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IMMUNOMODULATORY BENEFITS OF *ULVA LACTUCA*: A STUDY ON CELLULAR AND HUMORAL IMMUNE RESPONSES.

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ABSTRACT:
The immune system plays a critical role in protecting the body against infections and maintaining physiological homeostasis. Natural products, particularly marine algae, have gained increasing attention due to their diverse bioactive constituents and potential immunomodulatory properties. *Ulva lactuca*, commonly known as sea lettuce, is a green marine alga rich in phytoconstituents such as flavonoids, saponins, alkaloids, and steroids, which are known to exhibit various pharmacological activities. However, its role in modulating immune responses has not been comprehensively investigated. The present study was designed to evaluate the immunomodulatory potential of hydroalcoholic extract of *Ulva lactuca* (HUL) using established *in-vivo* models of cellular and humoral immunity. Immunomodulatory activity was assessed through delayed-type hypersensitivity response (DTH), hemagglutinating antibody titer assay, and cyclophosphamide-induced immunosuppression model. HUL at doses of 150 and 300 mg/kg significantly enhanced DTH response, restored leukocyte function in immunosuppressed animals, and increased antibody titers compared to the control group. Furthermore, HUL treatment resulted in a dose-dependent increase in spleen and thymus indices, indicating improved immune organ function. Histopathological examination of immune organs revealed protective effects against cyclophosphamide-induced immunological damage. These findings suggest that *Ulva lactuca* possesses significant immunomodulatory activity by enhancing both cellular and humoral immune responses. The observed effects may be attributed to the presence of bioactive phytoconstituents, supporting its potential as a natural immunotherapeutic agent.

KEYWORDS: *Ulva lactuca*, Immunomodulation, Cellular immunity, Humoral immunity, Antibody titer

INTRODUCTION:

The immune system is a well-coordinated defensive system in the body that safeguards it against infections, foreign bodies, and pathological conditions. It works in association with two major systems: the innate immune system, which offers immediate protection, and the adaptive immune system, which offers a specific response to antigens. The capacity of the immune system to distinguish between foreign bodies and self-components, while destroying the former and tolerating the latter, is critical for maintaining physiological equilibrium. Nevertheless, infections, stress, environmental toxins, and chemotherapeutic drugs can affect the immune system and make an individual more vulnerable to diseases. Consequently, substances that can modulate the immune system have assumed prominence in therapeutic research[1, 2] .
Natural products have recently been identified as potential sources of immunomodulators because of their safety, availability, and diverse pharmacological properties. Natural products, including medicinal plants, marine organisms, and their bioactive compounds, have been shown to possess the ability to stimulate the immune system by activating immune cells,

promoting the production of antibodies, and improving the immune functions of immune organs such as the spleen and thymus. Natural immunomodulators have the potential to serve as alternatives or complements to existing treatments, especially for conditions that result from immunosuppression[3–5].

Marine algae are a valuable source of biologically active compounds with great therapeutic potential. Among these, *Ulva lactuca*, also known as sea lettuce, is a green marine alga that is widely distributed in coastal areas around the world. It has been used traditionally as a nutritional and medicinal agent because of its valuable content of biologically active compounds such as flavonoids, alkaloids, saponins, steroids, polysaccharides, and phenolic compounds. These phytoconstituents have been reported to have antioxidant, anti-inflammatory, antimicrobial, antiviral, hepatoprotective, and anticancer activities. Besides its medicinal properties, *Ulva lactuca* is also used as a nutritional supplement and has applications in aquaculture and bioremediation[6].

Although the pharmacological properties of *Ulva lactuca* have been well documented, there is a lack of scientific evidence regarding its immunomodulatory properties, especially in relation to both cellular and humoral immunity. Cellular immunity can be activated through the stimulation of immune cells such as macrophages, lymphocytes, and neutrophils, whereas humoral immunity can be activated through the production of antibodies by B lymphocytes.

