Sustainable Food Technology

Accepted Manuscript

This article can be cited before page numbers have been issued, to do this please use: Z. Wang, Y. Zhou, L. Chen, T. Chen, Y. Wang, S. Ding, Y. Du, J. Hu and D. Wu, *Sustainable Food Technol.*, 2025, DOI: 10.1039/D5FB00446B.

View Article Online
DOI: 10.1039/D5FB00446B

Sustainability Spotlight Statement

1. First identification of *Ulva lactuca* lectin allergenicity *via* cellular/murine models.
2. UL lectin triggers IgE (45 ng/mL) & IgG1 (2888 ng/mL) surge and splenomegaly in mice.
3. UL lectin was regarded as hidden algal allergen requiring food safety assessment.

View Article Online
DOI: 10.1039/D5FB00446B

Molecular Properties and Allergenicity of Lectin from *Ulva lactuca*

Zhicong Wang [a, b], *Yingying Zhou* [a, b], *Lihang Chen* [a, b], *Tao Chen* [a, b], *Yuying Wang* [a, b], *Shanshan Ding* [a,b], *Yinan Du* [a, b], *Jiangning Hu* [a, b] *, *Di Wu* [a, b] *

[a] SKL of Marine Food Processing & Safety Control, National Engineering Research Center of Seafood, Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian, Liaoning, 116034, China

[b] School of Food Science and Technology, Dalian Polytechnic University, Dalian, 116034, China

Corresponding author: Prof. Jiangning Hu, Dr. Di Wu, SKL of Marine Food Processing & Safety Control, National Engineering Research Center of Seafood, Collaborative Innovation Center of Seafood Deep Processing, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China, Tel: +86-411-86332275, Fax: +86-411-86323262, E-mail: hujiangning2005@hotmail.com, m13039998695@163.com

View Article Online
DOI: 10.1039/D5FB00446B

ABSTRACT

Ulva lactuca, the type species of the genus algae, holds ecological significance in coastal ecosystems and has attracted considerable interest in food and medical applications due to its bioactive constituents. Proteins or peptides from algae that exhibit high abundance, stability, or significant homology to specific allergens were identified as antigens by immune cells. In response, the immune system mounts a variable reaction to each protein, ultimately leading to cellular degranulation and the manifestation of a range of diseases. Lectin, derived and purified from *Ulva lactuca* (*U. lactuca*), displaying distinct bands at molecular weights of 107.55, 75, 67.6, 35, 32.02, 28.75, 23.51, 18.7, and 10.7 kDa. Subsequent bioinformatic alignment against the PDB database revealed significant homology between *U. lactuca* lectin and documented allergenic lectins. *In vitro* evaluation using rat basophilic leukemia mast cells (RBL-2H3) demonstrated that *U. lactuca* lectin significantly promoted mast cell degranulation, whereas histamine (His) release rate was reached at 45.28 ± 2.40 ng mL^{-1}. *In vivo* experiments revealed that *U. lactuca* lectin induced splenomegaly, and promoted substantial elevation of His and mast cell proteases in mice. Moreover, *U. lactuca* lectin samples exhibited significantly upregulated levels of allergen-specific Immunoglobulin E (IgE), Immunoglobulin G1 (IgG1) inducing a Th2 immune response. These findings provide foundational evidence for the allergenic potential of *U. lactuca* lectin and contribute to safety evaluations of marine-derived proteins.

Keywords: *U. lactuca* lectin, allergenicity, mouse model

View Article Online
DOI: 10.1039/D5FB00446B

1. Introduction

Green algae have been recognized as one of the most significant allergenic sources in aquatic products. *U. lactuca*, a macroalga widely distributed in intertidal zones from temperate to tropical regions globally, was extensively studied due to its rapid growth, high biomass, and rich content of proteins, polysaccharides, minerals, and bioactive compounds. In recent decades, it has been regarded as a promising functional food ingredient and sustainable bioresource.[1] In Asian countries, *U. lactuca* was traditionally consumed as food for centuries. Its applications were also explored in bioenergy production, pharmaceuticals, and cosmetics.[1] The presence of umami amino acids in *U. lactuca* was identified, which contributed to its distinct marine flavor. This characteristic enabled its utilization in developing unique seafood-flavored products.[2] Furthermore, polyphenolic compounds extracted from *U. lactuca* were demonstrated to exhibit antioxidant and antimicrobial properties. These bioactive substances were proposed as natural food additives for meat preservation.[2] However, with the increasing industrial-scale application of *U. lactuca* and its derivatives, their potential allergenic risks have become a critical concern for both academia and the food industry.

The lectin from *U. lactuca* was characterized as carbohydrate-binding proteins with biological functions in cell signaling and pathogen defense through specific recognition of glycoconjugates.[3] Lectins were first isolated from castor beans (*Ricinus communis*) in 1888, designated as Ricin.[4] The development of affinity chromatography in the 1970s significantly advanced lectin research. However, Concanavalin A, crystallized from jack beans (*Canavalia ensiformis*) in 1919, was recognized as the first lectin to be systematically studied by Sumner and Howell.[4,5] Immunomodulatory, antiviral, and antitumor activities of lectins were documented, though their interaction

View Article Online
DOI: 10.1039/D5FB00446B

mechanisms with mammalian immune systems remained incompletely elucidated.[6] Notably, specific lectins (e.g., peanut agglutinin, soybean lectin) were confirmed to trigger IgE-mediated allergies by binding to intestinal epithelial receptors, potentially inducing anaphylaxis.[7] Compared to known allergens in other algae, allergic reactions related to lectins were on the rise. In China, lectins accounted for 36.59% of plant food allergy cases in 2017, attributed to their high abundance (2.4-5% of total proteins) in edible plants.[8, 9] Gastrointestinal symptoms including nausea, vomiting, and abdominal distension were observed in both children and adults. Current research predominantly focused on legume lectins, while systematic risk assessment of *U. lactuca* lectin allergenic potential remained unexplored. Thus, comprehensive evaluation of *U. lactuca* lectin allergenicity was urgently required to ensure the safety of algal-based food products.

Food allergy has been acknowledged as a worldwide food safety issue, and its prevalence has significantly increased over the past two decades. IgE-mediated type I hypersensitivity was established as the primary mechanism. Following gastrointestinal processing of allergens, epitopes were recognized by antigen-presenting cells.[10] Th2-type immune responses were subsequently activated. This cascade induced the production of allergen-specific IgE antibodies by B cells.[11] These antibodies were bound to mast cells and basophils via FcεRI receptors. Re-exposure to homologous allergens triggered cellular degranulation. Inflammatory mediators including His were released. Multisystem symptoms ranging from mild pruritus to anaphylactic shock were observed.[12] Lectin proteins were recognized as potential hidden allergens due to their gastrointestinal stability and capacity to enhance antigen presentation. The allergenic potential of *U. lactuca* lectin was therefore considered critical for ensuring the safety of Ulva-derived food products.

Internationally recognized frameworks for assessing protein allergenicity had been established by regulatory bodies including the European Food Safety Authority, Codex Alimentarius Commission, and World Health Organization. These guidelines encompassed animal models, cellular immunological assays, serological analyses, and bioinformatics approaches.[13, 14] Previous research efforts were predominantly directed toward developing cellular and animal experimental systems.[15] In this study, lectin was isolated from *U. lactuca* using ammonium sulfate precipitation. RBL-2H3 cell model was established to evaluate His release following *U. lactuca* lectin stimulation. Subsequently, BALB/c mice were sensitized with *U. lactuca* lectin. Clinical allergy symptoms, serum-specific antibody levels, and cellular degranulation responses were systematically measured. The allergenic potential of *U. lactuca* lectin was comprehensively investigated through this multidimensional experimental strategy. These findings were intended to support the safe utilization of algal resources and contribute theoretical insights for identifying marine-derived allergens.

2. Materials and Methods

2.1. Materials and Chemicals

Ulva lactuca were purchased from Daqin Island, Changdao, Yantai, Shandong Province. BCA Protein Concentration Detection Kit, Sodium dodecyl sulfate, Tris, Toluidine blue stain solution and DEAE-Sepharose Fast Flow were procured from Solarbio Science & Technology Co., Ltd. (Beijing, China). Coomassie Brilliant Blue R-250, glycerol, glycine, acrylamide, N, N'-methylenediacrylamide, TEMED, Ammonium persulfate, bromophenol blue, β-Mercaptoethanol, MTT, cholera toxin

View Article Online
DOI: 10.1039/D5FB00446B

(CT) and albumin(OVA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). H_2SO_4, NaCl, CH_3COOH and NaOH were procured from Tianjin Damao Chemical Reagent Co., Ltd. (Tianjin, China). $(NH_4)_2SO_4$, HCl were bought from Tianjin Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Advanced DMEM and Trypsin-EDTA were supplied by Gibco (Waltham, MA, USA). FBS was purchased from Procell Co., Ltd. (Wuhan, China). Penicillin-Streptomycin Solution was procured from Neyotime Co., Ltd. (Shanghai, China). RBL-2H3 cells were purchased from the Cell Resource Center (Shanghai, China). ELISA kits of His , IgE, IgG1 and Mouse mast cell protease 1 (mMCP-1) were bought from Jianglai Co., Ltd. (Shanghai, China).

