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## Molecular properties and allergenicity of lectin from *Ulva lactuca*

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*Ulva lactuca*, the type species of the green 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*), displayed 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 reached  $45.28 \pm 2.40 \text{ ng mL}^{-1}$ . *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) and 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.

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### Sustainability spotlight

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

## 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.<sup>1</sup> In Asian countries, *U. lactuca* was traditionally consumed as food for centuries. Its applications were also explored in bioenergy production, pharmaceuticals, and cosmetics.<sup>1</sup> 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.<sup>2</sup> 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.<sup>2</sup> 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 a carbohydrate-binding protein with biological functions in cell signaling and pathogen defense through specific recognition of glycoconjugates.<sup>3</sup> Lectins were first isolated from castor beans (*Ricinus communis*) in 1888, designated as Ricin.<sup>4</sup> 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 Tsaneva and Damme.<sup>4,5</sup> Immunomodulatory, antiviral, and antitumor activities of lectins were documented, though their interaction mechanisms with mammalian immune systems remained incompletely elucidated.<sup>6</sup>

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Notably, specific lectins (e.g., peanut agglutinin and soybean lectin) were confirmed to trigger IgE-mediated allergies by binding to intestinal epithelial receptors, potentially inducing anaphylaxis.<sup>7</sup> 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.<sup>8,9</sup> Gastrointestinal symptoms including nausea, vomiting, and abdominal distension were observed in both children and adults. Current research is predominantly focused on legume lectins, while systematic risk assessment of *U. lactuca* lectin allergenic potential remains unexplored. Thus, a comprehensive evaluation of *U. lactuca* lectin allergenicity is 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.<sup>10</sup> Th2-type immune responses were subsequently activated. This cascade induced the production of allergen-specific IgE antibodies by B cells.<sup>11</sup> These antibodies were bound to mast cells and basophils via Fc $\epsilon$ RI 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.<sup>12</sup> 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 have 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.<sup>13,14</sup> Previous research efforts were predominantly directed toward developing cellular and animal experimental systems.<sup>15</sup> In this study, lectin was isolated from *U. lactuca* using ammonium sulfate precipitation. The 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* was purchased from Daqin Island, Changdao, Yantai, Shandong Province. A 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,  $\beta$ -mercaptoethanol, MTT, cholera toxin (CT) and albumin (OVA) were purchased from Sigma-Aldrich (St. Louis, MO, USA). H<sub>2</sub>SO<sub>4</sub>, NaCl, CH<sub>3</sub>COOH and NaOH were procured from Tianjin Damao Chemical Reagent Co., Ltd (Tianjin, China). (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and 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 (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 per L NaCl (pH 7.0). The mixture was centrifuged at 9000 rpm for 20 min at 4 °C using a hybrid refrigerated centrifuge (Techcomp, CAX-571, China), after being extracted for 16 h at 4 °C. (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> with 20% saturation was added into the supernatant at 4 °C, and the mixture was stirred with a magnetic stirrer (YUHUA, 98-2, China) overnight (4 °C). After centrifugation at 11 000 rpm for 20 min at 4 °C, the precipitate was discarded and the supernatant was brought to 60% saturation with (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and stored for 12 h. The precipitate was dissolved in distilled water and was dialyzed for 36 h against the same buffer to eliminate residual (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>. Then, the solution was lyophilized and stored in a dryer, which was named lectin (L). The target proteins were purified using ion exchange chromatography (GE, AKTA avant 25, China) on a DEAE-Sepharose Fast Flow column (39 mL; Henghuibio, HC-0220-02A, China) with an eluting buffer of NaCl (0–1 mol L<sup>-1</sup>) in Tris-HCl (50 mM, pH 8.0).<sup>16</sup> Then, the penetrating peak (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.<sup>17</sup> In Native-PAGE, samples were prepared in a buffer containing 20% glycerol and 25 mM Tris-HCl (pH 8.0). And then, 10  $\mu$ L of each sample was loaded onto Native-PAGE gels. In SDS-PAGE, samples were dissolved to 4 mg mL<sup>-1</sup> with the sample buffer, which contains 25 mM Tris-HCl (pH 8.0), SDS, glycerol and  $\beta$ -mercaptoethanol. After boiling for 10 min, the samples (10  $\mu$ L) were centrifuged and loaded onto a stacking gel (3%) and a 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 with water. Imaging was performed using a GS-800 densitometer (DNR BioImaging Systems Ltd, Israel).<sup>18</sup>

