

Experimental Design

The experimental study was carried out under controlled laboratory conditions to evaluate the effect of ethanol exposure on the physiology and behaviour of *Channa marulius* and to assess the protective role of Aloe vera powder against ethanol-induced stress. The experimental groups and treatment conditions used during the study are presented in Table 1. The fishes were randomly divided into different experimental groups, including a control group and treatment groups. The control group was maintained in clean water without ethanol exposure, while treatment groups were exposed to different concentrations of ethanol prepared in aquarium water. Another treatment group was supplemented with Aloe vera powder after ethanol exposure to study its recovery-enhancing effect. Each experimental group was maintained in triplicate to ensure reliability and accuracy of observations. The exposure period included both short-term and medium-term observations to examine immediate as well as progressive toxic effects.

Preparation of Ethanol Solution

Analytical-grade ethanol was used for the preparation of experimental concentrations. Required concentrations were prepared by diluting ethanol in dechlorinated water before introduction into aquaria. Fresh ethanol solutions were prepared regularly to maintain uniform exposure conditions throughout the experiment.

Preparation of Aloe vera Powder

Fresh Aloe vera leaves were collected, washed thoroughly with distilled water, and shade dried under laboratory conditions. The dried plant material was ground into fine powder using a sterile grinder and stored in airtight containers until further use. The prepared Aloe vera powder was administered in treatment aquaria for evaluating its ameliorative effect against ethanol-induced stress.

Behavioural Observations

Behavioural responses of fishes were observed carefully during the exposure period. Parameters such as swimming pattern, opercular movement, feeding response, surface gulping, restlessness, lethargy, loss of equilibrium, and abnormal body movement were recorded systematically. Behavioural changes were compared between control and treated groups to determine the toxic effects of ethanol exposure. Photographic and visual observations were also used for documentation of fish responses, as behavioural biomarkers are considered sensitive indicators of environmental stress in aquatic organisms (Chukwuka et al., 2022).

Measurement of Opercular Movement

Respiratory activity was determined by counting opercular beats per minute. Individual fish were observed for one minute, and the number of opercular movements was recorded manually. Increased opercular activity was considered an indicator of respiratory stress and physiological disturbance caused by ethanol exposure. Observations were recorded at regular intervals during the experimental period.

Water Quality Parameters

Important water quality parameters including temperature, pH, dissolved oxygen, and ammonia concentration were monitored periodically throughout the experiment using standard laboratory methods. Proper aeration and water replacement were carried out to maintain suitable environmental conditions for fish survival.

Statistical Analysis

All experimental observations were recorded as mean $\pm$ standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by comparison between control and treatment groups. A probability value of $p < 0.05$ was considered statistically significant. Graphical representation of behavioural and physiological data was prepared using standard software tools.

RESULTS AND DISCUSSION

Effect of Ethanol on Opercular Movement

The present study revealed that ethanol exposure caused a significant increase in opercular movement in *Channa marulius* compared to the control group (Table 3). Fishes exposed to ethanol showed rapid opercular beats during the initial stages of treatment, indicating respiratory

distress and increased oxygen demand. The increase in opercular activity became more noticeable with prolonged exposure duration (fig 2). Ethanol may interfere with normal gill functioning and gaseous exchange, thereby forcing fishes to increase respiratory effort to maintain oxygen balance. Similar findings were reported by Chukwuka et al. (2022), who observed respiratory abnormalities in *Oreochromis mossambicus* exposed to toxic chemicals. Increased opercular frequency is widely considered an important physiological biomarker of aquatic stress and pollutant exposure (Bhuvaneswari et al., 2023).

