Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 4733651, 14 pages
https://doi.org/10.1155/2023/4733651

Research Article

The Polysaccharide from *Dendrobium officinale* Can Improve Mice with Sjögren's Syndrome by Regulating BAFF and Fas Expressions

Pengpeng Zhu,[1] **Guoteng Ou**,[2] **Ting Zhou**,[1] **Wenwen Zhu**,[1] **Wei Sun**,[2] **Xueping Wei**,[2] **Rongrong Huang**,[2] **Junsong Jing**,[2] **Sheng Zhang**,[3] **Jingjin Hu**,[2] **Yuanyuan Li**,[1] **Hua Liu**,[2] **Tangbiao Shen**,[2] **Zhengrong Zhao**,[1] **and Yueguo Wu**[1,4]

[1]*School of Pharmacy, Hangzhou Medical College, Hangzhou 310013, China*
[2]*School of Public Health, Hangzhou Medical College, Hangzhou 310013, China*
[3]*Center for Safety Evaluation and Research, Hangzhou Medical College, Hangzhou 310013, China*
[4]*Key Laboratory of Neuropsychiatric Drug Research of Zhejiang Province, Hangzhou Medical College, Hangzhou 310013, China*

Correspondence should be addressed to Tangbiao Shen; 2019000064@hmc.edu.cn, Zhengrong Zhao; 2020000334@hmc.edu.cn, and Yueguo Wu; 2020000320@hmc.edu.cn

Received 27 September 2023; Revised 6 December 2023; Accepted 19 December 2023; Published 28 December 2023

Academic Editor: Hongxun Tao

Exploring herbs and their compounds for potential drugs is a significant research focus due to the incurable nature of Sjögren's syndrome (SS), a prevalent chronic inflammatory autoimmune disease. *Dendrobium officinale* polysaccharide (DOP) has demonstrated diverse biological effects, particularly in immunomodulation. Nonetheless, limited research has been conducted on DOP concerning the treatment of SS. This research aims to examine the impacts of DOP on experimental SS (ESS) mice and its possible mechanisms. An ESS animal model was established using the immune induction method. Besides the control group, ESS mice were randomly assigned to the model (distilled water), the HCQ (60 mg/kg), the DOP (43 mg/kg), the DOP (130 mg/kg), and the DOP (390 mg/kg) groups. Drugs were treated for three weeks. The findings indicated that DOP enhanced saliva production of ESS mice and diminished lymphocyte infiltration and foci in the submandibular gland (SMG). DOP also significantly downregulated the mRNA content of proinflammatory factors (TNF-α, IL-1β, and IL-17) and the expression of genes and proteins of apoptotic factors (Bax and Caspase-3). Furthermore, DOP downregulated the BAFF and BAFF receptor (BAFFR) as well as Fas and Fas ligand (Fas-L) expressions in the SMG. Our study demonstrates that DOP may reduce inflammation and apoptosis by inhibiting BAFF/BAFFR and Fas/Fas-L signaling, thus producing therapeutic effects in ESS mice.

1. Introduction

Sjögren's syndrome (SS), a prevalent autoimmune disease, is characterized by chronic inflammation and affects the mouth and eyes, causing dryness. Primary SS (pSS) is distinguished by the presence of dry mucous membranes and tissue injury resulting from focal lymphadenitis, whereas secondary SS (sSS) may be accompanied by additional rheumatic conditions, including rheumatoid arthritis [1]. SS incidence rate ranks second among rheumatic immune diseases, with an epidemiological survey exhibiting a prevalence of 0.29~0.77% in China [2]. Scholars have long been interested in lymphocyte infiltration in affected tissues and abnormal T and B lymphocyte activation, which are significant immunological characteristics of SS because they cause inflammation and apoptosis in the gland [1]. One of the activities includes the BAFF (a TNF family B cell activation factor) synthesis, stimulating the B cell development, extending the lifespan of fully developed B cells, and activating nearby T cells. BAFF accomplishes these functions by attaching to BAFFR, a membrane-bound receptor on B cells [3]. However, BAFF overexpression leads to excessive B cell