## 2.4 RBL-2H3 cell model experiment

**2.4.1. RBL-2H3 cell viability assay.** The cell viability test was measured in accordance with the MTT cytotoxicity assay utilizing RBL-2H3 cells (Shanghai Cell Bank of Chinese Academy of Sciences). The medium for cell culture was Minimum Essential Medium (Hyclone, Logan, UT, USA) with the addition of 1% penicillin-streptomycin (Hyclone, Logan, UT, USA) and 15% fetal bovine serum (PAN-Biotech, UK). Typically, RBL-2H3 cells (200  $\mu$ L) were suspended in cell culture medium and seeded into 96-well plates at a density of  $5 \times 10^4$  cells per mL and incubated for 24 h at 37 °C in 5% CO<sub>2</sub> to obtain a monolayer of cells.<sup>19</sup> After 24 h of incubation, 200  $\mu$ L of L, A and B in different concentrations (0, 50, 100, 200, 300, 400, 500, 700 and 1000  $\mu$ g mL<sup>-1</sup>) were added for 24 h of stimulation. The sample solution was removed after incubation and the cells were further cultured with MTT (5 mg mL<sup>-1</sup>, 15  $\mu$ L) solution for 4 h. Finally, the culture medium was substituted with DMSO (150  $\mu$ L) to dissolve the formazan, and the absorbance of the DMSO solution at 490 nm was tested using a micro plate reader (Infinite<sup>TM</sup> M200, TECAN, 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  $5 \times 10^4$  cells per mL and incubated for 24 h at 37 °C in 5% CO<sub>2</sub> to obtain a monolayer of cells.<sup>19</sup> After 24 h of incubation, 200  $\mu$ L of A, B and lectin (300  $\mu$ g mL<sup>-1</sup>) were added for 24 h of stimulation. At the end of excitation, the supernatants were collected and centrifuged (1000 rpm, 20 min). Then the supernatant was collected, and His content was determined using ELISA kits,<sup>20</sup> 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 a toluidine blue stain solution.

## 2.5 Allergenicity assessment

**2.5.1. Mouse 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 to the antigen.<sup>21</sup> 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 the corresponding samples by intragastric administration. OVA, BL, and LL groups were gavaged with a 200  $\mu$ L sample at a concentration of 2 mg mL<sup>-1</sup>. BH, LH, and UL groups were gavaged with a 200  $\mu$ L sample at a concentration of 20 mg mL<sup>-1</sup>. The mice were sensitized weekly by intraperitoneal injection with samples plus CT (5  $\mu$ g) per mouse, and the other two groups were gavaged with saline and CT, respectively. For the stage of challenge, OVA and sample groups of mice were given 5 times the dose of OVA and samples, respectively, and sacrificed on Day-36.<sup>22</sup> The experimental design is shown in Fig. 3a. 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 the Dalian Polytechnic University Ethical Committee for Animal Experimentation (approval number: DLP2024077).

**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:<sup>23</sup>

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

**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.<sup>24</sup> 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 using 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 using a Mouse IgE ELISA Kit, Mouse IgG<sub>1</sub> 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  $\mu$ m thickness were made and prepared for staining of Hematoxylin and Eosin (H&E) to observe the histopathological changes.<sup>25</sup> 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.<sup>26</sup>