MATERIAL AND METHODS:

Collection and Authentication of Plant Material

The fresh samples of *Ulva lactuca* were collected from the coastal area of Okha, Gujarat, India. The collected sample was properly washed and shade-dried at room temperature for a few days to retain the phytoconstituents. The plant was identified by Dr. A. M. Patel, Principal and Taxonomist, J and J College of Science, Nadiad, Gujarat, India. A voucher specimen was also preserved.

Preparation of Extract

The powdered form of *Ulva lactuca* was taken for extraction using the maceration technique with 70% ethanol as the solvent. The activated charcoal treatment step was carried out to remove chlorophyll from the extract. The filtrate was then concentrated under reduced pressure using a rotary evaporator to obtain a semi-solid hydroalcoholic extract. The dried extract was stored in an airtight container [7].

Preliminary phytochemical screening

The hydroalcoholic extract of *Ulva lactuca* (HUL) was analysed for qualitative phytochemical screening to determine the presence of major phytoconstituents such as alkaloids, flavonoids, saponins, tannins, steroids, glycosides, and phenolics. Standard phytochemical screening methods were employed to detect the presence of these phytoconstituents[2,7].

Animals

Female Swiss albino mice, weighing 20-25 g, were obtained from Syncbio Research Centre, Ahmedabad, Gujarat, India. The animals were kept in polypropylene cages under standard laboratory conditions, with a temperature of 23 ± 2°C, relative humidity of 55-60%, and a 12-hour light and dark cycle. The animals were acclimatized to the laboratory conditions for a period of seven days before the experiment. The animals were given standard pellet diet and water ad libitum. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) (Protocol No: RPCP/IAEC/2022-23/R1).

Experimental Design

The animals were randomly divided into four groups, with six animals in each group:

Group I Normal (Vehicle)

Group II Cyclophosphamide (50 mg/kg, i.p.) treated control (immunosuppressed group)

Group III *Ulva lactuca* (UL) extract low (150 mg/kg, p.o.)
Group IV *Ulva lactuca* (UL) extract (300 mg/kg, p.o.)

The extract was given orally once a day for 14 consecutive days. Immunosuppression was induced in the designated group using cyclophosphamide, an established immunosuppressive drug that has been shown to suppress the immune system by inhibiting the proliferation of leukocytes and damaging the immune organs[8–10].

Induction of Immunosuppression

The immunosuppressive drug cyclophosphamide was given intraperitoneally at a dose of 30 mg/kg body weight to induce immunosuppression. This model is commonly used to assess immunomodulatory drugs since it simulates conditions of suppressed immunity by decreasing the white blood cell count and weakening the immune response.

Assessment of Cellular Immune Response- Delayed-Type Hypersensitivity (DTH) Response

The cell-mediated immunity was assessed by the delayed-type hypersensitivity (DTH) reaction elicited by sheep red blood cells (SRBCs). On day 0, the animals were immunized by subcutaneous injection of SRBC suspension into the right hind footpad. On day 7, the animals were challenged with SRBC suspension into the left hind footpad. The thickness of the footpad was measured before and 24 hours after antigen challenge with the digital vernier caliper. The increase in footpad thickness was taken as an index of cell-mediated immunity[8,9,11].

Assessment of Humoral Immune Response- Hemagglutinating Antibody (HA) Titer Assay

The humoral immune response was determined by hemagglutinating antibody titers against SRBC antigen. The animals were immunized with the SRBC suspension intraperitoneally. Blood was collected after the completion of the experimental period, and the serum was separated by centrifugation. The serum was then serially diluted and mixed with the SRBC suspension in microtiter plates. The plates were then incubated at room temperature, and the antibody titer was determined by the highest dilution that gave visible hemagglutination. This assay is based on the immune response mediated by antibodies [1,11,12].