Behavioural Alterations in Ethanol-Exposed Fish

Behavioural observations showed clear signs of stress in ethanol-treated fishes. The major behavioural changes observed during ethanol exposure and Aloe vera treatment are summarized in Table 2. During the early exposure phase, fishes exhibited hyperactivity, erratic swimming, rapid movement, restlessness, and repeated surface gulping. These responses indicated immediate physiological stress and neurobehavioural disturbance caused by ethanol toxicity. Ethanol exposure is known to affect the nervous system and muscular coordination of fishes, leading to abnormal swimming patterns and locomotory imbalance (Kaur & Kaur, 2023). With increasing exposure duration, fishes gradually became lethargic and displayed reduced swimming activity, sluggish movements, and loss of equilibrium. Feeding response was also significantly reduced in the treated groups. Some fishes exhibited excessive mucus secretion and temporary body imbalance under higher ethanol exposure conditions. Similar behavioural abnormalities have also been reported in fishes exposed to industrial pollutants, pesticides, and organic contaminants (Patel et al., 2022; Rani et al., 2024). These behavioural changes serve as early indicators of toxic stress and impaired physiological functioning in aquatic organisms (Fig 1).

Comparison with Control Group

The control group maintained under normal laboratory conditions did not show any abnormal behavioural or respiratory changes throughout the experimental period. The fishes remained active, maintained normal swimming patterns, and exhibited stable opercular movement and feeding behaviour. This indicates that the physiological and behavioural alterations observed in treatment groups were directly associated with ethanol exposure rather than environmental variations or handling stress (Singh et al., 2022; Al-Ghanim et al., 2023).

Protective Effect of Aloe vera Powder

An important finding of the study was the protective and recovery-enhancing effect of Aloe vera powder against ethanol-induced stress. Fishes treated with Aloe vera showed gradual normalization of swimming behaviour and respiratory activity (table 4). Opercular movement rate decreased toward normal levels compared to untreated ethanol-exposed fishes. Improvement in feeding activity and reduction in stress-related responses were also observed during the recovery phase (fig 3). The beneficial effect of Aloe vera may be attributed to the presence of bioactive compounds such as flavonoids, phenolics, vitamins, enzymes, and polysaccharides that possess antioxidant and anti-inflammatory properties. These compounds help reduce oxidative stress and improve physiological recovery in stressed organisms. Similar protective effects of Aloe vera and other herbal supplements have been reported in aquatic toxicology studies (Nirmala et al., 2022; Yang et al., 2022).

Significance of Behavioural and Respiratory Biomarkers

Behavioural and respiratory parameters proved to be highly sensitive indicators for assessing ethanol toxicity in *Channa marulius*. Alterations in swimming behaviour, opercular movement, and feeding response were observed even during early stages of exposure, suggesting that these biomarkers can be effectively used for rapid ecotoxicological assessment. Respiratory and behavioural biomarkers are widely recognized as reliable tools for monitoring aquatic pollution because they reflect physiological disturbances before severe pathological damage occurs (Yusuf et al., 2023; Abdel-Tawwab et al., 2022; Rahman et al., 2024).

Role of *Channa marulius* as a Bioindicator Species

The present findings indicate that *Channa marulius* responds sensitively to ethanol-induced stress and may serve as an effective bioindicator species for freshwater pollution studies. Due to its ecological importance, adaptability, and measurable physiological responses, this fish species can be utilized in aquatic toxicology and environmental monitoring programs. Similar studies have emphasized the importance of freshwater fishes as indicators of ecosystem health and pollutant toxicity (Mousaviyon et al., 2025).

thus, the present investigation demonstrated that ethanol exposure significantly affects the normal physiology and behaviour of *Channa marulius*. Increased opercular movement, abnormal swimming patterns, reduced feeding behaviour, and lethargy were major symptoms observed under ethanol stress. The recovery observed after Aloe vera treatment suggests its potential application as a natural protective agent against aquatic

toxicants. The study contributes valuable information to aquatic toxicology research and highlights the importance of herbal-based approaches for stress management in freshwater fishes.