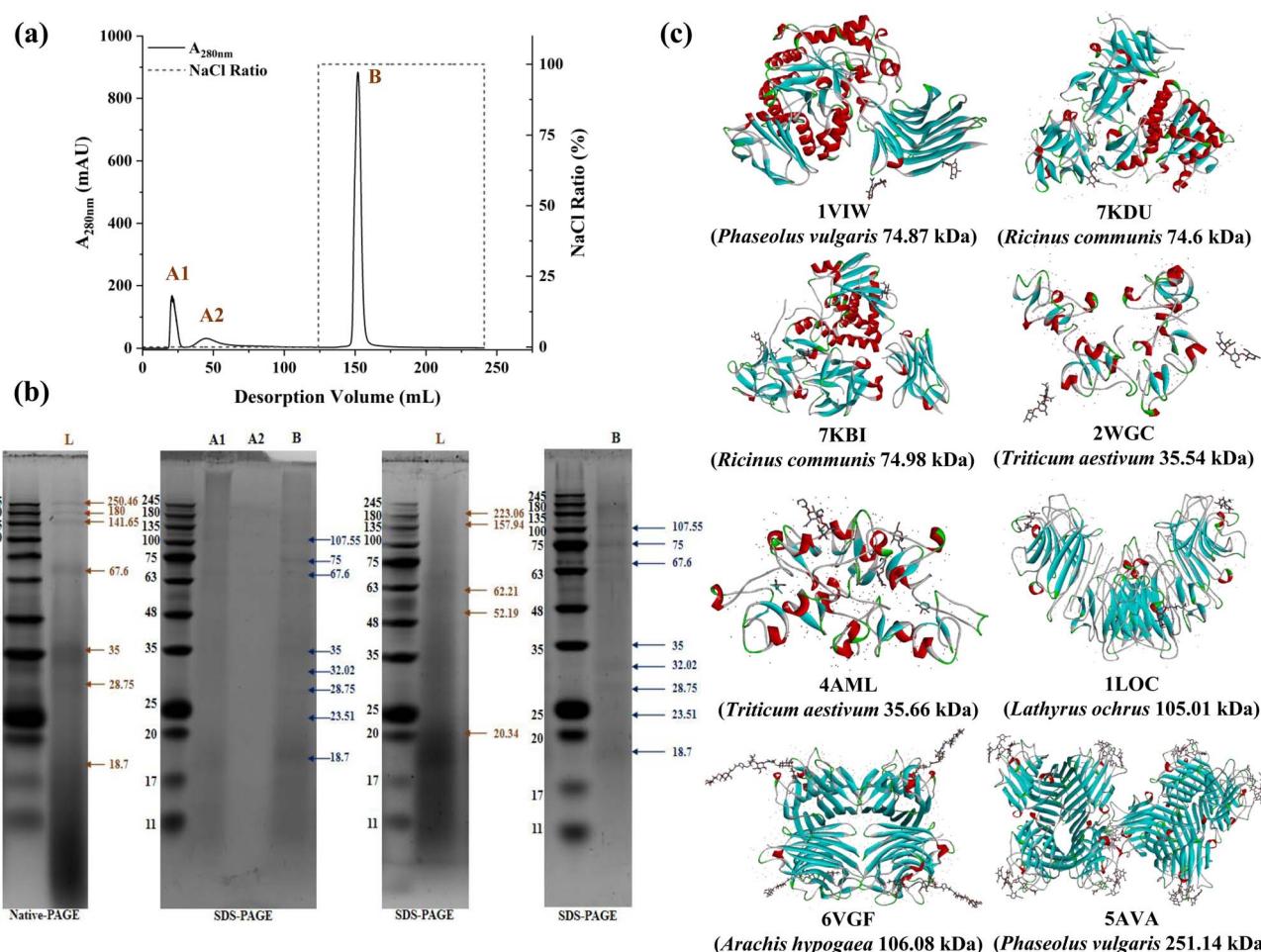
## 2.6 Statistical analysis

The mean  $\pm$  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 Tukey's HSD *post hoc* test with SPSS software (SPSS 16.0 Inc, Chicago, USA). The significance level was set at  $p < 0.05$ .

## 3 Results and discussion

### 3.1 Preparation of *U. lactuca* lectin

*U. lactuca* L. was extracted by salting out with NaCl and NH<sub>4</sub>SO<sub>4</sub> 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 (Fig. 1b). Serial paraffin sections with 4–6  $\mu$ m thickness were made and prepared for staining of Hematoxylin and Eosin (H&E) to observe the histopathological changes.<sup>25</sup> 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.<sup>26</sup>



**Fig. 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. The 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 Tris–HCl at a flow rate of 2.0  $\text{mL min}^{-1}$ . 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 the Protein Data Bank.



and 7KBI (*Ricinus communis*) were distributed at 74.87, 74.6 and 74.98 kDa (Fig. 1c).<sup>3,28,29</sup> 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.<sup>30-32</sup> Molecular structures of these lectins, which had similar molecular weights to those 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).

### 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 an RBL-2H3 cell model.<sup>33</sup> Selection of the appropriate drug concentration for subsequent experiments was first made by MTT experiments; the concentration of 300  $\mu\text{g mL}^{-1}$  was

identified as the optimal concentration for cell stimulation.<sup>34</sup> As shown in Fig. 2e, the His content of RBL-2H3 was determined. His release rate of RBL-2H3 cells was significantly increased after induction by B and L, reaching  $42.40 \pm 1.47 \text{ ng mL}^{-1}$  and  $45.28 \pm 2.40 \text{ ng mL}^{-1}$ , 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.<sup>35</sup> The results show that B and L were more sensitizing. This result can also be seen in the cellular morphology of Fig. 2e-h. Effects of control (Fig. 2e), lectin (Fig. 2f), A (Fig. 2g), and B (Fig. 2h) on RBL-2H3 cells were observed and photographed using a microscope. After being stimulated for 24 hours, compared with the control group, the cell morphology changed and became more