Haematological Analysis

Blood samples were obtained from the animals for the determination of haematological parameters such as total leukocyte count. The parameters were used to assess the protective effect of the extract against cyclophosphamide-induced immunosuppression[3,13].

Determination of Immune Organ Index

At the end of the experimental period, the animals were sacrificed under ethical conditions. The spleen and thymus were dissected, cleaned, and weighed immediately. The index of the organs was calculated using the following formula:

$$\text{Organ index} = \text{Organ weight (mg)} / \text{Body weight (g)}$$

The indices of the spleen and thymus were used as immune status and immunomodulatory effects[14] .

Histopathological Examination

The spleen and thymus tissues were excised and fixed using 10% formalin solution. The sections were stained with hematoxylin and eosin dyes and observed using a light microscope for histopathological alterations. This was done to assess the structural changes and the protective role of the extract on the immune organs[14] .

Statistical analysis

All experimental results were presented in the form of mean ± standard error of mean (SEM) for six animals in each group (n = 6). Statistical analysis was done by one-way analysis of

variance (ANOVA), and then Tukey's multiple comparison test was used to check the significance of the differences among the experimental groups. The analysis was done by Graphpad Prism software (version 8.0.1). The experimental groups were Normal control (N), cyclophosphamide-treated control (CP), and *Ulva lactuca*-treated groups at doses of 150 mg/kg (UL150) and 300 mg/kg (UL300). The significance of the results was considered at $p < 0.05$. Highly significant differences were denoted by ***$p < 0.001$ when UL150 and UL300 groups were compared with the CP group, and ###$p < 0.001$ when the CP group was compared with the Normal control group.

RESULTS:

Phytochemical Screening

Preliminary phytochemical screening of the HUL revealed the presence of several important bioactive constituents. The extract showed positive results for carbohydrates, saponin glycosides, flavonoids, alkaloids, tannins, and phenolic compounds. These phytoconstituents are well known for their diverse pharmacological properties, including immunomodulatory and antioxidant activities. However, proteins, amino acids, cardiac glycosides, and steroids were not detected in the extract. The presence of these bioactive compounds suggests that the HUL possesses significant therapeutic potential and may contribute to its observed immunomodulatory effects.

Effect of HUL on WBC Count

Cyclophosphamide treatment caused a significant decrease in WBC count compared to the normal group ($p < 0.001$), which validated the immunosuppressive effect. Oral dosing of HUL at 150 mg/kg and 300 mg/kg caused a significant increase in WBC count compared to the cyclophosphamide-treated group ($p < 0.001$). The effect was more pronounced at 300 mg/kg, which was dose-dependent (Figure 1).

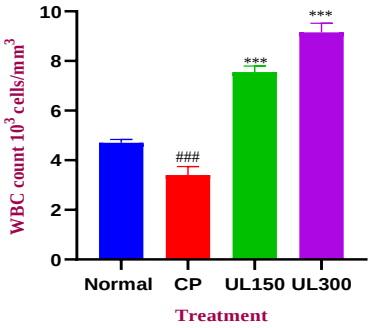


Figure 1. Effect of HUL on WBC Count. The values represent mean ± SEM (n=6); N, Normal, CP-Cyclophosphamide, UL150-HUL 150 mg/kg, UL300-HUL 300 mg/kg, ***p<0.001 comparison of UL150 and UL300 vs CP group, ###p<0.001 comparison of CP versus Normal group.

Effect of HUL on Footpad Thickness

The cyclophosphamide-treated group had a significant increase in footpad thickness compared to the normal group ($p < 0.001$). The HUL treatment at 150 mg/kg had a significant reduction in footpad thickness compared to the cyclophosphamide group ($p < 0.001$), and the 300 mg/kg dose had an even greater reduction. This indicates a successful recovery of the cellular immune response (Figure 2a and 2b).

