Effect of sea lettuce (*Ulva rigida*) extract on histology and oxidative stress in whiteleg shrimp (*Litopenaeus vannamei*)

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Abstract

Sea lettuce (*Ulva rigida*) has been widely used as a feed additive for various aquatic animals, as previous research indicates that it can enhance oxidative stress responses and immunity. However, scientific data regarding its application in whiteleg shrimp remains limited. Therefore, this study aimed to evaluate the effects of dietary supplementation with sea lettuce extract on histology and oxidative stress parameters in whiteleg shrimp. Shrimp were fed diets supplemented with sea lettuce extract at doses of 0, 0.5, 1.5, and 5.0 g/kg for 14 days. At the end of the experimental period, samples of the hepatopancreas, muscle, intestine, and gill were collected for histological examination and oxidative stress assessment. Hemolymph samples were also collected to further oxidative stress analysis. Histological examination revealed no tissue alterations in any of the examined organs after 14 days of feeding the extract-supplemented diets. Oxidative stress was evaluated by measuring malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and glutathione peroxidase (GPx). The findings indicated that the sea lettuce extract significantly reduced lipid peroxidation ($P < 0.05$) as indicated by decreased MDA levels across all organs, maintaining levels comparable to those of the control group. Shrimp fed the 0.5 g/kg diets showed increased GSH content in the hepatopancreas and significantly elevated CAT activity in the intestine. Moreover, GR activity in the hemolymph significantly increased at all supplementation levels. Among the tested doses, 0.5 g/kg demonstrated the greatest antioxidant efficacy in mitigating oxidative stress in whiteleg shrimp. In conclusion, these findings suggest the sea lettuce (*U. rigida*) extract has potential as a functional feed additive to enhance oxidative resilience in whiteleg shrimp without adverse histological effects.

Introduction

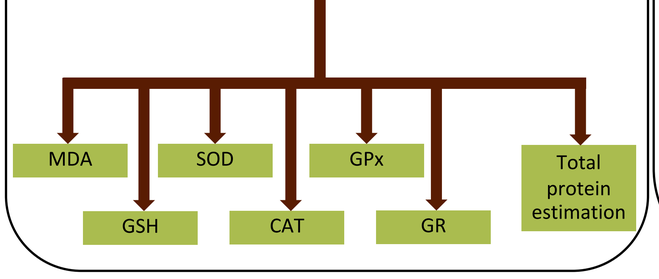
Oxidative stress is a condition resulting from excessive oxygen utilization by various organs within the body. Oxidative stress significantly impact health, primarily arising from an imbalance between free radicals and antioxidants. This equilibrium disruption can lead to systemic damage. Currently, shrimp farmers face substantial economic losses due to mass mortality caused by infectious and oxidative stress related diseases. High-density pond environments facilitate the rapid transmission of pathogens once a vector enters the system. Common shrimp pathogens include both bacteria and viruses, such as *Vibrio parahaemolyticus*, *Enterocytozoon hepatopenaei* (EHP), and White Spot Syndrome Virus (WSSV) (Babu *et al*., 2024). Traditional management relies heavily on antibiotics; however, frequent or excessive use can lead to antimicrobial resistance (AMR), rendering shrimp more vulnerable to disease and increasing cultivation difficulty. This has prompted a shift toward identifying natural bioactive compounds as sustainable alternatives to antibiotics. Various herbal extracts have been evaluated for their efficacy in pathogen resistance and health promotion in *L. vannamei*. Examples include White Wormwood (*Artemisia herba-alba*), Japanese Honeysuckle (*Lonicera japonica*), Madonna Lily (*Lilium candidum*), and Carry-me-seed (*Phyllanthus amarus*). The search for natural extracts often focuses on locally available and accessible resources. For instance, research on *Spirulina platensis* has demonstrated its ability to enhance immune responses against *Vibrio parahaemolyticus* (Ahmed *et al*., 2021). In addition, previous research in Asian Sea Bass (*Lates calcarifer*) indicated that *Ulva rigida* extracts help reduce *Vibrio vulnificus* infections and normalize oxidative stress markers (Klongklaew *et al*., 2025). These findings suggest that *Ulva rigida* may bolster the physiological resilience of *L. vannamei* through its antioxidant properties. Building on this foundation, the present study aimed to examine the effects of extract of sea lettuce (*Ulva rigida*) on oxidative stress parameters and histological alteration in whiteleg shrimp (*Litopenaeus vannamei*).