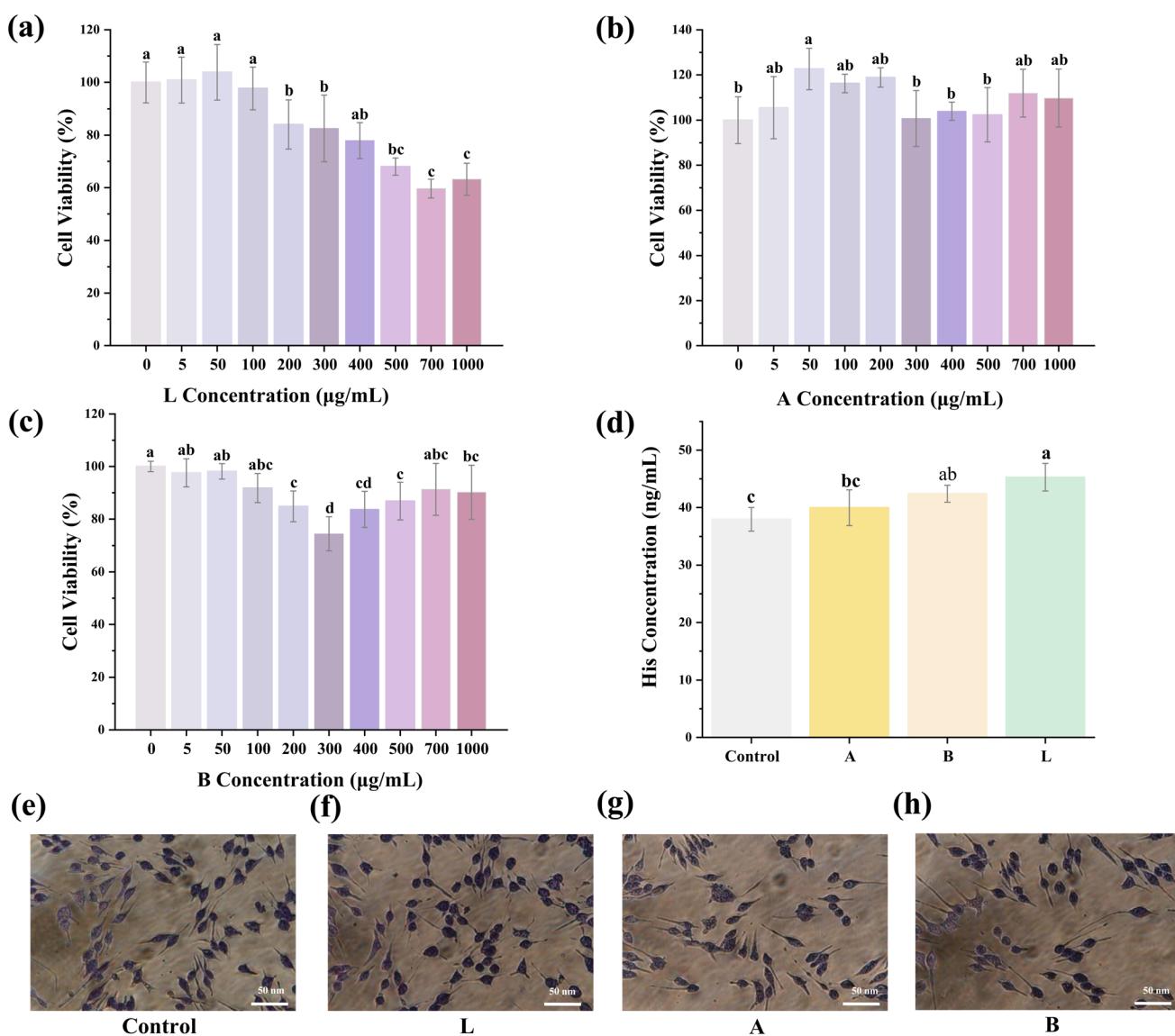


Fig. 2 Proliferation and histamine content on RBL-2H3 cells treated by lectin. Lectin (a), A (b), and B (c) toxicity for 24 on RBL-2H3 cells. (d) Histamine release rate of RBL-2H3 cells induced by lectin. Effects of control (e), lectin (f), A (g), and B (h) on RBL-2H3 cells were observed and photographed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