CONCLUSION

The present study demonstrated that ethanol exposure causes significant physiological and behavioural disturbances in *Channa marulius* under controlled laboratory conditions. The fishes exposed to ethanol showed noticeable alterations in opercular movement, swimming behaviour, feeding activity, and stress responses. Increased opercular activity, erratic swimming, hyperactivity, lethargy, and loss of equilibrium were identified as major indicators of ethanol-induced toxicity. These findings clearly suggest that ethanol adversely affects respiratory efficiency and normal metabolic functioning in freshwater fishes. The study further revealed that behavioural and respiratory parameters are highly sensitive biomarkers for evaluating aquatic toxic stress. Early changes in fish behaviour and opercular movement provided rapid indications of physiological imbalance before the occurrence of severe damage. Therefore, such biomarkers may serve as useful tools for environmental monitoring and ecotoxicological assessments. An important outcome of the investigation was the protective role of Aloe vera powder against ethanol-induced stress. Treatment with Aloe vera improved behavioural responses, reduced respiratory stress, and promoted recovery in exposed fishes. The beneficial effect may be associated with the antioxidant and anti-inflammatory properties of the plant. The findings indicate that *Channa marulius* can be considered a suitable bioindicator species for freshwater pollution studies, while Aloe vera may offer a natural and eco-friendly approach for reducing toxic stress in aquatic organisms.

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Conflict of Interest

The authors declare no conflict of interest with anyone.

Data Availability Statement

The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Ethical Considerations

The experimental work was conducted according to standard ethical guidelines for handling aquatic organisms. Fish were handled carefully throughout the study to minimize stress and unnecessary suffering. Exposure concentrations were maintained within sublethal limits, and all laboratory procedures were performed under controlled conditions.

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freshwater fish exposed to aquatic contaminants.

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Tables and figures:

Table 1. Experimental Setup for Ethanol Exposure Study in *Channa marulius*

Experimental Group	Treatment Condition	Expected Observation
Control Group	Fish maintained in clean water without ethanol exposure	Normal swimming and respiration
Low Ethanol Exposure	Fish exposed to low ethanol concentration	Mild increase in opercular movement and slight behavioural alteration
Moderate Ethanol Exposure	Fish exposed to moderate ethanol concentration	Erratic swimming, stress response, and altered respiration
High Ethanol Exposure	Fish exposed to higher ethanol concentration	Loss of balance, sluggish movement, severe respiratory stress
Ethanol + Aloe vera Treatment	Ethanol-exposed fish treated with <i>Aloe vera</i> powder	Recovery in behaviour and opercular movement

Table 2. Behavioural Responses of *Channa marulius* Under Ethanol Exposure

Behavioural Parameters	Control	Ethanol-Treated Fish	Aloe vera Treated Fish
Swimming Pattern	Normal	Erratic and irregular	Gradually normalized
Restlessness	Absent	Increased	Reduced
Surface Gulping	Absent	Frequent	Reduced
Feeding Response	Normal	Reduced	Improved
Loss of Equilibrium	Absent	Observed in higher exposure	Reduced
Sluggish Movement	Absent	Present	Reduced
Stress Response	Normal	Severe	Mild

Behavioural observations were recorded through manual monitoring and visual examination.

Table 3. Respiratory Responses Based on Opercular Movement

Experimental Condition	Opercular Movement	Respiratory Condition	Interpretation
Control Group	Normal	Stable respiration	Healthy physiological state
Ethanol Exposure	Increased	Respiratory stress	Ethanol-induced physiological disturbance
Higher Ethanol Concentration	Rapid opercular movement	Severe stress condition	Altered breathing rate
Aloe vera Treatment	Gradual normalization	Recovery phase	Protective effect of <i>Aloe vera</i>

Opercular movement was assessed by counting gill cover movement per minute.

Table 4. Overall Physiological Impact of Ethanol and Recovery by Aloe vera

Parameters Observed	Ethanol Exposure	After <i>Aloe vera</i> Treatment
Respiratory Activity	Increased significantly	Reduced toward normal
Swimming Stability	Disturbed	Improved
Activity Level	Reduced	Increased
Stress Symptoms	Severe	Mild
Physiological Functioning	Altered	Partially restored
Fish Condition	Weak and stressed	Recovery observed