2.2. Extraction and Purification of *U. lactuca* Lectin

A mixer grinder equipment (Dade, DFY-1000C, China) was applied to produce *U. lactuca* powder. *U. lactuca* powder (1:15, w/v) was dissolved in 10 mM PBS with 0.15 mol L^{-1} NaCl (pH 7.0). The mixture was centrifuged at 9000 rpm for 20 min at 4 °C by hybrid refrigerated centrifuge (Techcomp, CAX-571, China), after extracted for 16 h at 4 °C. $(NH_4)_2SO_4$ with 20% saturation were added into the supernatant at 4 ℃, the mixture was stirred at magnetic stirrer (YUHUA, 98-2, China) overnight (4 ℃). After centrifugation at 11000 rpm for 20 min at 4 °C, the precipitate was discarded and the supernatant was brought to 60% saturation with $(NH_4)_2SO_4$ and stored for 12 h. The precipitation was dissolved in distilled water, and was dialyzed for 36 h against the same buffer to eliminate residual $(NH_4)_2SO_4$. Then, the solution was lyophilized and stored in a dryer, which was named as lectin (L). The target proteins were purified using ion exchange chromatography (GE, AKTA avant 25, China) in a DEAE-Sepharose Fast Flow, with a 39 mL column (Henghuibio, HC-0220-02A, China) with the eluting buffer of NaCl (0-1 mol L^{-1}) in the Tris-HCl (50 mM, pH 8.0).[16] Then, the penetrating peak

View Article Online
DOI: 10.1039/D5FB00446B

(A) and elution peak (B) of L were obtained. All purification procedures were conducted at 4 °C, with subsequent lectin aliquots stored at -20 °C pending downstream assays.

2.3. SDS and Native Polyacrylamide Gel Electrophoresis

Native-PAGE and SDS-PAGE analyses were performed using the following methods with some modifications.[17] In Native-PAGE, samples were prepared in a buffer containing 20% glycerol and 25 mM Tris-HCl (pH 8.0). And then, 10 μL of each sample was loaded onto Native-PAGE gels. In SDS-PAGE, samples were dissolved to 4 mg mL^{-1} with the sample buffer which contains 25 mM Tris-HCl (pH 8.0), SDS, glycerinum and β-mercaptoethanol. After boiling for 10 min, the samples (10 μL) were centrifuged and loaded onto a stacking gel (3%) and separating gel (12.5%). The electrophoresis was performed under constant flow conditions of 10 mA. After electrophoresis, the gel was fixed with a solution of 100 mM ammonium acetate dissolved in methyl alcohol: acetic acid (5:1, v/v) for 2 h. After fixing, gels were stained with Coomassie Brilliant Blue R-250 and destained with 10% acetic acid solution for 12 h, and washed by water. Imaging was performed using a GS-800 densitometer (DNR BioImaging Systems Ltd., Israel).[18]

2.4. RBL-2H3 Cell Model Experiment

2.4.1. RBL-2H3 Cell Viability Assay

The cell viability test was measured in accordance to MTT cytotoxicity assay utilizing RBL-2H3 cells (Shanghai cell bank of Chinese Academy of Sciences). Medium for cells culture was Minimum Essential Medium (Hyclone, Logan, UT, USA)

View Article Online
DOI: 10.1039/D5FB00446B

with addition of 1% penicillin-streptomycin (Hyclone, Logan, UT, USA) and 15% fetal bovine serum (PAN-Biotech, UK). Typically, RBL-2H3 cells (200 μL) were suspended in cell culture medium and seeded into 96-well plates at a density of 5×10^4 cells per mL, being incubated for 24 h at 37 °C in 5% CO_2 to obtain a monolayer of cells.[19] After 24 h of incubation, 200 μL of L, A and B in different concentrations (0, 50, 100, 200, 300, 400, 500, 700 and 1000 μg/mL) were added for 24 h of stimulation. The sample solution was removed after incubation and the cells were further cultured with of MTT (5 mg mL^{-1}, 15 μL) solution for 4 h. Finally, the culture medium was substituted with DMSO (150 μL) to dissolve the formazan and the absorbance of the DMSO solution at 490 nm was tested by a micro plate reader (Infinite™ M200, TECANE, Switzerland).

2.4.2. Degranulation and Morphological Observation of RBL-2H3 Cells

RBL-2H3 cells (1 mL) were suspended in cell culture medium and seeded into 12-well plates at a density of 5×10^4 cells mL^{-1}, being incubated for 24 h at 37 °C in 5% CO_2 to obtain a monolayer of cells.[19] After 24 h of incubation, 200 μL of A, B and lectin (300 μg mL^{-1}) were added for 24 h of stimulation. At the end of excitation, the supernatants were collected and centrifuged (1000 rpm, 20min). Then the supernatant was collected, and His content were determined using ELISA kits,[20] which were purchased from Jianglai Co., Ltd. (Shanghai, China). At the end of the excitation, the cells were observed under a microscope and photographed after staining with toluidine blue stain solution.

View Article Online
DOI: 10.1039/D5FB00446B

2.5. Allergenicity Assessment

2.5.1. Mice Allergy Model

Female BALB/c mice (three to four weeks) were purchased from Changsheng Biotechnology Co., Ltd (Benxi, Liaoning, China). All mice were housed in an air-conditioned room (22 ± 2 °C, with a 12 h/12 h dark/light cycle, 50 ± 15% relative humidity) with free access to water and sample-free chow. All animal experimentation was approved by the Ethics Committee of Dalian Polytechnic University.

2.5.2. Sensitization and Challenge

All BALB/c mice were distributed to 8 groups (n = 10 for each group) as follows: saline control group (Control), mice gavaged with CT (CT), mice gavaged with CT + OVA (OVA), mice gavaged with CT + high concentration of B (BH), mice gavaged with CT + low concentration of B (BL), mice gavaged with CT + high concentration of L (LH), mice gavaged with CT + low concentration of L (LL), and mice gavaged with CT + *U. lactuca* powder (UL). CT as an adjuvant can stimulate the immune system and effectively enhance the immune response of the antigen.[21] To mitigate potential confounding effects of CT on immune responses, a dedicated control group receiving CT-only gavage was established in this study. For the stage of basic sensitization, mice in each group were given different doses of corresponding samples by intragastric administration. OVA, BL, and LL groups were gavaged 200 μL sample with a concentration of 2 mg mL^{-1}. BH, LH, and UL groups were gavaged 200 μL sample with a concentration of 20 mg mL^{-1}. The mice were sensitized weekly by intraperitoneal injection with samples plus CT (5 μg) per mice, and other two groups were gavaged with saline and CT, respectively. For the stage of challenge, OVA and sample groups

View Article Online
DOI: 10.1039/D5FB00446B

mice were given 5 times the dose of OVA and samples respectively sacrificed on Day-36.[22] All the experimental design of animal were shown in **Figure 3 a**. In this study, all animal operations were conducted by following the National Institutes of Guide for the Care and Use of Laboratory Animals, and approved by Dalian Polytechnic University Ethical Committee for Animal Experimentation (approval number: DLPU2024077).

2.5.3. Serum Collection

Blood samples were collected by intraocular canthal vein puncture at 30, 33, 35 and 36 days. Serum samples were centrifuged at 4000 rpm for 20 min at 4 °C. All serum samples were kept at -80 °C until analysis

2.5.4. Analysis of Spleen Index

On day 36, the target organs (spleen) were removed and weighed. The spleen index was calculated according to the formula :[23]

$$\text{Spleen index} = \frac{Weight\ of\ spleen\ (g)}{Body\ weight\ (g)}$$

2.5.5. Measurement of Vascular Permeability

The levels of albumin in the peritoneal fluid of BALB/c mice were determined 40 min after challenge.[24] Mice were intraperitoneally injected with 1 mL of PBS. After 1 min of abdominal massage, the abdominal massage-derived fluids were processed through refrigerated centrifugation (4 °C, 500×g, 6 min). The supernatant was stored at -80 °C. Albumin concentration in the supernatant was quantified by a BCA kit. The BCA working fluid and standard solution were prepared according to the requirements of the kit. The sample or standard solution was mixed with the BCA working fluid. The sample or standard solution was placed at 37 °C for 30 min and cooled to room

temperature. The absorbance of each sample was measured at 562 nm. The protein concentration was calculated according to the standard curve.

2.5.6. ELISA Assays for Specific IgE, IgG1, His and mMCP-1 Levels

The serum from each mouse was collected, and IgE, IgG1, mMCP-1 and His concentrations were detected by ELISA assay using a Mouse IgE ELISA Kit, Mouse IgG_1 ELISA Kit, Mouse HIS ELISA Kit and mMCP-1 ELISA Kit according to the manufacturer's instructions. Absorbance was measured at 450 nm using a microplate reader, and serum concentrations were calculated based on standard curves.

2.5.7. Histopathology of Lung, Spleen, Jejunum and Duodenum

Resected lung, spleen, jejunum and duodenum tissues were removed after euthanasia and the jejunum was washed with normal saline. Then, the lung, spleen jejunum and duodenum tissues were fixed in 4% paraformaldehyde and embedded in paraffin. Serial paraffin sections with 4-6 μm thickness were made and prepared for staining of Hematoxylin and Eosin (H&E) to observe the histopathological.[25] To measure the mast cell degranulation, 5 spleen sections in each group were stained with toluidine blue (TB) and the number of intact and degranulated mast cells in each section was counted under a microscope in 5 randomly chosen fields.[26]

2.6. Statistical Analysis

The mean ± standard deviations (SD) of three sets of data from three independent experiments and technical replicates were calculated. Data were assessed using one-way ANOVA and the post hoc test HSD Tukey with the SPSS software (SPSS 16.0 Inc, Chicago, USA). The significance level was set at $p < 0.05$.