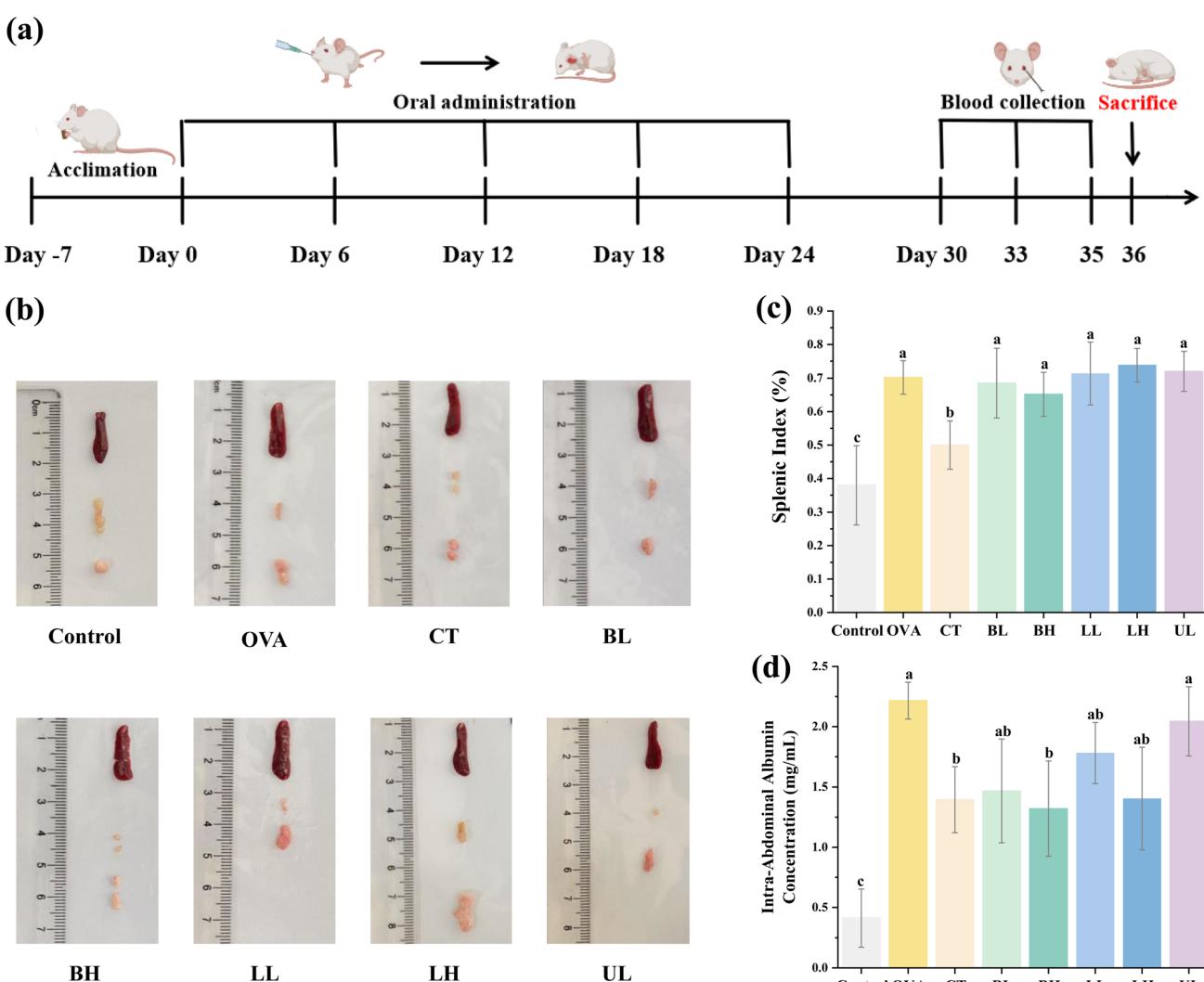


rounded in lectin (Fig. 2f) and B (Fig. 2 h), whereas there was no significant difference between the control and A (Fig. 2g).

### 3.3 Changes in splenic index in BALB/c mice

The experimental protocol for mice is shown in Fig. 3a. 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 conditions were good. Furthermore, there were no deaths or significant toxicological symptoms throughout the whole experiment. The spleen is the largest and most important immune tissue in the body, and after antigenic stimulation, it 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.<sup>36</sup> As shown in Fig. 3b, mice treated with OVA, BL, LL, and LH showed a larger spleen size than the 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 not only to participate in immune regulation but also to 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.<sup>37</sup> As demonstrated in Fig. 3c, the spleen indices in OVA, BL, BH, LL, LH, and UL groups were significantly higher than those in the control and CT groups ( $p < 0.05$ ), whereas the LH group exhibited the highest index of  $0.74 \pm 0.05\%$ . A notable increase in splenic index was also detected in the CT group relative to the control group ( $p < 0.05$ ), which might be attributed to CT-induced inflammatory responses leading to structural alterations in the splenic architecture.<sup>38</sup> This result suggested that lectins (L and B) in *U. lactuca* induce a significant increase in splenic index in mice, causing swelling of immune tissues. Comparative analysis of images of the spleen revealed



**Fig. 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 (d).

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.

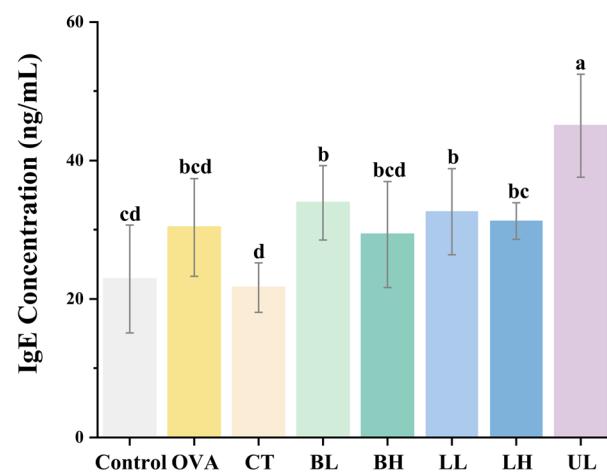
### 3.4 Changes in vascular permeability in BALB/c mice

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.<sup>39</sup> 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.<sup>40</sup> Therefore, vascular permeability in mice was quantified by determining the level of peritoneal albumin in each group of mice. As shown in Fig. 3d, the highest intra-

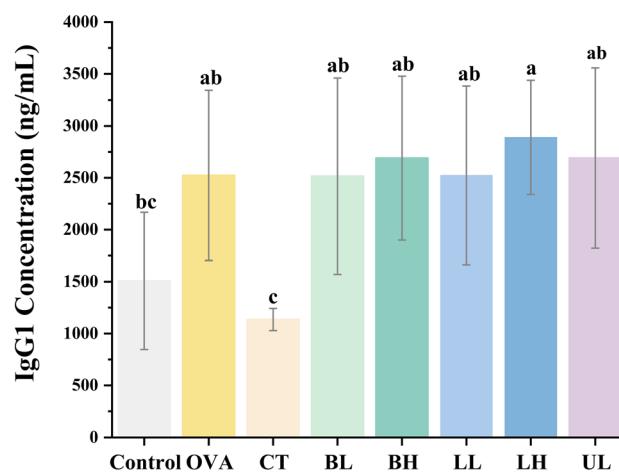
abdominal albumin concentrations in peritoneal fluid were detected in both OVA ( $2.21 \pm 0.15 \text{ mg mL}^{-1}$ ) and UL ( $2.04 \pm 0.29 \text{ mg mL}^{-1}$ ) ( $p < 0.05$ ) groups. There was no significant difference in abdominal albumin content between BL, BH, LH and CT groups. The results of 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