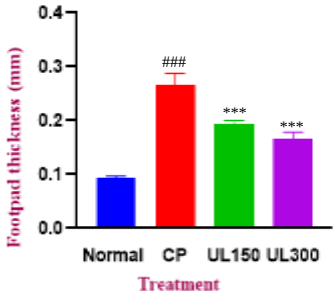


Figure 2a. Effect of HUL on Footpad Thickness. The values represent mean ± SEM (n=6); N, Normal, CP-Cyclophosphamide, UL150-HUL 150 mg/kg, UL300-HUL 300 mg/kg, ***p<0.001 comparison of UL150 and UL300 vs CP group, ###p<0.001 comparison of CP versus Normal group.

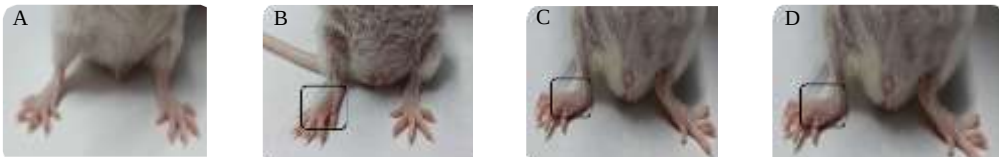


Figure 2b. Effect of HUL on Footpad Thickness. A) Normal, B) CP-Cyclophosphamide, C) UL150-HUL 150 mg/kg and D) UL300-HUL 300 mg/kg.

Effect of HUL on Antibody Titre

Cyclophosphamide caused a significant reduction in antibody titre compared to the normal group ($p < 0.001$), showing that humoral immunity was suppressed. The treatment with Ulva lactuca extract at 150 mg/kg showed a significant increase in antibody titre compared to the cyclophosphamide group ($p < 0.001$). The extract at 300 mg/kg showed a greater augmentation in antibody production, showing that humoral immunity was improved in a dose-dependent manner (Figure 3).

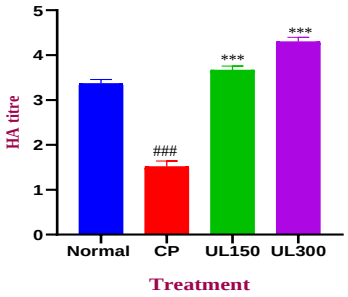


Figure 2. Effect of HUL on Antibody titre. The values represent mean ± SEM (n=6); N, Normal, CP-Cyclophosphamide, UL150-HUL 150 mg/kg, UL300-HUL 300 mg/kg, ***p<0.001 comparison of UL150 and UL300 vs CP group, ###p<0.001 comparison of CP versus Normal group.

Effect of HUL on Spleen and Thymus Index

The treatment with cyclophosphamide caused a significant reduction in the spleen and thymus indices compared to the normal group ($p < 0.001$), which indicated the atrophy of immune organs. The administration of HUL at a dose of 150 mg/kg caused a significant increase in the spleen and thymus indices compared to the cyclophosphamide group ($p < 0.001$). The effect was more pronounced at a dose of 300 mg/kg (Figure 4).

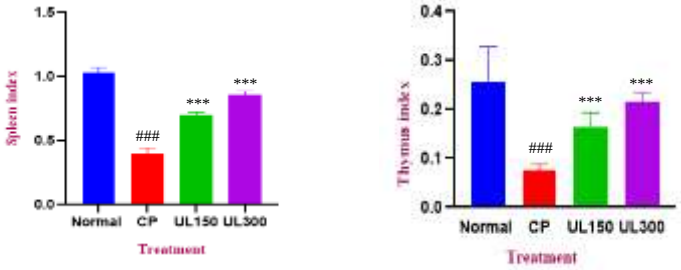
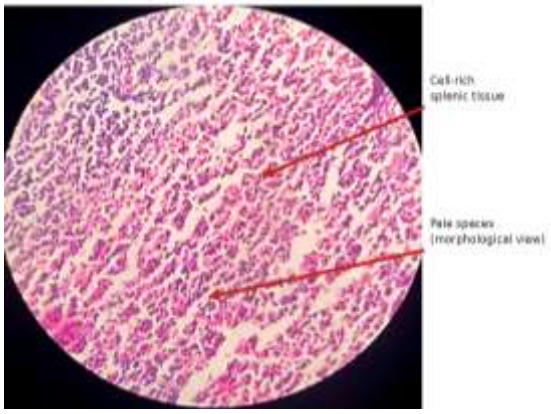