View Article Online
DOI: 10.1039/D5FB00446B

3. Results and Discussions

3.1 Preparation of *U. lactuca* Lectin

The *U. lactuca* L was extracted by salting out with NaCl and NH_4SO_4 and purified by DEAE-Sepharose Fast Flow chromatography. The subunits of L were composed at 250.46, 180, 141.65, 67.6, 35, 28.72, and 18.7 kDa in Native-PAGE (**Figure 1 b**). During the process of ion exchange chromatography, with the ascending of the concentration of NaCl, peaks were collected. One single elution peak B and A1, A2 were observed during the elution process (**Figure 1 a**) and subjected to SDS-PAGE. From the results of the SDS-PAGE (**Figure 1 b**), the molecular weights of B were at 107.55, 75, 67.6, 35, 32.02, 28.75, 23.51 and 18.7 kDa. Lectins are carbohydrate-binding proteins that recognize and selectively bind to specific sugar structures. Developments in bioinformatics reveal that the plant's proteome includes a range of putative amino acid sequences encoding lectins with diverse carbohydrate-recognition domains.[27] As reported by the molecular weight of lectins 1VIW (*Phaseolus vulgaris*), 7KDU (*Ricinus communis*) and 7KBI (*Ricinus communis*) were distributed at 74.87, 74.6 and 74.98 kDa (**Figure 1 c**).[3, 28, 29] Lectins 2WGC (*Triticum aestivum*), 4AML (*Triticum aestivum*), 1LOC (*Lathyrus ochrus*), 6VGF (*Arachis hypogaea*) and 5AVA (*Phaseolus vulgaris*) were distributed at 35.54, 35.66, 105.01, 106.08 and 251.14 kDa.[30-32] Molecular structures of these lectins, which had the similar molecular weight in *U. lactuca* L were downloaded from the Protein Data Bank (https://www.rcsb.org). Allergenic protein might be contained in *U. lactuca* L and purified lectin (B).

View Article Online
DOI: 10.1039/D5FB00446B

3.2 Experimental Allergenicity Assessment of RBL-2H3 cells

To explore the allergenicity of the *U. lactuca* L at the cellular level, we evaluated the effects of L, A and B on cell degranulation with RBL-2H3 cell model.[33] Selection of the appropriate drug concentration for subsequent experiments was first made by MTT experiments, the concentration of 300 μg/mL was identified as the optimal concentration for cell stimulation.[34] As shown in **Figure 2 e**, the His content of RBL-2H3 were determined. His release rate of RBL-2H3 cells was significantly increased after induction by B and L, reaching at 42.40 ± 1.47 ng mL^{-1} and 45.28 ± 2.40 ng mL^{-1}, respectively. Mast cells and basophils were activated through allergen stimulation, triggering exocytotic release of granular contents. His was identified as a key biomarker of cellular degranulation. Therefore, His levels in RBL-2H3 cell culture supernatants were quantified.[35] The results show that B and L were more sensitizing. This result can also be seen in the cellular morphology of the **Figure 2 e-h**. Effects of control (**Figure 2 e**), lectin (**Figure 2 f**), A (**Figure 2 g**), and B (**Figure 2 h**) on RBL-2H3 cells were observed and photographed by microscope. After being stimulated for 24 hours, compared with the control group, the cell morphology changed and became more rounded in lectin (**Figure 2 f**) and B (**Figure 2 h)**, whereas there was no significant difference between control and A (**Figure 2 g**).

3.3 Changes in Splenic Index in BALB/c Mice

The experimental protocol for mice was shown in the **Figure 3 a**. During this study, all groups of mice were kept under the same conditions and allowed to drink and eat freely. The hair of all mice remained shiny without shedding, no obvious fighting or biting behavior was observed, and the general condition was good. Furthermore, there

were no deaths or significant toxicological symptoms throughout the whole experiment. Spleen is the largest and most important immune tissue in the body, and after antigenic stimulation causes an immune response in which macrophages and lymphocytes in the spleen phagocytose the exogenous material, which in turn leads to swelling of the splenic tissue.[36] As shown in **Figure 3 b**, mice treated with OVA, BL, LL, and LH showed a larger size of spleen than control. During allergic responses, the immune system was activated, and the spleen, as a critical immune organ, was observed to exhibit substantial proliferation of immune cells including T lymphocytes, B lymphocytes, and dendritic cells. Concurrently, a variety of inflammatory cytokines such as interleukin-4 and interleukin-12 were induced. These mediators were found to not only participate in immune regulation but also contribute to structural and functional alterations in splenic tissues. Subsequent cellular activation and hyperplasia were triggered in the spleen, ultimately resulting in a measurable increase in splenic volume.[37] As demonstrated in the **Figure 3 c**, the spleen indices in OVA, BL, BH, LL, LH, and UL groups were significantly higher than control and CT groups ($p < 0.05$), whereas the LH group exhibiting the highest index of $0.74 \pm 0.05\%$. A notable increase in splenic index was also detected in the CT group relative to control group ($p < 0.05$), which might be attributed to CT-induced inflammatory responses leading to structural alterations in splenic architecture.[38] This result suggested that lectins (L and B) in *U. lactuca* induce significant increase in splenic index in mice, causing swelling of immune tissues. Comparative analysis of images of the spleen revealed significant splenomegaly in experimental groups relative to the control and CT groups. This morphological deviation was pathologically associated with hypersensitivity manifestations, providing histopathological confirmation of allergic reactivity in the sample-administered groups.

View Article Online
DOI: 10.1039/D5FB00446B

3.4 Changes in Vascular Permeability in BALB/c Mice

When an allergic reaction occurs, mast cells and basophils release mediators such as His, which can lead to inflammatory responses such as vasodilation and increased permeability.[39] Increased vascular permeability is one of the hallmarks of inflammation, and measuring its changes can reflect the severity of inflammation and help determine the intensity of an allergic reaction.[40] Therefore, vascular permeability in mice was quantified by determining the level of peritoneal albumin in each group of mice. As shown in **Figure 3 d**, highest intra-abdominal albumin concentrations in peritoneal fluid were detected in both OVA (2.21 ± 0.15 mg mL^{-1}) and UL (2.04 ± 0.29 mg mL^{-1}) ($p < 0.05$). There was no significant difference in abdominal albumin content between BL, BH, LH and CT. The results of the vascular permeability experiments described above indicated that there was a significant vascular leakage response in L-treated and B-treated mice, causing them to develop an allergic reaction.

3.5 Analysis of Specific Antibody Levels in the Serum of BALB/c Mice

Most food allergic reactions were mediated by IgE,[41] so an increase in allergen-specific IgE is a distinctive feature of the occurrence of allergic reactions. It is typical for serum antibody levels to be elevated in the presence of food allergy.[42] As shown in **Figure 4**, mice in the control group did not exhibit allergic reactions. The levels of various specific antibodies in the mouse serum were then detected. Serum levels of specific IgE antibodies in mice were shown in **Figure 4 a**, serum-specific IgE antibody level in the adjuvant (CT) group was maintained at a lower concentration of 21.65 ± 3.58 ng mL^{-1}, indicating that CT adjuvant was specifically employed to enhance the immune response to allergens without inducing allergic responses itself. Compared

View Article Online
DOI: 10.1039/D5FB00446B

with CT groups, serum-specific IgE levels ($p < 0.05$) were significantly elevated in BL (33.88 ± 5.38 ng mL^{-1}), LL (32.59 ± 6.23 ng mL^{-1}), LH (31.24 ± 2.65 ng mL^{-1}), and UL (45.02 ± 7.43 ng mL^{-1}) groups ($p < 0.05$). The results showed that UL, LL and LH induced B cells to produce specific IgE antibodies. Serum-specific IgG1 antibodies can also induce an allergic reaction in the organism.[43] The serum-specific IgG1 antibody levels in each group of mice were presented in the **Figure 4 b**, compared with control and CT groups, LH group showed the highest concentration (2888.82 ± 549.43 ng mL^{-1}) in serum-specific IgG1 antibody levels. The serum specific IgE and IgG1 antibody levels in mice revealed that *U. lactuca* powder and lectins in *U. lactuca* triggered a Th2 type of immune response and sensitized the mice.[44]

3.6 Changes in Serum His and mMCP-1 levels in BALB/c Mice

His was a key allergic mediator released during mast cell degranulation, and changes in its levels also reflect the degree of sensitization of the organism.[41] mMCP-1 was stored and secreted by intestinal mucosal mast cells, which was predominantly expressed in mucosal mast cells.[41] Researchers studied mast cell degranulation also causes elevated levels of mMCP-1 and increased permeability of intestinal epithelial cells, leading to food allergic diseases.[45] As demonstrated in **Figure 4 c-d**, there were no significant differences between control and CT groups in serum His and serum mMCP-1 levels. Compared with the BALB/c mice challenged with OVA (100.18 ± 13.32 ng mL^{-1}), there were no significant differences in BH (84.72 ± 12.54 ng mL^{-1}), LH (98.55 ± 11.59 ng mL^{-1}) and UL (95.20 ± 8.90 ng mL^{-1}) groups ($p > 0.05$) in His. Compared with control and CT groups, mMCP-1 concentrations were significantly elevated in the OVA, BH, LL, LH, and UL groups, with the highest level (6.77 ± 2.32 ng mL^{-1}) being recorded in the BH group ($p < 0.05$). The above results indicate that

lectins in *U. lactuca* induced an allergic reaction in mice, resulting in the rupture of mast cells in the spleen and the release of large amounts of inflammatory mediators. His and mMCP-1, which were transported to various tissues and organs through the bloodstream, causing severe allergic reactions in mice. As a result, His and mMCP-1 were detected in large quantities in the serum of *U. lactuca* lectins-sensitized mice, and their levels were significantly higher than those of non-sensitized mice (control).