activation, and the exocrine gland tissue is infiltrated by a significant amount of autoantibodies and immune complexes, ultimately leading to inflammation and dysfunction of the gland. Studies have revealed that mice with BAFF transgenes are prone to develop autoimmune diseases resembling SS [4]. Therefore, BAFF and BAFFR are crucial in developing SS. Furthermore, submandibular salivary gland (SMG) epithelial cell apoptosis is a significant factor in developing SS [5]. The augmentation of apoptosis in SMG epithelial cells of mice leads to the emergence of pSS-like autoimmune disorders [6]. Among them, Fas and its ligand Fas-L, crucial regulators of apoptosis, are considered significant members of the regulatory induction family, involved in the clearance of activated T cells, and play a vital role in the induction of autoimmune diseases [7]. Hence, it is crucial to investigate potential treatments that can decrease inflammation and apoptosis related to the glands to treat SS effectively.

Western medicine has limited and incurable drug options for SS, which can treat the disease while also causing serious adverse reactions to patients, such as hydroxychloroquine (HCQ), causing visual field loss, gastrointestinal reactions, and liver toxicity [2]. According to traditional Chinese medicine, SS is classified as "dry evidence." Treatments are designed to enhance the gland secretion in the mouth, nose, and eyes, ultimately strengthening the body's ability to fight against illnesses [8]. Recent studies have indicated that natural medicines or active components exhibit positive outcomes in immunomodulation [9], reducing inflammation [2] and anti-apoptosis [10] while posing minimal side effects.

D. officinale, also known as *Dendrobium officinale* Kimura et Migo, is a dried orchid stem that has beneficial effects on the stomach and the production of fluid. It can be used clinically for patients with fluid deficiency, dry mouth and thirst, and deficiency heat after illness [11]. In our investigation for herbal remedies against SS, we previously examined the salivary-stimulating impact of *D. officinale*. We discovered that this extract could enhance saliva production in rats from the dry mouth syndrome model group [12]. Recent studies revealed that *D. officinale* polysaccharides have many pharmacological activities. Wang et al. [13] discovered that *D. officinale* polysaccharide (DOP) reduced TNF-α levels, enhanced IL-10 production, and promoted the Bcl-2 protein and other factor expressions to preserve intestinal balance. Simultaneously, it suppressed the Bax and Caspase-3 protein expressions to safeguard against liver fibrosis. Jiang et al. [14] discovered that DOP could hinder vascular calcification in chronic kidney disease patients by exerting anti-inflammatory and anti-apoptotic properties. During the clinical treatment of pSS patients, DOP improves patients' symptoms, especially in improving dry mouth symptoms [15]. In contrast, studies on DOP for SS and its specific mechanisms are scarce and poorly defined.

Therefore, the objective of this study is to use DOP and establish an experimental SS animal (ESS) model using the immune induction method. The aim is to explore the therapeutic impact of DOP on SS and its potential mechanism by assessing the salivary flow rate, lymphocyte infiltration in SMG, and inflammatory and apoptotic factors in mice.

2. Materials and Methods

2.1. Materials and Reagents. D. officinale was provided by Zhejiang Tiefengtang Biotechnology Co., Ltd. (Wenzhou, China) and identified by researcher Zhiguo Zhang. HCQ was obtained from Shangyao Zhongxi Pharmaceutical Co., Ltd. (Shanghai, China). Pilocarpine was purchased from Shandong Boshilun Furuida Pharmaceutical Co., Ltd. (Shandong, China). Pentobarbital sodium was acquired from Sigma (MO, USA). Freund's Complete Adjuvant (FCA) and Freund's Incomplete Adjuvant (FIA) were purchased from Biofroxx (Einhausen, Germany).

2.2. DOP Preparation. Fresh or dehydrated (dehydrated at a temperature of 70°C, crushed, and powdered through a No. 4 sieve) *D. officinale* was treated with 95% ethanol and extracted with water. The aqueous extract was filtered and concentrated under reduced pressure, ethanol was added to 80% alcohol content, stirred and left overnight, and centrifuged, and the precipitated part was dried to obtain DOP. The phenol-sulfuric acid method [16] was used to detect the polysaccharide content. The high polysaccharide yield was taken as the subsequent experimental drug, and the solution was prepared to the corresponding concentration before use.