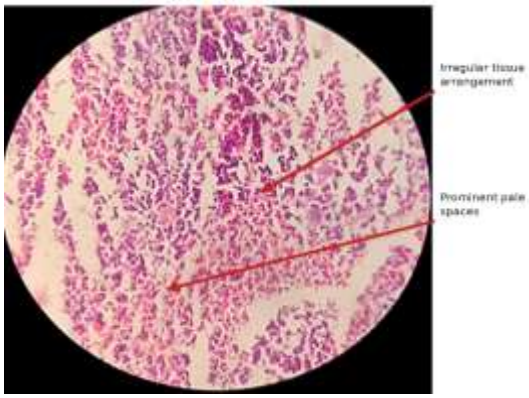
Figure 4. Values represent mean ± SEM (n=6); N, Normal, CP-Cyclophosphamide, UL150-Ulva lactuca 150 mg/kg, UL300-Ulva lactuca 300 mg/kg, ***p<0.001 comparison of UL150 and UL300 vs CP group, ###p<0.001 comparison of CP versus Normal group.

Effect of HUL on Histopathology of Spleen

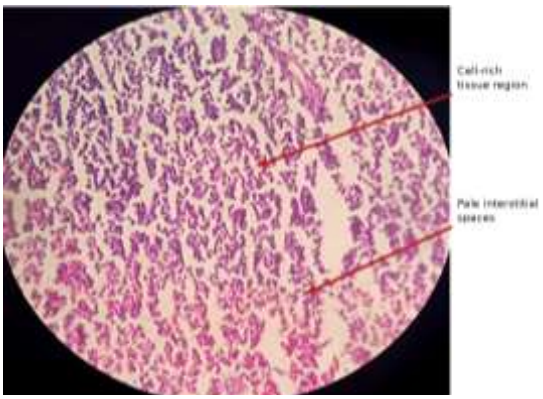
Histopathological examination of the spleen revealed variations in tissue architecture among the experimental groups (Figure 5). The normal control group exhibited a relatively organized tissue appearance, whereas the cyclophosphamide-treated group showed an irregular distribution of cellular regions and prominent pale areas. The HUL-treated groups (150 and 300 mg/kg) also displayed variations in cellular distribution and interstitial spacing. These observations indicate differences in the histological appearance of splenic tissue across the treatment groups. However, the extent of histological improvement following HUL treatment could not be conclusively established from the available microscopic images alone (Figure 5).



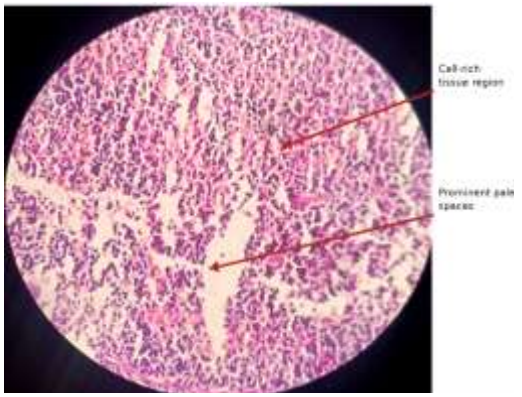
(a)



(b)



(c)