3.7 Histopathological Changes in BALB/c Mice

A systematic histopathological evaluation of multiple organ systems was conducted to comparatively assess the pathophysiological effects induced by differential dietary allergen exposure in murine models. Histopathological evaluation examined by H&E staining of jejunal sections across experimental groups (**Figure 5 a**) revealed preserved intestinal architecture in control and CT groups, characterized by intact mucosal integrity, well-organized villous alignment, and maintenance of crypt-villus axis proportions. As shown in **Figure 5 a**, distinct pathological alterations were observed in the OVA, BL, BH, LL, LH, and UL experimental cohorts, where jejunal villus architecture exhibited marked disruption, including villous fragmentation and goblet cell hyperplasia. As shown in **Figure 5 b**, the intestinal villi of duodenum sections in OVA group and sample groups were damaged and broken, and the intestinal barrier was damaged. In contrast, well-organized alignment of intestinal villi with preserved structural integrity was observed in both control and CT groups. The duodenal architecture and histological features were maintained without detectable pathological damage or necrotic changes. Consistent histopathological alterations in the gastrointestinal tract are commonly observed in conjunction with IgE-mediated food hypersensitivity reactions.[46, 47] This leads to the conclusion that lectins in *U. lactuca*

View Article Online
DOI: 10.1039/D5FB00446B

caused an allergic reaction in the intestine. Normal alveolar septal architecture without significant histopathological alterations was observed in pulmonary tissues of control and CT groups. In contrast, OVA-sensitized mice exhibited marked bronchial epithelial hyperplasia and alveolar septal thickening. Although less pronounced than in the OVA group, similar pathological features were detected in BL, BH, LL, LH, and UL groups (**Figure S1**). This study revealed that alveolar septal thickening might contribute to airway obstruction, potentially exacerbating allergic responses in murine models.[48]

3.8 Changes in the spleen of BALB/c mice

Splenic tissues from allergic mice demonstrated marked white pulp hyperplasia, accompanied by dilated and congested red pulp with extensive immune cell infiltration, widened marginal zones containing densely packed lymphocytes, and vascular dilation with blood stasis compared to normal controls.[49] As shown in **Figure 6 a**, splenic architecture was examined using H&E staining. Well-demarcated white pulp compartments with preserved periarteriolar lymphoid sheaths were observed in both control and CT groups, accompanied by loosely arranged red pulp exhibiting intact sinusoidal networks. Distinct demarcation between the marginal zone and red pulp was maintained across all specimens. Distinct white pulp hyperplasia with compromised interface integrity between white and red pulp compartments was observed in OVA-sensitized, BL, BH, LL, LH, and UL groups. The experimental findings demonstrated that dietary lectins exposure was associated with hypersensitivity reactions in murine models, which subsequently triggered immune activation characterized by augmented lymphoproliferative responses.

View Article Online
DOI: 10.1039/D5FB00446B

Mast cells, which are ubiquitously distributed throughout somatic tissues, were demonstrated to play pivotal roles in allergic pathogenesis.[50,51] Allergic mice exhibited a significantly increased splenic mast cell population with prominent degranulation, whereas normal controls showed sparse distribution of morphologically intact mast cells retaining cytoplasmic granules. As illustrated in **Figure 6 b**, TB staining was systematically performed on splenic tissue sections to evaluate morphological characteristics and degranulation activity of mast cells within this primary immune organ. Mast cells infiltrations were significant observed in splenic tissues of OVA and experimental groups, with evident membrane disintegration and perivascular dispersion of cytoplasmic granules, indicative of advanced degranulation processes. Elevated mast cell populations were observed in the BL, BH, and UL groups compared to the LL and LH groups, with concurrent degranulation activity detected. In contrast, minimal mast cell presence was detected in control and CT groups. In addition, the morphometric analysis of degranulated mast cells in the spleen was presented in **Figure S2**. The Control and CT groups showed a relatively low level of mast cells degranulation, with an average of approximately 6.67% and 11.11%. Compared to mice challenged with normal saline and CT, mice stimulated with OVA, B and L showed a notable increase in the percentage of degranulated mast cells ($p < 0.05$), with an average exceeding 60.71%. The results showed that B and L could induce the degranulation of mast cells in the spleen of BALB/c mice. Thus, the *U. lactuca* lectin has potential high allergenic.

4. Conclusions

Within the broader context of simultaneous social development and escalating allergic disease burdens, pronounced limitations and asymmetries were recognized in

View Article Online
DOI: 10.1039/D5FB00446B

existing allergen recognition frameworks. Researches have long been predominantly confined to terrestrial plant-derived allergens (e.g., peanuts and soybeans), whereas insufficient attention has been paid to potential allergens present in marine flora, particularly in macroalgae species that are utilized as food or food additives. The allergenic potential of these components, which may trigger human immune responses, has not been thoroughly investigated, and related research remained in its infancy, constituting a critical knowledge gap urgently requiring further investigation. Therefore, the allergenic potential of lectin, a significant allergen from *U. lactuca* (a common edible macroalga), was investigated and was considered essential for a comprehensive understanding of seaweed allergy. Previous researches have confirmed that lectins in the diet could interfere with nutrient digestion and absorption, and high dietary levels of lectins could seriously threaten the growth and health of consuming animals. Compelling evidence was provided in this study demonstrating that the lectin purified from the green alga (*U. lactuca*) represents a novel marine-derived allergen with significant functional properties. Systematic investigation revealed that its immunomodulatory characteristics underlie the allergenic potential. These findings offered critical insights for fundamental research in allergology and the applied utilization of marine resources.

This study systematically investigated the allergenic potential of *U. lactuca* lectin, establishing it as a novel marine-derived allergen with distinct immunomodulatory properties. Experimental validation demonstrated that highly purified *U. lactuca* lectin significantly induced mast cell degranulation and His release *in vitro*, mechanistically linked to the activation of Th2-polarized immune responses. *U. lactuca* lectin elevated serum levels of allergen-specific IgE and IgG1 antibodies, His, and mMCP-1 in

BALB/c mice. These findings revealed that *U. lactuca* lectin induced type I hypersensitivity through Th2-biased immune activation and mast cell degranulation. This study emphasized the necessary to evaluate allergenic risks during the development of marine-algae resources. The study provided critical experimental evidence for safety assessments of *U. lactuca* in food and pharmaceutical applications, while established a method for identifying and characterizing marine-lectin allergens. Future research should focus on developing desensitization strategies to enable the safe utilization of *U. lactuca* lectin in food research development.

Conflicts of interest

There are no conflicts of interest to declare.

ACKNOWLEDGEMENTS

This study was financially supported by the National Key Research and Development Program of China (2023YFF1104001), the National Natural Science Foundation of China (32302004), Youth Science and Technology Star Program of Dalian (2023RQ092), and the Special Fund of Basic Scientific Research Expenses of Undergraduate Universities in Liaoning Province (LJ212510152042).

Abbreviations

His: histamine

IgE: immunoglobulin E

IgG1: immunoglobulin G1

View Article Online
DOI: 10.1039/D5FB00446B

RBL-2H3: rat basophilic leukemia mast cells

CT: cholera toxin

OVA: albumin

mMCP-1: mouse mast cell protease 1

L: lectin

A: target protein A, the penetrating peak of ion exchange chromatography in Figure 1a

B: target protein B, the elution peak of ion exchange chromatography in Figure 1a

BL: low concentration of B

BH: high concentration of B

LL: low concentration of L

LH: high concentration of L

UL: *Ulva lactuca* powder

H&E: hematoxylin and Eosin

TB: toluidine blue

5. REFERENCES

[1] Lahaye, M., Robic, A. Structure and functional properties of ulvan, a polysaccharide from green seaweeds[J]. *Biomacromolecules*, 2007, 8(6), 1765-1774.

[2] Qi, H. M., Sun, et al. Antioxidant activity of high sulfate content derivative of ulvan in hyperlipidemic rats[J]. *International Journal of Biological Macromolecules: Structure, Function and Interactions*, 2015, 7(6), 326-329.

[3] Elumalai, P., Rubeena, A. S., Arockiaraj, J., et al.The Role of Lectins in Pinfish: A Review[J]. *Reviews in Fisheries Science & Aquaculture*, 2019, 27(2), 152-169.

[4] Tsaneva M, Damme, E. J. M. V. 130 years of Plant Lectin Research[J]. *Glycoconjugate Journal*, 2020, 37, 1594074.

[5] Damme, E. V. V. Purification of GNA-Related Lectins from Natural Sources[J]. *Methods in Molecular Biology*, 2020, 2132, 413-419.

[6] Hopper, Jonathan, T. S., Stephen, et al. The Tetrameric Plant Lectin BanLec Neutralizes HIV through Bidentate Binding to Specific Viral Glycans[J]. 2017, 5(25), 773-782.

[7] Geijtenbeek, T. B. H., Gringhuis, S. I. C-type lectin receptors in the control of T helper cell differentiation[J]. *Nature Reviews Immunology*, 2016, 16, 433--448.

[8] Damme, E. J. M. V., Peumans, W, J., Barre, A, et al. Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles[J]. *Critical Reviews in Plant Sciences*, 1998, 17(6), 575-692.

[9] Van, Damme, E. J. M., Lannoo, N., Peumans, W. J. Plant lectins [M]. *Advances in botanical research.* Academic Press, 2008, 48, 107-209.