2.3. Animal Ethics and Treatment. Eight-week-old female C57BL/6J mice (body mass: 18–22 g) were obtained from Hangzhou Medical College Experimental Animal Center (Hangzhou, China) and housed in a specific-pathogen-free-(SPF-) grade animal practical laboratory. The ESS mouse model was established, referring to Guggino et al. [17]. SMGs were removed from C57BL/6J mice and homogenized under aseptic conditions. The antigen concentration was determined by diluting PBS buffer to 5 mg/mL, emulsifying it to 2.5 mg/mL with the same volume of FCA, and setting it aside. After one week of adaptive feeding, the mice were injected with antigen at multiple points on the dorsal and caudal roots on day 0, with a total of 1 mL/each, and another booster injection on day seven. On day 14, SMG antigen was emulsified to 2.5 mg/mL in FIA and injected as before. At the fifth week, 50 mice of the ESS model with successful modeling were evaluated based on their water intake and salivary flow rate. Besides the control group, ESS mice were randomly assigned to the model, the HCQ, the DOP (43 mg/kg), the DOP (130 mg/kg), and the DOP (390 mg/kg) groups. Each group was given distilled water, 60 mg/kg HCQ, 43 mg/kg DOP, 130 mg/kg DOP, and 390 mg/kg DOP via gavage. The control group was given distilled water via gavage. The mice were gavaged continuously for three weeks and euthanized at the end of the eighth week. The Animal Ethics Committee of Hangzhou Medical College granted approval for all experimental procedures (Number: 2021-054).

2.4. General Observation of the Situation. The mice's health and hair loss were observed at the beginning of the experiment. The mice were weighed and recorded weekly.

2.5. Feeding and Drinking Rate Measurement. The feeding and drinking rates (g/h) of each group of mice were measured, recorded, and calculated weekly.

2.6. Salivary Flow Rate Measurement. The salivary flow rate (mg/min) was measured weekly. Mice were anesthetized and stimulated with an intraperitoneal injection of 0.5 mg/kg of pilocarpine to measure salivary secretion. Saliva was collected for 15 min.

2.7. Determination of Organ Indices. The mice's SMG, spleen, and thymus were removed from the ice box and washed with saline at 4°C, and then the excess water and blood were blotted on filter paper and weighed.

2.8. SMG Histological Analysis. After 48 h of immersion in 4% paraformaldehyde, the SMGs were embedded in paraffin. The wax blocks were sectioned, stained with hematoxylin and eosin (H&E), and evaluated using light microscopy. Two methods were employed to determine the level of lymphocyte infiltration in SMGs. First, histological grading determined the following: 1 = 1–5 leukocytic lesions; 2 = 5 or more lesions with no significant damage to the parenchyma; 3 = multiple foci of fusion with moderate degeneration of parenchymal tissue; and 4 = extensive lymphocyte infiltration and parenchyma destruction. Second, the labial gland biopsy was graded using the labial biopsy rubric in three discrete areas of SMG sections: grade 0, no lymphocytic infiltrate; grade I, mild lymphocytic infiltration; grade II, moderate lymphocytic infiltration but no infiltrative foci were formed; grade III, one lymphocytic infiltrate foci per 4 mm^2; and grade IV, two or more foci of lymphocytic infiltration per 4 mm^2 (note: ≥50 lymphocytes per 4 mm^2 of gland area refer to one focus of infiltration) [18].

2.9. Changes in Serum BAFF Levels Detected by Enzyme-Linked Immunosorbent Assay (ELISA). An assay was performed using a BAFF ELISA kit (Proteintech, Wuhan, China). The absorbance was measured at 450 nm and 630 nm using an enzyme marker (Thermo Fisher Scientific, Shanghai, China).

2.10. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR). The total RNA was extracted from SMGs using Trizol reagent, followed by reverse transcription into cDNA using the reagent kit from TaKaRa (Japan, #RR036A). The qRT-PCR was performed using SYBR qPCR Master Mix (Vazyme Biotech, Nanjing, China, #Q711). Primers for polymerase chain reaction are shown in Table 1. The $2^{-\Delta\Delta CT}$ method was used for relative quantification.