Methodology

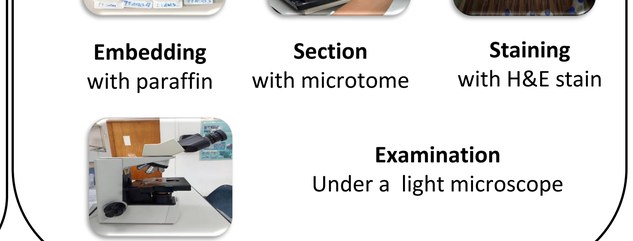
Extraction of *U. rigida*



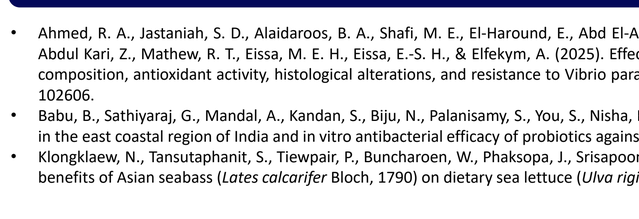
Experimental groups



Tissue Extraction



Oxidative stress process



Histological process

Result and Discussion

Oxidative Status

Intestine

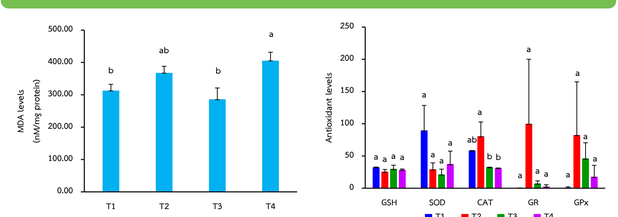


Fig. 1 MDA and GSH levels and SOD, CAT, GR and GPx activities in intestine of whiteleg shrimp treated with sea lettuce extracts (T2, T3 and T4) compared to control (T1). Superscript letters represent mean ± SE. Significant differences at $p < 0.05$.

Hepatopancreas

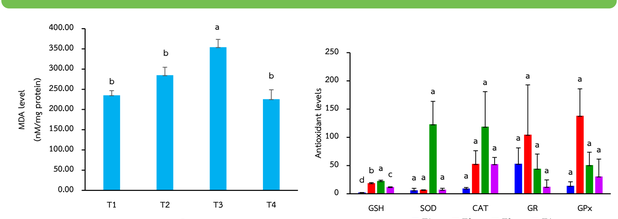


Fig. 2 MDA and GSH levels and SOD, CAT, GR and GPx activities in hepatopancreas of whiteleg shrimp treated with sea lettuce extracts (T2, T3 and T4) compared to control (T1). Superscript letters represent mean ± SE. Significant differences at $p < 0.05$.

Gill

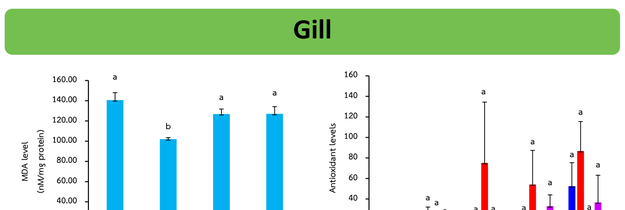


Fig. 3 MDA and GSH levels and SOD, CAT, GR and GPx activities in gill of whiteleg shrimp treated with sea lettuce extracts (T2, T3 and T4) compared to control (T1). Superscript letters represent mean ± SE. Significant differences at $p < 0.05$.

Muscle

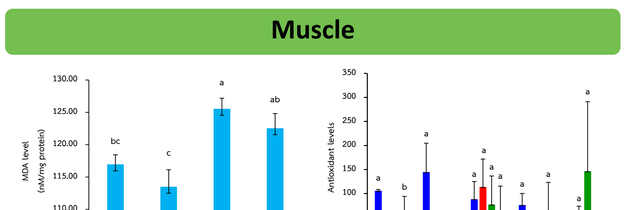


Fig. 4 MDA and GSH levels and SOD, CAT, GR and GPx activities in muscle of whiteleg shrimp treated with sea lettuce extracts (T2, T3 and T4) compared to control (T1). Superscript letters represent mean ± SE. Significant differences at $p < 0.05$.

Hemolymph

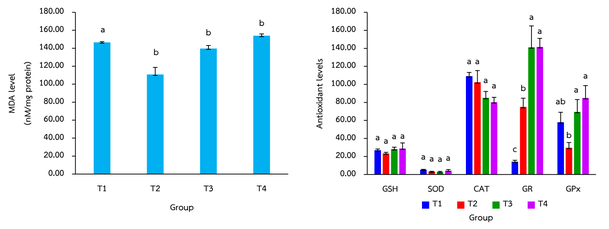


Fig. 5 MDA and GSH levels and SOD, CAT, GR and GPx activities in hemolymph of whiteleg shrimp treated with sea lettuce extracts (T2, T3 and T4) compared to control (T1). Superscript letters represent mean ± SE. Significant differences at $p < 0.05$.