[10] Sampson, H, A., O'Mahony, L., Burks, A. W., et al. Mechanisms of food allergy[J]. *The Journal of Allergy and Clinical Immunology*, 2018, 141(1), 11-19.

[11] Gowthaman, U., Chen, J. S., Zhang, B., et al. Identification of a T follicular helper cell subset that drives anaphylactic IgE[J]. *Science*, 2019, 365(6456), eaaw6433.

[12] Sicherer, S. H., Sampson, H. A. Food Allergy: A review and update on epidemiology, pathogenesis, diagnosis, prevention and management[J]. *Journal of Allergy and Clinical Immunology*, 2017, 141(1), 41-58.

[13] Herman, R. A., Ladics, G. S. Allergenic sensitization versus elicitation risk criteria for novel food proteins[J]. *Regulatory Toxicology & Pharmacology*, 2018, 94, 283-285.

[14] Kedar, O., Golberg, A., Obolski, U., Confino-Cohen, R. Allergic to bureaucracy? Regulatory allergenicity assessments of novel food: Motivations, challenges, compromises, and possibilities[J]. *Comprehensive Reviews in Food Science and Food Safety*, 2024, 23, e13300.

[15] Han, K., Xie, F., Phoo, M. T., et al. Inulin-gel-based oral immunotherapy remodels the small intestinal microbiome and suppresses food allergy[J]. *Nature Materials,* 2024, 23(10), 30.

[16] Li, H., Yuan, D., Wang, C., Wang, Y., Zhang, J., Li, Z., et al. Zwitterionic peptide pendant as a homing agent to achieve lymphatic targeting antigen-specific immunotherapy of allergenic protein[J]. *Chemical Engineering Journal*, 2024, 485, 149315.

[17] Zang, J. C., Chen, H., Zhang, X. R., Zhang, C. X., Guo, J., Du, M., Zhao G. H. Disulfide-mediated conversion of 8-mer bowl-like protein architect

ure into three different nanocages[J]. *Nature Communications*, 2019, 10(1), 778.

[18] Li, Y. J., Wang, Z. C., Liu, H. X., Chen, T., Du, Y. N., Hu, J. N., Wu, D. Controlled ionic-induced self-assembly of phycocyanin: Structure, binding mechanism, and molecular dynamics simulation[J]. *Food Chemistry*, 2025, 477, 143603, 1-9.

[19] Liu, Y., Lin, S., Liu, K., Wang, S., Li, W., & Sun, N. Insights into sensitizing and eliciting capacity of gastric and gastrointestinal digestion products of shrimp (*penaeus vannamei*) proteins in balb/c mice[J]. *Food Science and Human Wellness*, 2024, 13(1), 10.

[20] Wang, Y., Zhang, K., Chen, W. M., Mao, J. H., Shao, Y. H., & Tu, Z. C., et al. Allergenicity assessment of β-lactoglobulin ferulic acid-glucose conjugates[J]. *Food Chemistry*, 2024, 460, 140605.

[21] Scott, C. L., Bain, C. C., Wright, P. B., Sichien, D., Kotarsky, K., Persson, E. K., Luda, K., Guilliams, M., Lambrecht, B. N., Agace, W. W., Milling, S. WF., & Mowat, A. M. Ccr2(+)cd103(-) intestinal dendritic cells develop from dc-committed precursors and induce interleukin-17 production by t cells[J]. *Mucosal Immunology*, 2015, 8, 327-339.

[22] Liu, Y., Lin, S. Y., Liu, K. X., Wang, S., Liu, Q. Z., Sun., N., Exploration of digestion-resistant immunodominant epitopes in shrimp (*Penaeus vannamei*) allergens[J]. *Food Chemistry*, 2024, 438, 137920, 1-14.

[23] Xue, L., Li, Y., Li, T., Pan, H., & Wang, L. Phosphorylation and enzymatic hydrolysis with alcalase and papain effectively reduce allergic reactions to gliadins in normal mice[J]. *Journal of Agricultural and Food Chemistry*, 2019, 67(22), 6313-6323.

[24] Jiang, T., He, F., Han, S., et al. Characterization of cAMP as an anti-allergic functional factor in Chinese jujube (*Ziziphus jujuba Mill.*)[J]. *Journal of Functional Foods*, 2019, 60, 103414.

[25] Yu, B., Bi, D., Yao, L., Li, T., Gu, L., Xu, H., et al. The inhibitory activity of alginate against allergic reactions in an ovalbumin-induced mouse model[J]. *Food & Function*, 2020, 11, 2704-2713.

[26] Luo, C., Chen, G., Ahmed, I., Sun, L., Li, W., & Pavase, T. R., et al. Immunostimulatory and allergenic properties of emulsified and non-emulsified digestion products of parvalbumin (*scophthalmus maximus*) in RBL-2H3 cells and balb/c mouse models[J]. *Food & Function*, 2021, 12, 5351-5360.

[27] Naithani, S., Komath, S. S., Nonomura, A., et al. Plant lectins and their many roles: Carbohydrate-binding and beyond[J]. *Journal of Plant Physiology*, 2021, 266, 153531.

[28] Bourne, Y., Ayouba, A., Rougé, P., Cambillau, C. Interaction of a legume lectin with two components of the bacterial cell wall. a crystallographic study[J]. *Journal of Biological Chemistry*, 1994, 269(13), 9429.

563 [29] Christine, S. W. 2·2 Å resolution structure analysis of two refined N-acet
564 ylneuraminyl-lactose-Wheat germ agglutinin isolectin complexes[J]. *Journal*
565 *of Molecular Biology*, 1990, 4(215), 635-651

566 [30] Cagnoni, A. J., Primo, E. D., Klinke, S., Cano, M. E., Giordano, W., Ma
567 riño, K. V., Kovensky, J., Goldbaum, F. A., Uhrig, M. L., Otero, L. H. C
568 rystal structures of peanut lectin in the presence of synthetic β-N- and β-S
569 -galactosides disclose evidence for the recognition of different glycomimeti
570 c ligands[J]. *Acta Crystallographica Section D*, 2020, 76, 1080-1091

571 [31] David, S., Caroline, M., Johannes, G. B., Sonja, S., Kay, D., Heiko, M.
572 M., Wolfram, W., Valentin, W. Structural Basis of Multivalent Binding to
573 Wheat Germ Agglutinin[J]. *Journal of the American Chemical*, 2010, 132,
574 25

575 [32] Michael, J. R., Amanda, Y. P., Simona, K., Anne, G. M., Claire, R. P.,
576 Simon, A. D., Kirsten, S., David, J. V., Nicholas, J. M. Structural Analysi
577 s of Toxin-Neutralizing, Single-Domain Antibodies that Bridge Ricin's A-B
578 Subunit Interface[J]. *Journal of Molecular Biology*, 2021, 15(433) 167086

579 [33] Hung, C. F. Anti-allergic and anti-inflammatory effects of neferine on RB
580 L-2H3 cells[J]. *International Journal of Molecular Sciences*, 2021, 22(20),
581 10994.

582 [34] Yang, M., Xu, Z., Wu, D., Dong, Y., Wang, Z. Y., Du, M. Characterizati
583 ons and the Mechanism Underlying Osteogenic Activity of Peptides from
584 Enzymatic Hydrolysates of Stichopus japonicus[J]. *Journal of Agricultural*
585 *and Food Chemistry*, 2021, 69(51), 15611-15623.

586 [35] Sahid, M., Kiyoi, T. Mast Cell Activation Markers for in Vitro Study[J].
587 *Journal of Immunoassay & Immunochemistry*, 2020, 41(4): 778-816.

588 [36] Morafo, V., Srivastava, K., Huang, C. K., Kleiner, G., Lee, S. Y., Samps
589 on, H. A., et al. Genetic susceptibility to food allergy is linked to differen
590 tial th2-th1 responses in c3h/hej and balb/c mice[J]. *Journal of Allergy an*
591 *d Clinical Immunology*, 2003, 111(5), 1122-1128.

592 [37] Lewis, M. S.,Williams, A., Eisenbarth, C. S. Structure and function of the
593 immune system in the spleen[J]. *Science Immunology*, 2019, 4(33), eaau6
594 085.

595 [38] Benedetti, R., Lev, P., Massouh, E., et al. Oral administration of one dose
596 of cholera toxin induces a systemic immune response prior to a mucosal
597 immune response by a direct presentation in the spleen[J]. *Immunology Let*
598 *ters*, 1998, 60(2-3), 149-156.

599 [39] Sun N, Wang J, Zhou C, et al. Cell-based immunological assay: complem
600 entary applications in evaluating the allergenicity of foods with FAO/WHO
601 guidelines[J]. *Food Research International*, 2014, 62, 735-745.

602 [40] Medzhitov, R. Origin and physiological roles of inflammation[J]. *Nature*, 2
603 008, 454(7203), 428-435.

604 [41] Bai, J., Hui, J., Lu, Q., Yang, A., Yuan, J., & Gao, J., et al. Effect of tr
605 ansglutaminase cross-linking on the allergenicity of tofu based on a balb/c
606 mouse model[J]. *Food & Function*, 2020, 11, 1-10.

[42] Liu, K., Lin, S., Liu, Y., Wang, S., Liu, Q., Sun, K., Sun, N. Mechanism of the Reduced Allergenicity of Shrimp (*Macrobrachium Nipponense*) by Combined Thermal/Pressure Processing: Insight into Variations in Protein S tructure, Gastrointestinal Digestion and Immunodominant Linear Epitopes[J]. *Food Chemistry*,2023, 405, 134829.