2.11. Western Blotting (WB). After extracting proteins from SMGs, they were separated using 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to polyvinylidene difluoride membranes (Millipore, USA). The blots were incubated with primary antibodies, including Anti-Bax (1 : 1000; Abcam, USA, #ab32503), Anti-Bcl-2 (1 : 1000; Abcam, USA, #ab182858), Anti-Cleaved Caspase-3 (1 : 5000; Abcam, USA, #ab214430), and β-Actin Mouse Monoclonal Antibody (1 : 1000; Biyuntian, China, #AF0003), at 4°C overnight, after being closed with 5% (w/v) skim milk for 1 h at room temperature. After 1 h of incubation with goat anti-rabbit antibody at room temperature, the signal was detected using an enhanced chemiluminescence kit (Epizyme, China, #SQ202) reagent.

2.12. Immunohistochemical Analysis. BAFF (1 : 200; Bioss, #bs-2431R), BAFFR (1 : 200; Bioss, #bs-2472R), Fas (1 : 100; Proteintech, #13098-1-AP), and Fas-L (1 : 200; Bioss, #bs-0216R) expressions were detected in mouse SMG tissue using immunohistochemistry. The positive expression cells were identified by the brownish color of cytoplasm or cytosol. The representative value of the section was determined for statistical analysis by taking the average absorbance from four randomly chosen fields of view.

2.13. Statistical Analysis. All data were expressed as mean ± standard deviation and statistically analyzed using GraphPad Prism software version 9.5. Statistical differences among groups were calculated with a one-way ANOVA followed by the Dunnett posttest at a 95% confidence interval. A significance level of less than 0.05 was deemed statistically significant.

3. Results

3.1. DOP Extraction. The polysaccharides were obtained from the fresh or dehydrated (dehydrated at 70°C, crushed, and powdered through No. 4 sieve) *D. officinale* by the water extraction and alcohol precipitation method (Figures 1(a)–1(c)). The phenol-sulfuric acid method [16] was used to detect the polysaccharide content. Based on the mass of herbal samples, the polysaccharide yields were 10.16% and 34.89%, respectively. The dried product yielded 3.43 times more than the fresh product (Figures 1(d) and 1(e)), and the dried product of *D. officinale* is easier to preserve, which can improve the stability of the experimental samples. Therefore, the polysaccharides obtained from the dry product preparation were used in subsequent experiments. DOP composition has been studied, and it is a crude polysaccharide composed of many different monosaccharides with different substance ratios [14, 16, 19, 20]. We summarized that these homogeneous polysaccharides are composed of glucose, mannose, galacturonic acid, glucuronic acid, and galactose in different molar ratios via specific structures (Figure 1(f)).

Table 1: Primers for polymerase chain reaction.

Primer	Forward	Reverse
TNF-α	CCACGCTCTTCTGTCTACTG	ACTTGGTGGTTTGCTACGA
IL-1β	GCAGCAGCACATCAACAAGAGC	AGGTCCACGGGAAAGACACAGG
IL-17	TGACGCCCACCTACAACATC	CATCATGCAGTTCCGTCAGC
Bax	AGCTGCAGAGGATGATTGCTG	CTGATCAGCTCGGGCACTTTA
Bcl-2	GAACTGGGGGAGGATTGTGG	GCATGCTGGGGCCATATAGT
Caspase-3	GAGATGGCTTGCCAGAAGATACC	AAGGGACTGGATGAACCACGAC
BAFF	CCACCGTGCCTCTGTTTTTG	CTTCTGCGGAGTGATGGGAT
Fas	AGGCCGCCCGCTGTTTTC	ACGAACCCGCCTCCTCAGC
Fas-L	GCCGCCACTGACCCCTCTAA	CCACACTCCTCGGCTCTTTT
GAPDH	TCCCACTCTTCCACCTTC	CTGTAGCCGTATTCATTGTC