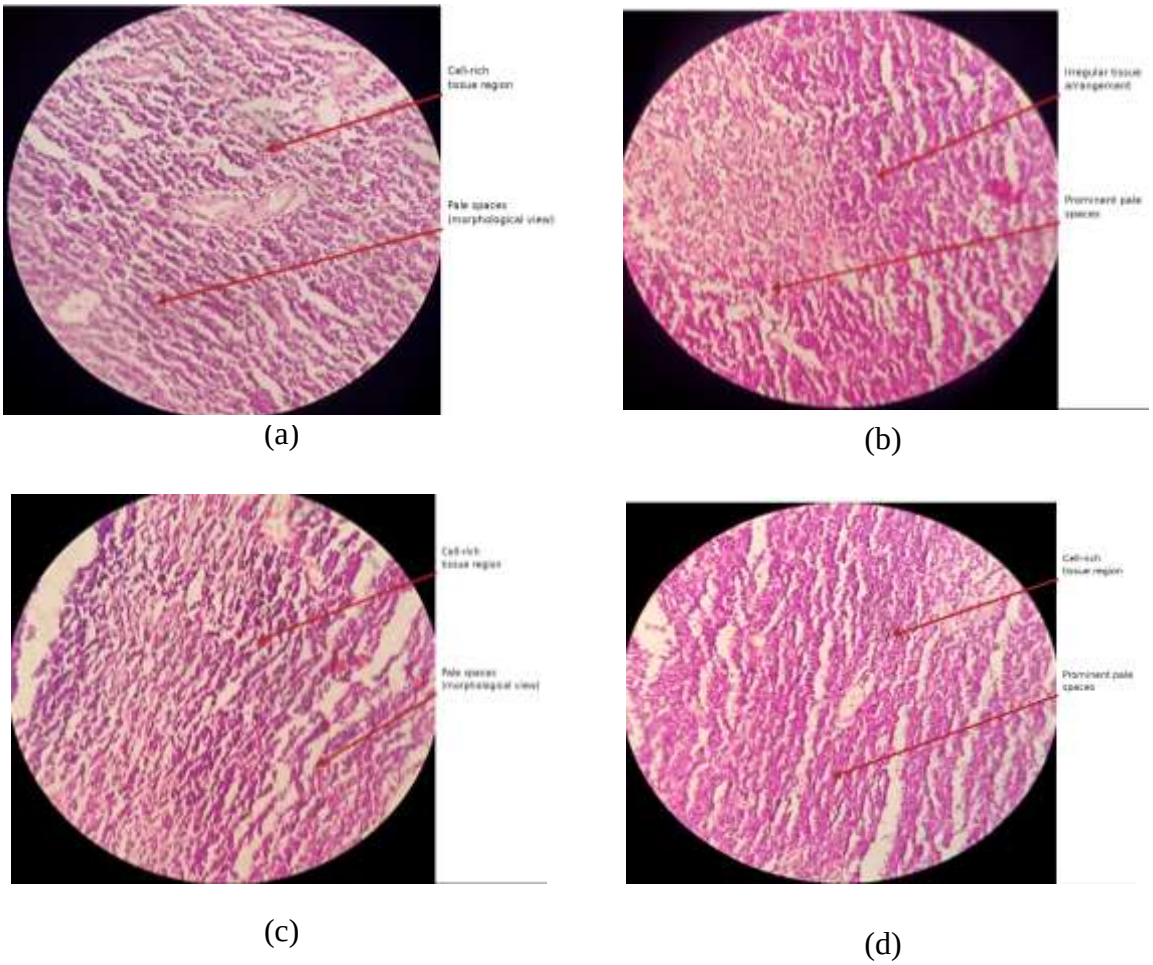


(d)

Figure 5: Effect of HUL on Histopathology of Spleen. Images were taken using Fluorescence microscope with 45X magnification. (a) Normal control (b) CP group (c) UL150 group (d) UL300 group; CP: Cyclophosphamide, UL150: HUL 150mg/kg, UL300: HUL 300mg/kg.