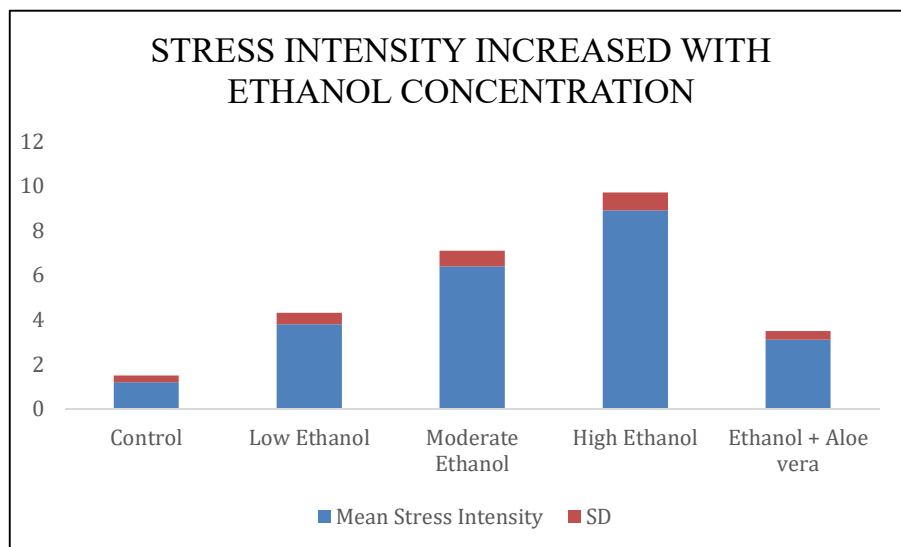


Figure 1 Stress Intensity Increased with Ethanol Concentration

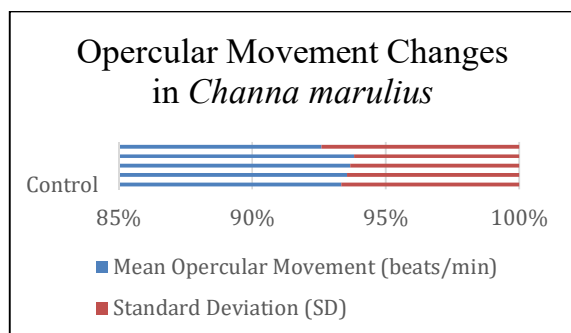


Figure 2. Opercular Movement Changes in *Channa marulius*

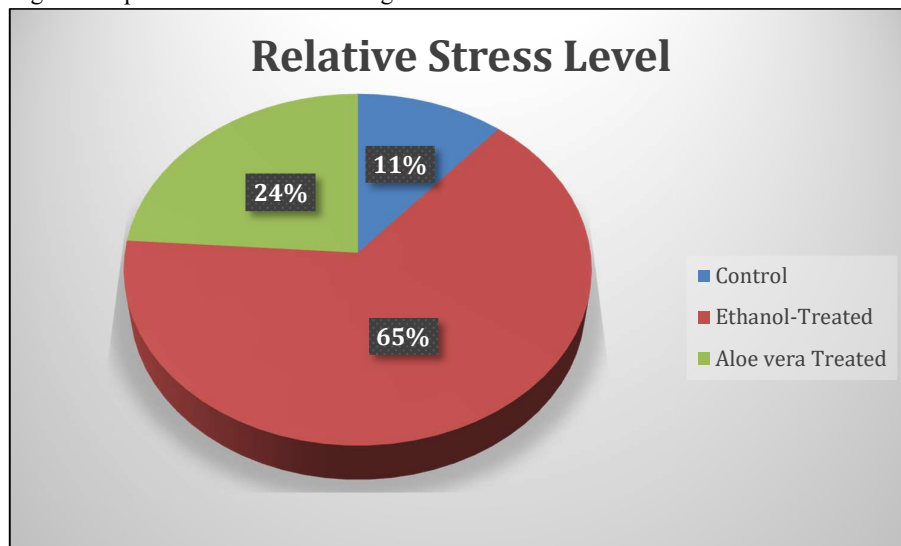


Figure 3. Comparative Recovery Graph