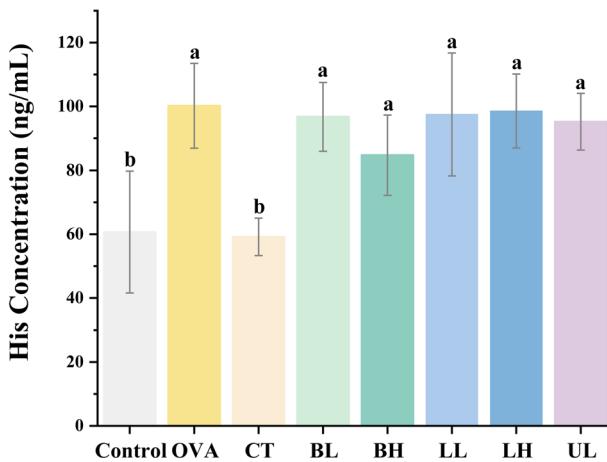
Most food allergic reactions are mediated by IgE,<sup>41</sup> so an increase in allergen-specific IgE is a distinctive feature of the occurrence of allergic reactions. It is typical for serum antibody levels to be elevated in the presence of food allergy.<sup>42</sup> As shown in Fig. 4, mice in the control group did not exhibit allergic reactions. The levels of



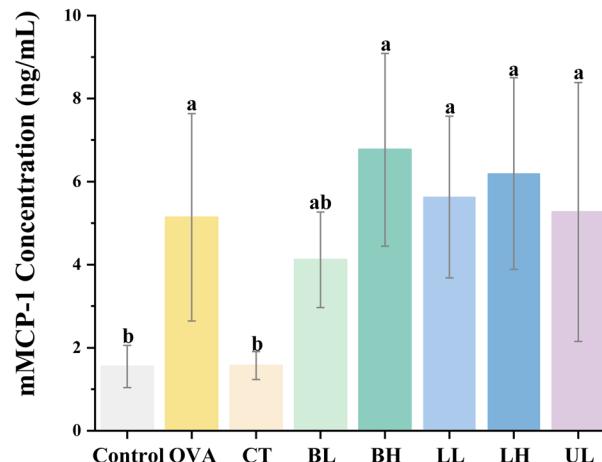
(a)



(b)



(c)



(d)

Fig. 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 mMCP-1 in each BALB/c mouse was determined using a commercial ELISA kit ( $n = 5$ , data presented as mean  $\pm$  SD, different letters above the bars represented significant differences, with a significance level of 0.05).



various specific antibodies in the mouse serum were then detected. Serum levels of specific IgE antibodies in mice are shown in Fig. 4a. The serum-specific IgE antibody level in the adjuvant (CT) group was maintained at a lower concentration of  $21.65 \pm 3.58 \text{ ng mL}^{-1}$ , indicating that the CT adjuvant was specifically employed to enhance the immune response to allergens without inducing allergic responses itself. Compared with CT groups, serum-specific IgE levels ( $p < 0.05$ ) were significantly elevated in BL ( $33.88 \pm 5.38 \text{ ng mL}^{-1}$ ), LL ( $32.59 \pm 6.23 \text{ ng mL}^{-1}$ ), LH ( $31.24 \pm 2.65 \text{ ng mL}^{-1}$ ), and UL ( $45.02 \pm 7.43 \text{ ng mL}^{-1}$ ) 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.<sup>43</sup> The serum-specific IgG1 antibody levels in each group of mice are presented in Fig. 4b. Compared with the control and CT groups, the LH group showed the highest concentration ( $2888.82 \pm 549.43 \text{ ng mL}^{-1}$ ) 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.<sup>44</sup>

### 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.<sup>41</sup> mMCP-1 was stored and secreted by intestinal mucosal mast cells and was predominantly expressed in mucosal mast cells.<sup>41</sup> Researchers studied that mast cell degranulation also causes elevated levels of mMCP-1 and increased permeability of intestinal epithelial cells, leading to food allergic diseases.<sup>45</sup> As demonstrated in Fig. 4c and d, there were no significant differences between the control and CT groups in serum His and serum mMCP-1 levels. Compared with the BALB/c mice challenged with OVA ( $100.18 \pm 13.32 \text{ ng mL}^{-1}$ ), there were no significant differences in BH ( $84.72 \pm 12.54 \text{ ng mL}^{-1}$ ), LH ( $98.55 \pm 11.59 \text{ ng mL}^{-1}$ ) and UL ( $95.20 \pm 8.90 \text{ ng mL}^{-1}$ ) groups ( $p > 0.05$ ) in His. Compared with the control and CT groups, mMCP-1 concentrations were significantly elevated in the OVA, BH, LL, LH, and UL groups, with the highest level ( $6.77 \pm 2.32 \text{ ng mL}^{-1}$ ) 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, caused severe allergic reactions in mice. As a result, His and mMCP-1 were detected in large quantities in the serum of *U. lactuca* lectin-sensitized mice, and their levels were significantly higher than those of non-sensitized mice (control).