[43] Zhang, T. T., Hu, Z. Y., Cheng, Y. W., Xu, H. X., Velickovic, T. C., H e, K., Sun, F., He, Z. D., Liu, Z. G., Wu, X. L. Changes in allergenicity of ovalbumin in vitro and in vivo on conjugation with quercetin[J]. *Jour nal of Agricultural and Food Chemistry*, 2020, 13(68), 4027−4035.

[44] Kanagaratham, C., Ansari, Y. S. E., Lewis, O. L., et al. IgE and IgG Ant ibodies as Regulators of Mast Cell and Basophil Functions in Food Allerg y[J]. *Frontiers in Immunology*, 2020, 11, 603050.

[45] Rupa, P., & Mine, Y., Comparison of glycated ovalbumin–monosaccharide s in the attenuation of ovalbumin-induced allergic response in a balb/c mo use model[J]. *Journal of Agricultural and Food Chemistry*, 2019, 29(67), 1 -11.

[46] Roth-Walter, F., Berin M. C., et al. Pasteurization of milk proteins promot es allergic sensitization by enhancing uptake through Peyer's patches[J]. *All ergy*, 2008, 63(7), 882-890.

[47] He, S., Zhao, J., Zhang, Y., et al. Effects of Low-pH Treatment on the Allergenicity Reduction of Black Turtle Bean (*Phaseolus vulgaris L.*) Lecti n and Its Mechanism[J]. *Journal of Agricultural and Food Chemistry*, 202 1, 69(4), 1379−1390.

[48] Kim, D. E., Lee, Y., Kim, M. G., et al. Bilirubin nanoparticles ameliorate allergic lung inflammation in a mouse model of asthma[J]. *Biomaterials*, 2017, 140, 37-44.

[49] Rajaura, S., Babu, R., Bhardwaj, N., et al. Aflatoxin B1 administration ca uses inflammation and apoptosis in the lungs and spleen[J]. *Toxicon*, 2024, 238(000), 7.

[50] Kumar, S., Sharma, A., Gupta, R., et al. Allergenicity assessment of Buch anania lanzan protein extract in BALB/c mice[J]. *International Immunophar macology*, 2018, 63, 170-182.

[51] C. H. O. Mast cells in food allergy: Inducing immediate reactions and sh aping long-term immunity[J]. *The Journal of Allergy and Clinical Immunol ogy*, 2022, 151(1), 21-25.

View Article Online
DOI: 10.1039/D5FB00446B

Figure Captions

Figure 1. Elution of DEAE-Sepharose Fast Flow chromatography of *Ulva lactuca* lectin and electrophoresis of purified lectin. (a) Elution of DEAE-Sepharose Fast Flow chromatography of *Ulva lactuca* lectin. Crude protein sample was subjected to an anion exchange column pre-equilibrated with 50 mM Tris-HCl buffer (pH 8.0). The column was eluted with a linear gradient of NaCl (0-1.0 M) in the Tris-HCl at a flow rate of 2.0 mL/min. The volume of each fraction was 5 mL. Protein was monitored by measuring absorbance at 280 nm. (b) Native-PAGE and SDS-PAGE of lectin and purified lectin. (c) Overview of lectins from Protein Data Bank.

Figure 2. Proliferation and histamine content on RBL-2H3 cells treated by lectin. Lectin (a), A (b), and B (c) toxicity for 24 on RBL-2H3 cells. (d) Histamine release rate of RBL-2H3 cells induced by lectin. Effects of control (e), lectin (f), A (g), and B (h) on RBL-2H3 cells were observed and photographed by microscope.

Figure 3. Protocol of food allergic model and hypersensitive reaction. (a) Illustration of mice allergenicity scheme. Images of the spleen (b), splenic index (c) and intra-abdominal albumin concentration in different groups.

Figure 4. Anaphylaxis in the BALB/c mice sensitized and challenged in different groups. Levels of IgE (a), IgG1 (b), histamine (c), and mMCP-1 (d) in serum from mice. The concentration of IgE, IgG1, histamine, and mMCPT-1 in each BALB/c mice was determined by a commercial ELISA kit (n = 5, data presented as mean ± SD, different

View Article Online
DOI: 10.1039/D5FB00446B

663 letters above the bars represented significant differences, with a significance level of
664 0.05).

665 **Figure 5.** Histopathology alterations of the jejunal (a) and duodenum (b) sections of
666 the BALB/c mice administrated with different groups. Images were taken by a
667 microscope at 20× magnification.

668 **Figure 6.** Hematoxylin-eosin (H&E, a) and toluidine blue (TB, b) staining of splenic.
669 Images were taken by a microscope at 20× magnification. In the H&E staining, the
670 white pulp with a clear boundary from the red pulp were indicated by white arrows in
671 (a). In the TB staining,the white arrows denoted intact mast cells and the red arrows
672 denoted degranulated mast cells in (b).

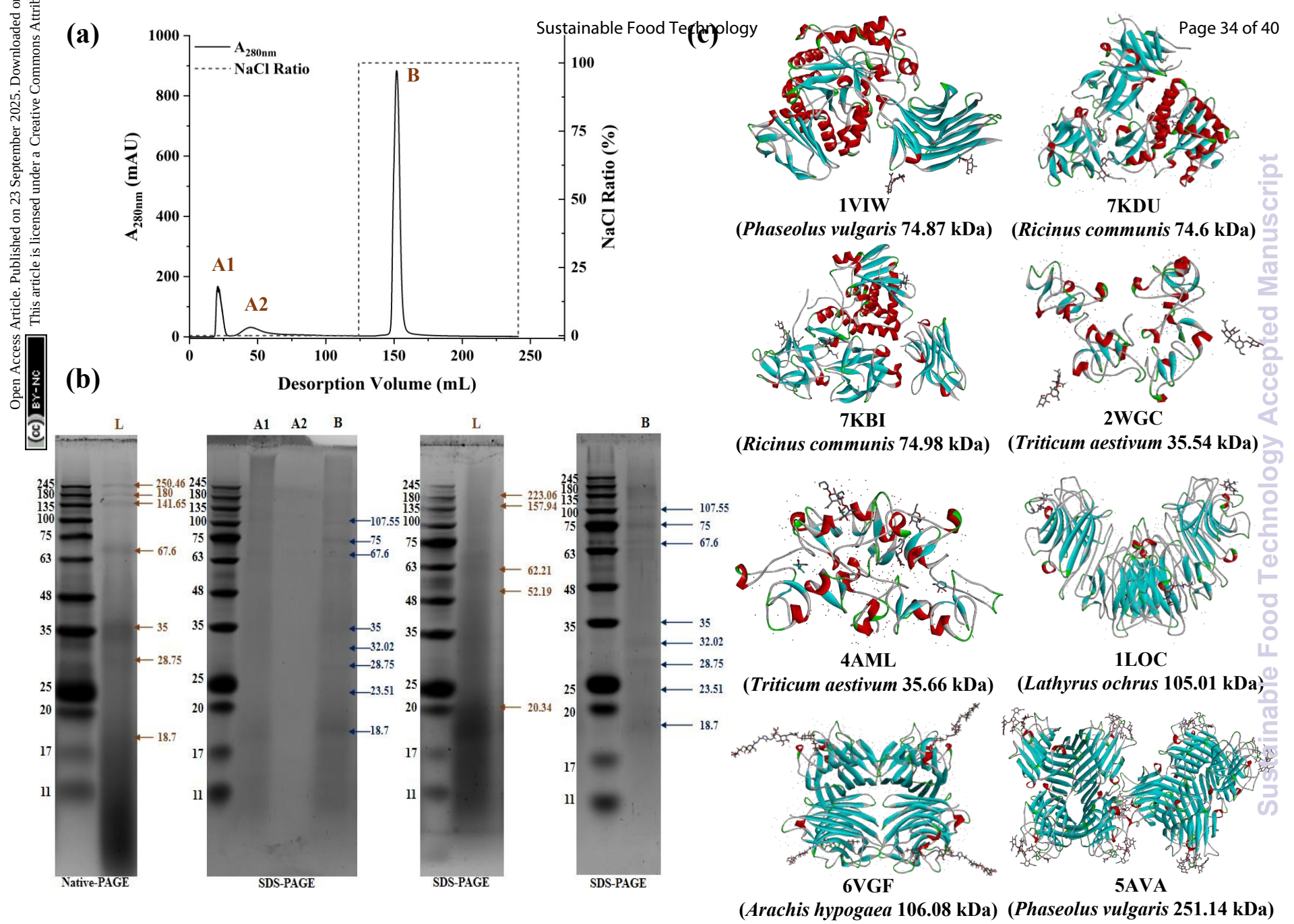

Figure 1. Elution of DEAE-Sepharose Fast Flow chromatography of *Ulva lactuca* lectin and electrophoresis of purified lectin.