Effect of HUL on Histopathology of Thymus

Histopathological examination of the thymus revealed variations in tissue architecture among the experimental groups (Figure 10). The normal control group exhibited a relatively organized tissue appearance with a distribution of cell-rich regions and pale spaces. In comparison, the cyclophosphamide-treated group showed an irregular distribution of cellular regions and prominent pale areas. The HUL-treated groups (150 and 300 mg/kg) also displayed variations in cellular distribution and interstitial spacing. Although differences in tissue appearance were observed among the treatment groups, the extent of histological improvement following HUL treatment could not be conclusively established from the available microscopic images alone. (Figure 10).



(a) (b)

(c) (d)

Figure 10: Effect of Hydroethanolic extract of Ulva lactuca on Histopathology of Thymus. Images were taken using Fluorescence microscope with 45X magnification. (a) Normal control (b) CP group (c) UL150 group (d) UL300 group; CP: Cyclophosphamide, UL150: HUL 150mg/kg, UL300: HUL 300mg/kg.

DISCUSSION:

The current study assessed the immunomodulatory effect of hydroethanolic extract of *Ulva lactuca* (HUL) using the cyclophosphamide-induced immunosuppression model.

Cyclophosphamide is known to cause immunosuppression by decreasing the production of leukocytes, impairing the antibody response, and damaging the immune organs like the spleen and thymus. Hence, the normalization of these parameters suggests the immunoprotective effect of the test extract[2,13,15]. Phytochemical analysis has shown the presence of valuable bioactive compounds like flavonoids, phenolic compounds, saponins, alkaloids, and carbohydrates in *Ulva lactuca*. These phytochemicals have been shown to have antioxidant and immunomodulatory activities. Flavonoids and phenolic compounds have been shown to increase the activity of immune cells and protect the immune system from damage. These compounds are also known to have antioxidant properties.

In the current study, cyclophosphamide administration significantly decreased the WBC count, indicating the suppression of immune function. Administration of HUL significantly increased the WBC count, indicating the ability of the extract to protect the function of the bone marrow and stimulate the production of leukocytes.

Delayed-type hypersensitivity, a measure of cellular immunity, was also impacted by cyclophosphamide treatment. The use of HUL significantly enhanced the DTH reaction, indicating the restoration of T-cell-mediated immunity. This implies that the extract enhances cellular immune mechanisms[15].

Likewise, the suppression of humoral immunity by cyclophosphamide was significant, as evidenced by the lowered antibody titre. The treatment with HUL significantly elevated the production of antibodies, indicating its potential to stimulate humoral immunity.

Cyclophosphamide also induced a significant reduction in spleen and thymus indices, which indicated immune organ atrophy. However, the treatment with HUL significantly improved both spleen and thymus indices, which indicated protection and restoration of the immune organ structure and function. The histopathological observations also supported these findings, which indicated improved cellular arrangement and reduced tissue damage in the extract-treated groups, especially at the higher dose.

In general, the results of this study clearly show that the HUL was effective in restoring the immune system by increasing the number of leukocytes, the production of antibodies, and the protection of the immune organs from cyclophosphamide damage. This was more evident in the higher dose, which showed a dose-dependent effect. These findings clearly indicate that *Ulva lactuca* has great potential as an immunomodulatory agent.

CONCLUSION:

The results obtained from the current study clearly show that the hydroethanolic extract of *Ulva lactuca* has potent immunomodulatory properties. The immunomodulatory effect of the extract was evident as it significantly enhanced both cellular and humoral immunity in cyclophosphamide-induced immunosuppressed animals at doses of 150 mg/kg and 300 mg/kg. The extract also helped in the restoration of leukocyte counts, stimulated the production of antibodies, and protected the spleen and thymus from damage. The histopathological studies also confirmed the protective action of the extract on immune organs. The 300 mg/kg dose was more effective in bringing about improvement, which clearly indicates that the action is dose-dependent.

CONFLICT OF INTEREST:

None.

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