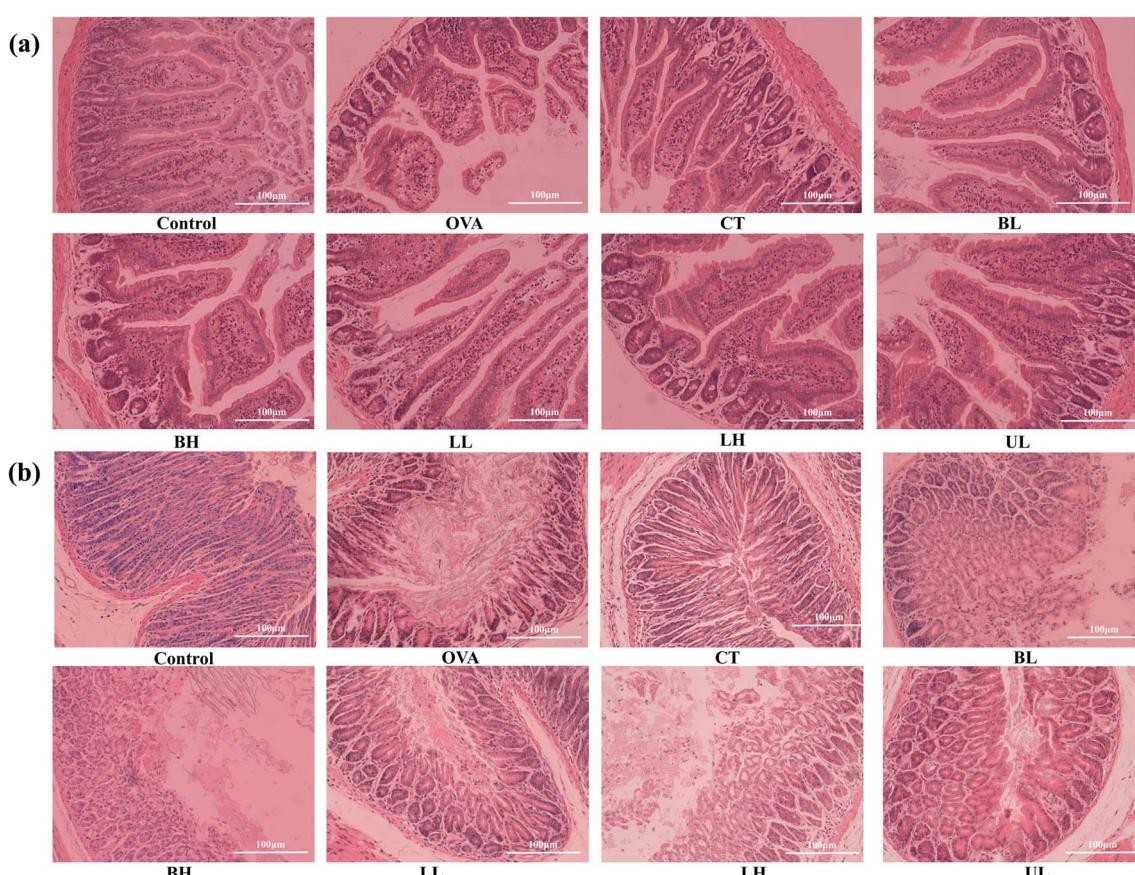


Fig. 5 Histopathology alterations of the jejunal (a) and duodenum (b) sections of the BALB/c mice administered with different groups. Images were taken using a microscope at  $20\times$  magnification.

### 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 (Fig. 5a), 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 Fig. 5a, distinct pathological alterations were observed in the OVA, BL, BH, LL, LH, and UL experimental cohorts, where the jejunal villus architecture exhibited marked disruption, including villous fragmentation and goblet cell hyperplasia. As shown in Fig. 5b, the intestinal villi of duodenum sections in the 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.<sup>46,47</sup> This leads to the conclusion that lectins in *U. lactuca* caused an allergic reaction in the intestine. A 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 (Fig. S1). This study revealed that alveolar septal thickening might contribute to airway obstruction, potentially exacerbating allergic responses in murine models.<sup>48</sup>

### 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.<sup>49</sup> As shown in Fig. 6a, the 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. A distinct demarcation between the marginal zone and red pulp was