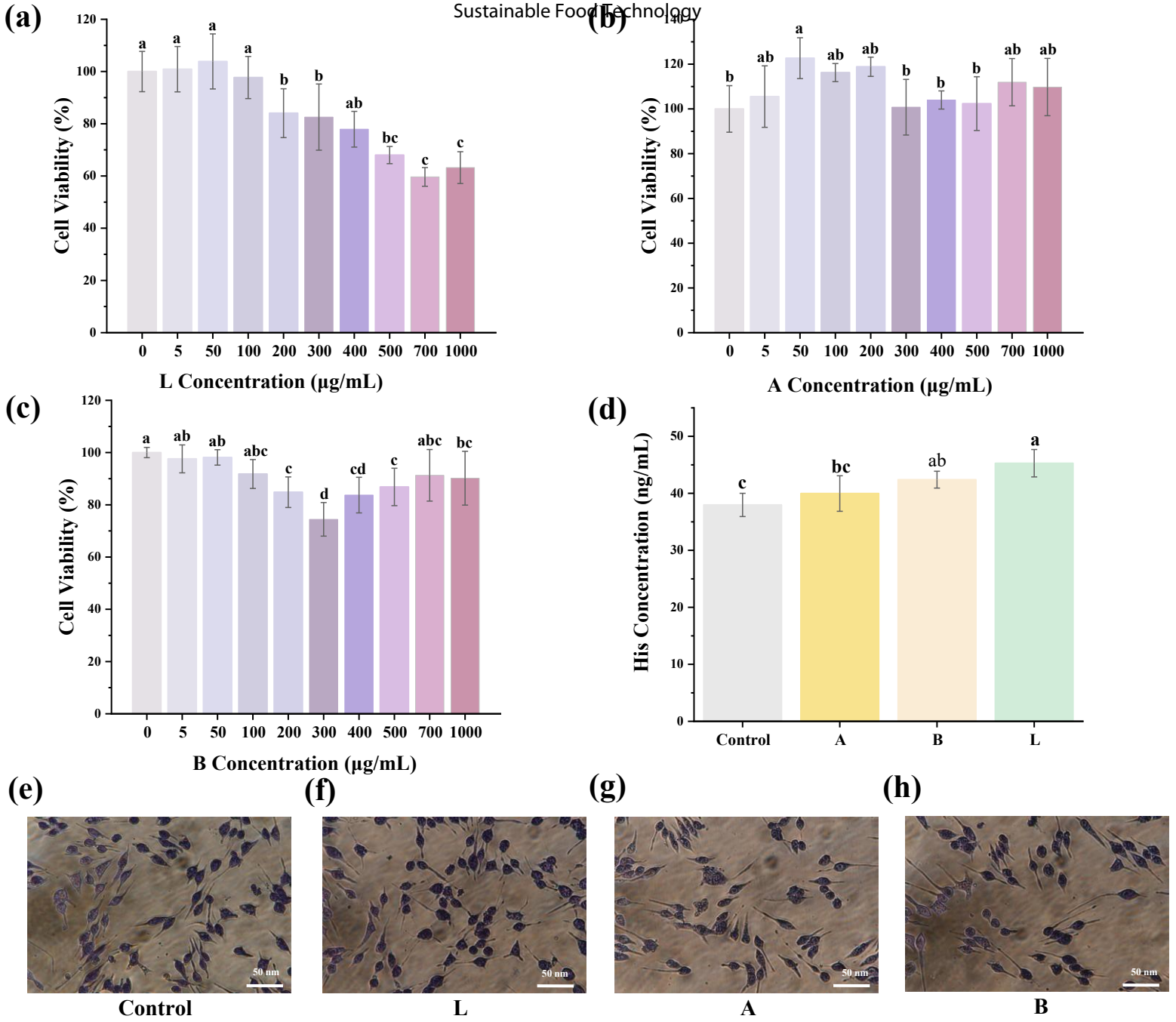

Figure 2. Proliferation and histamine content on RBL-2H3 cells treated by lectin.

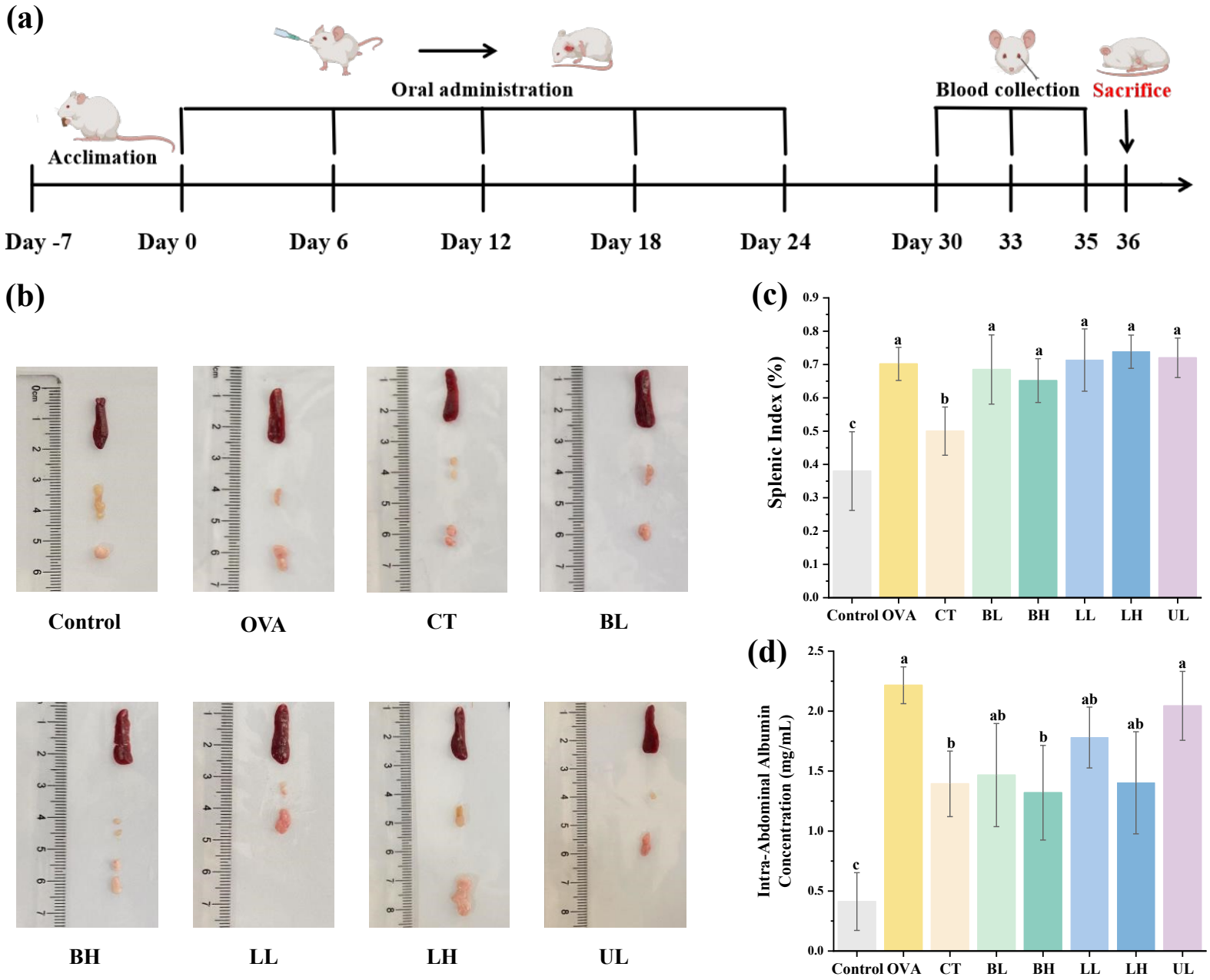

Figure 3. Protocol of food allergic model and hypersensitive reaction.