Histology

Intestine

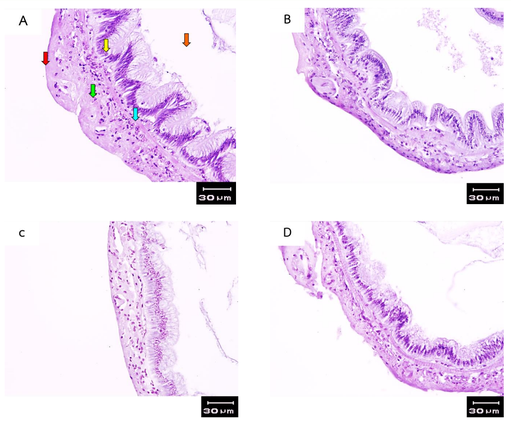


Fig. 6 Intestinal tissues of whiteleg shrimp fed with sea lettuce extract: (A) Control group; (B) Group treated with 0.5 g/kg extract; (C) Group treated with 1.5 g/kg extract; and (D) Group treated with 5.0 g/kg extract. H&E stain, 20x magnification.
➪Mucosa, ➪ Basement membrane, ➪Muscularis layer, ➪ Serosa and ➪ Lumen

Hepatopancreas

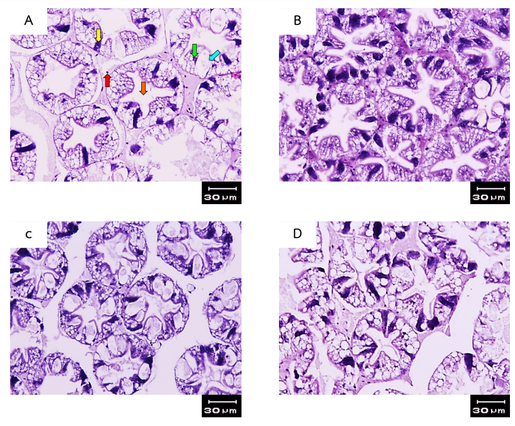


Fig. 7 Hepatopancreas of whiteleg shrimp fed with sea lettuce extract: (A) Control group; (B) Group treated with 0.5 g/kg extract; (C) Group treated with 1.5 g/kg extract; and (D) Group treated with 5.0 g/kg extract for 14 days. H&E stain, 20x magnification.
➪F-cells, ➪B-cells, ➪ R-cells, ➪E-cells and ➪Lumen

Gill

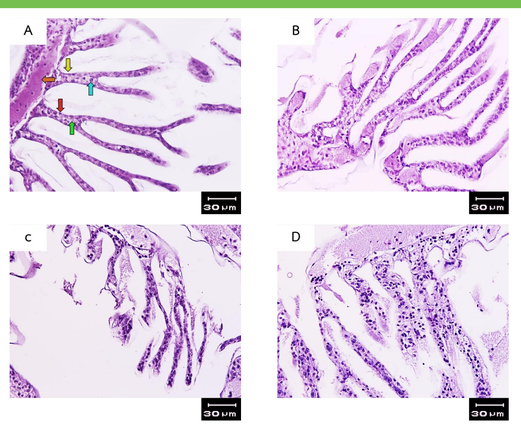


Fig. 8 Gill of whiteleg shrimp fed with sea lettuce extract: (A) Control group; (B) Group treated with 0.5 g/kg extract; (C) Group treated with 1.5 g/kg extract; and (D) Group treated with 5.0 g/kg extract for 14 days. H&E stain, 20x magnification.
➪ Gill filament, ➪ Hemocyte, ➪ Hemolymphatic lacuna, ➪ Pillar cells and ➪ Gill lamella

Muscle

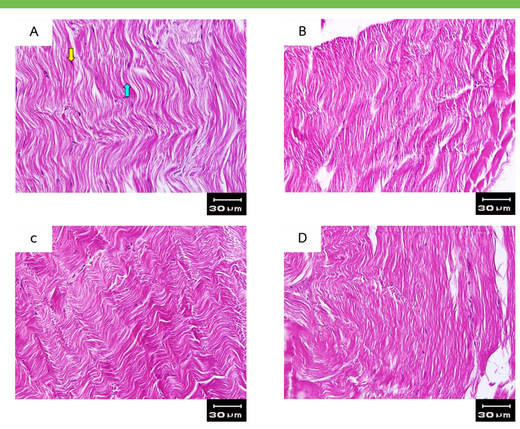


Fig. 9 muscle of whiteleg shrimp fed with sea lettuce extract: (A) Control group; (B) Group treated with 0.5 g/kg extract; (C) Group treated with 1.5 g/kg extract; and (D) Group treated with 5.0 g/kg extract for 14 days. H&E stain, 20x magnification.
➪Nucleus and ➪Muscle fiber

Conclusion

In conclusion, these findings suggest that the aqueous sea lettuce (*U. rigida*) extract has potential as a functional feed additive to enhance oxidative resilience in whiteleg shrimp without adverse histological effects. However, the greatest dose of sea lettuce extract in reducing oxidative stress was 0.5 g/kg.

Acknowledgements

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