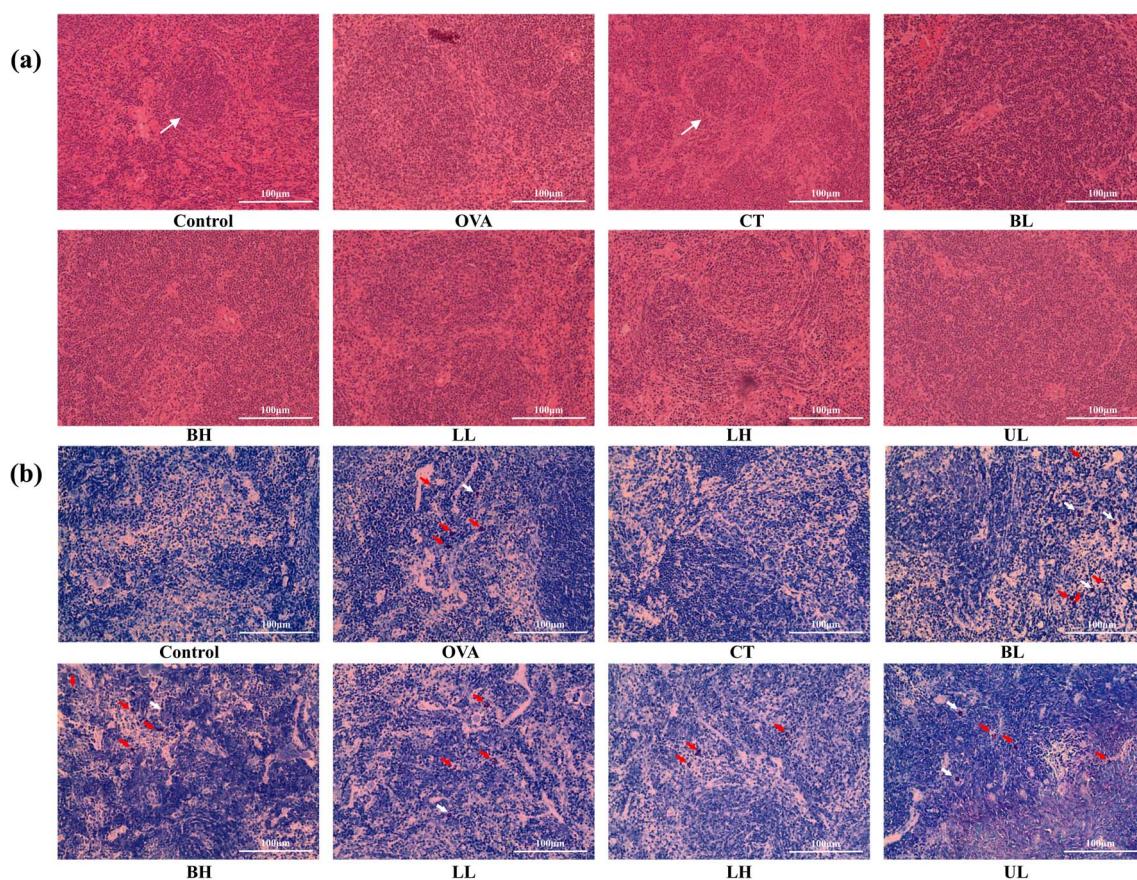


Fig. 6 Hematoxylin–eosin (H&E, a) and toluidine blue (TB, b) staining of the spleen. Images were taken using a microscope at 20 $\times$  magnification. In the H&E staining, the white pulp with a clear boundary from the red pulp is indicated by white arrows in (a). In the TB staining, the white arrows denote intact mast cells and the red arrows denote degranulated mast cells in (b).



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 lectin exposure was associated with hypersensitivity reactions in murine models, which subsequently triggered immune activation characterized by augmented lymphoproliferative responses.

Mast cells, which are ubiquitously distributed throughout somatic tissues, were demonstrated to play pivotal roles in allergic pathogenesis.<sup>50,51</sup> 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 Fig. 6b, 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 cell infiltrations were significantly observed in the 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 the control and CT groups. In addition, the morphometric analysis of degranulated mast cells in the spleen is presented in Fig. S2. The control and CT groups showed a relatively low level of mast cell 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 high allergenic potential.

## 4 Conclusions

Within the broader context of simultaneous social development and escalating allergic disease burdens, pronounced limitations and asymmetries were recognized in existing allergen recognition frameworks. Research has 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 remains 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 research has confirmed that lectins in the diet can interfere with nutrient digestion and absorption, and high dietary levels of

lectins can 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 its 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 necessity 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 establishing 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.

## Abbreviations

His	Histamine
IgE	Immunoglobulin E
IgG1	Immunoglobulin G1
RBL-	Rat basophilic leukemia mast cells
2H3	
CT	Cholera toxin
OVA	Albumin
mMCP-	Mouse mast cell protease 1
1	
L	Lectin
A	Target protein A, the penetrating peak of ion exchange chromatography in Fig. 1a
B	Target protein B, the elution peak of ion exchange chromatography in Fig. 1a
BL	Low concentration of B
BH	High concentration of B
LL	Low concentration of L
LH	High concentration of L
UL	<i>Ulva lactuca</i> powder
H&E	Hematoxylin and eosin
TB	Toluidine blue

## Conflicts of interest

There are no conflicts of interest to declare.



## Data availability

The authors confirmed that the data supporting the findings of this study are available within the article and its supplementary information (SI). Supplementary information is available. See DOI: <https://doi.org/10.1039/d5fb00446b>.

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