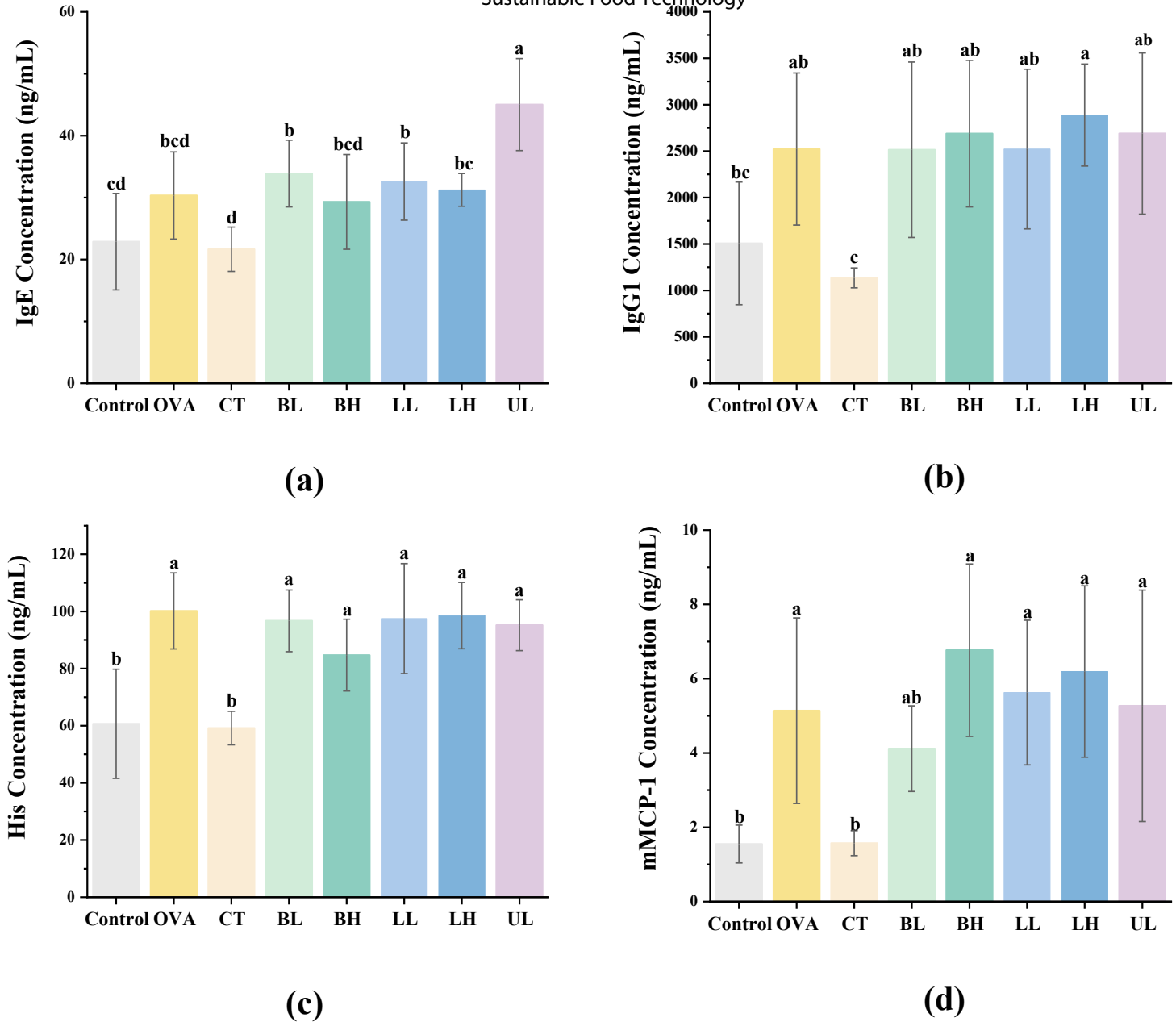

Figure 4. Anaphylaxis in the BALB/c mice sensitized and challenged in different groups.

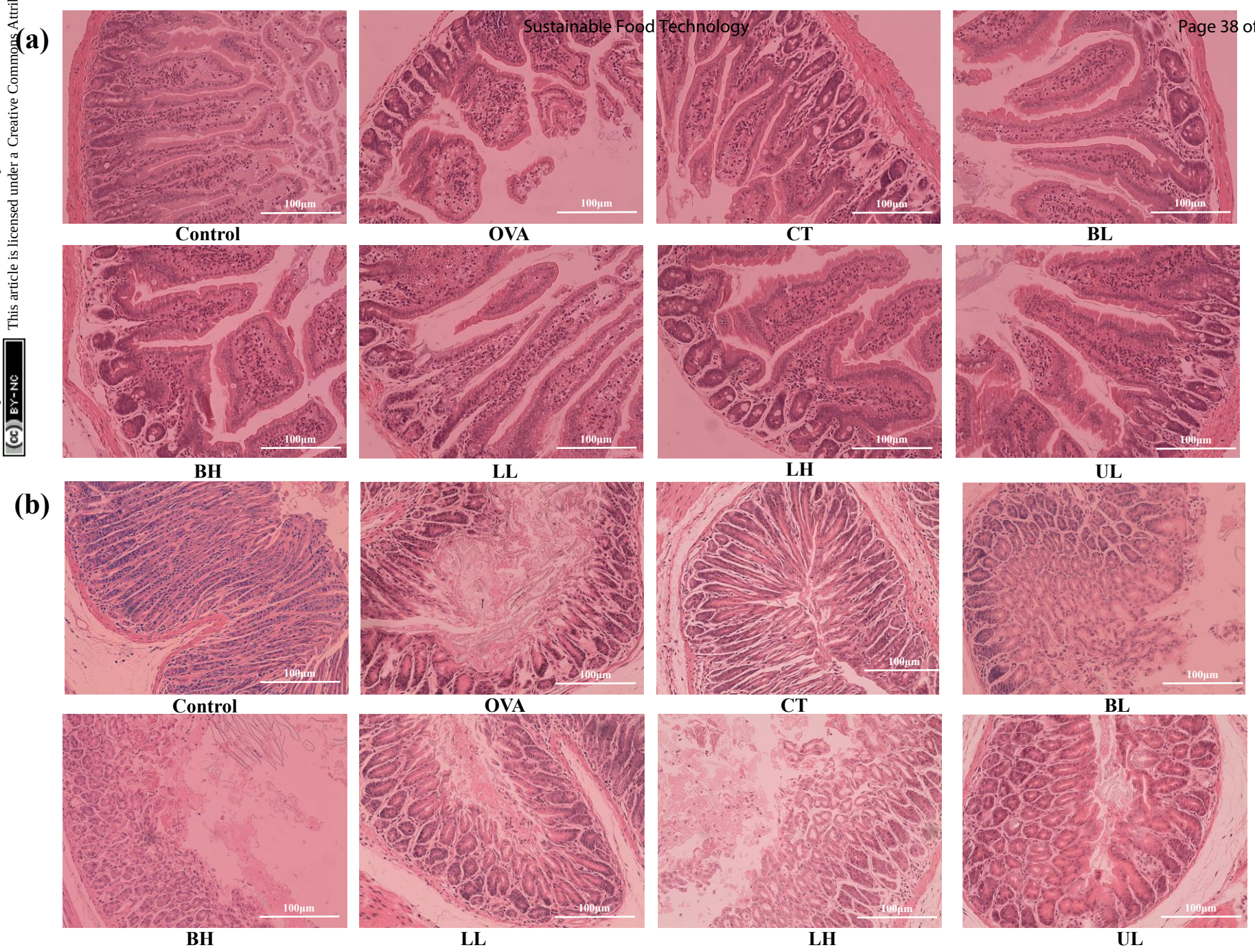

Figure 5. Histopathology alterations of the jejunal and duodenum sections of the BALB/c mice administrated with different groups.

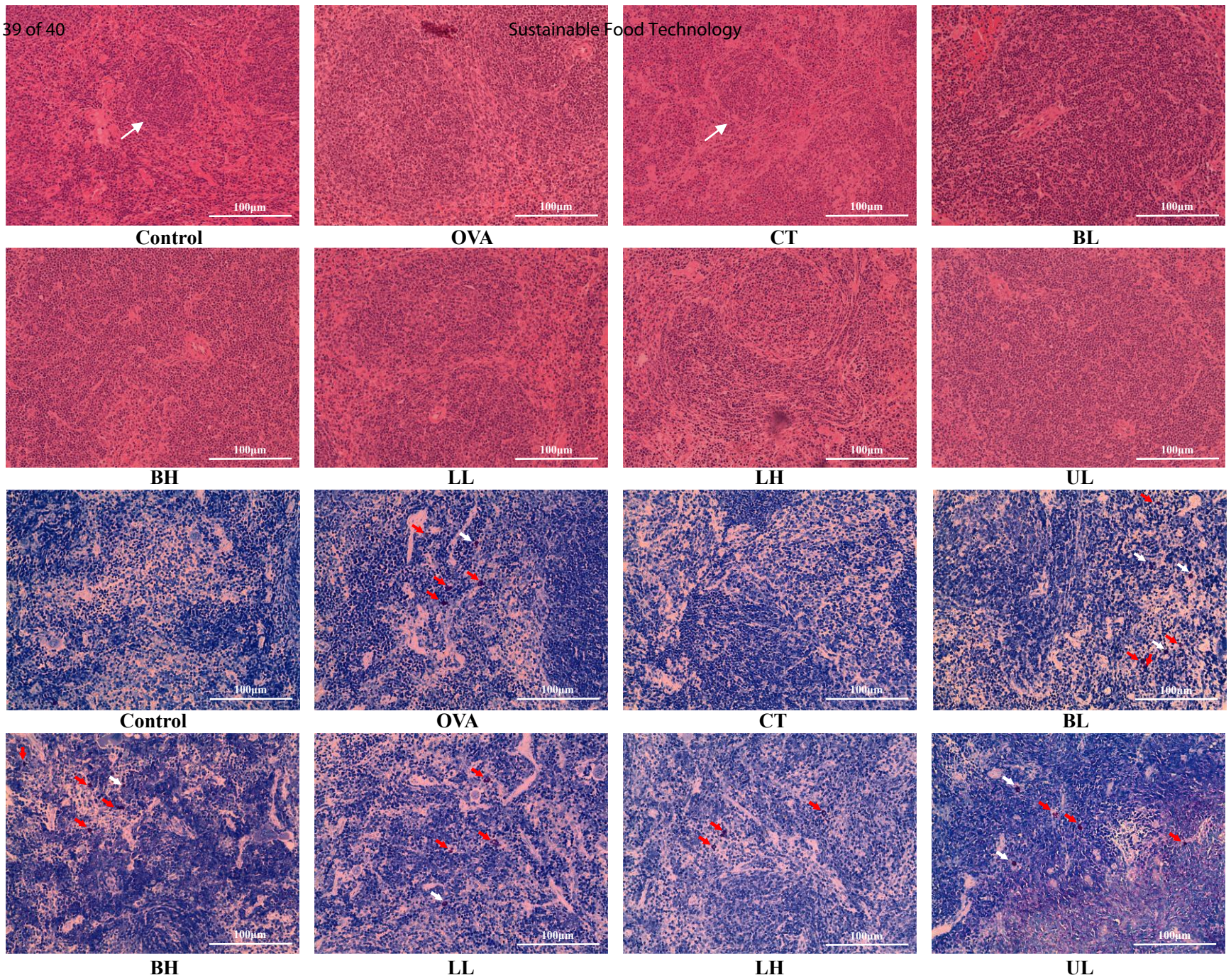

Figure 6. Hematoxylin-eosin (H&E, a) and toluidine blue (TB, b) staining of splenic.

View Article Online
DOI: 10.1039/D5FB00446B

Data Availability Statement

The authors confirmed that the data supporting the findings of this study are available within the article and its supplementary materials.