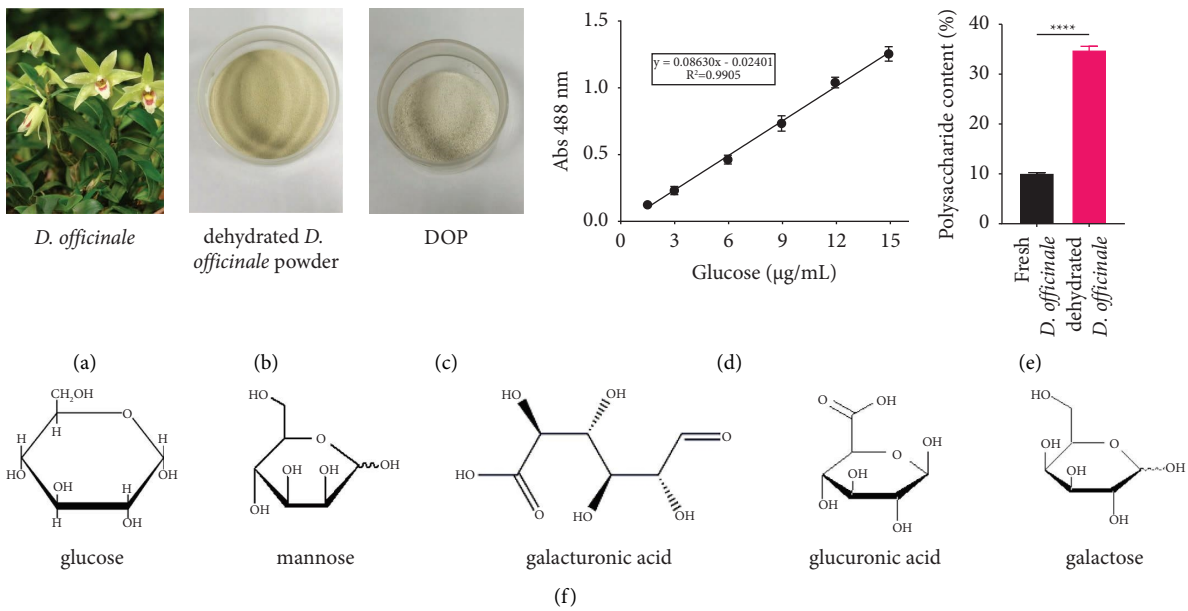

Figure 1: DOP extraction. (a) *D. officinale*. (b) Dehydrated *D. officinale* powder. (c) DOP powder. (d) Glucose standard curve. (e) DOP content in fresh and dehydrated products. (f) Structural formula of glucose, mannose, galacturonic acid, glucuronide, and galactose. **** $p < 0.0001$.

3.2. DOP Improves the General Condition of ESS Mice. The ESS mouse model was established, referring to Guggino et al. [17] (Figure 2(a)). From the third week onwards, the antigen-injected mice exhibited dull hair color, hair loss, tongue licking, mouth and lip scratching, and reduced activity. As the disease progressed, this phenomenon became more evident by the fifth week, and mice given antigen immunization induction showed increased drinking rate, significantly reduced feeding rate and salivary flow rate, and petechiae on the back and tail with progressive severity. Some mice also had skin ulceration (Figures 2(d)–2(f)). After gavage administration of the drug, the hair loss and skin ulceration of mice in each administration group improved to different degrees compared to the model group (Figure 2(b)). During the experiment, body weight did not change in drug administration groups compared to the model group (Figure 2(c)). Furthermore, after three weeks of continuous administration, the feeding rate of the model group mice decreased while the drinking rate increased, exhibiting a significant disparity at week eight ($p < 0.001$). Following one week of treatment, the HCQ ($p < 0.05$), DOP (130 mg/kg, $p < 0.05$), and DOP (390 mg/kg, $p < 0.01$) groups displayed a noteworthy augmentation in food intake compared to the model group. After two weeks of treatment, the HCQ, DOP (130 mg/kg), and DOP (390 mg/kg) groups exhibited a significant reduction in the drinking rate ($p < 0.05$). After three weeks of treatment, all dosing groups exhibited therapeutic effects, with the HCQ and DOP (390 mg/kg) groups demonstrating the best results ($p < 0.001$, $p < 0.0001$, Figures 2(d) and 2(e)). The findings indicated a significant decrease in salivary flow rate in the model group, gradually worsening as the disease advanced. After one week of treatment, the DOP (390 mg/kg) group exhibited a significant increase in salivary flow rate ($p < 0.05$). This increase continued in the DOP group after three weeks of treatment, and the salivary flow rate in the HCQ and DOP (390 mg/kg) groups ($p < 0.0001$) approached those of the control group (Figure 2(f)). This indicates that DOP can improve the hair and salivary secretion of SS mice and has a certain improvement effect on

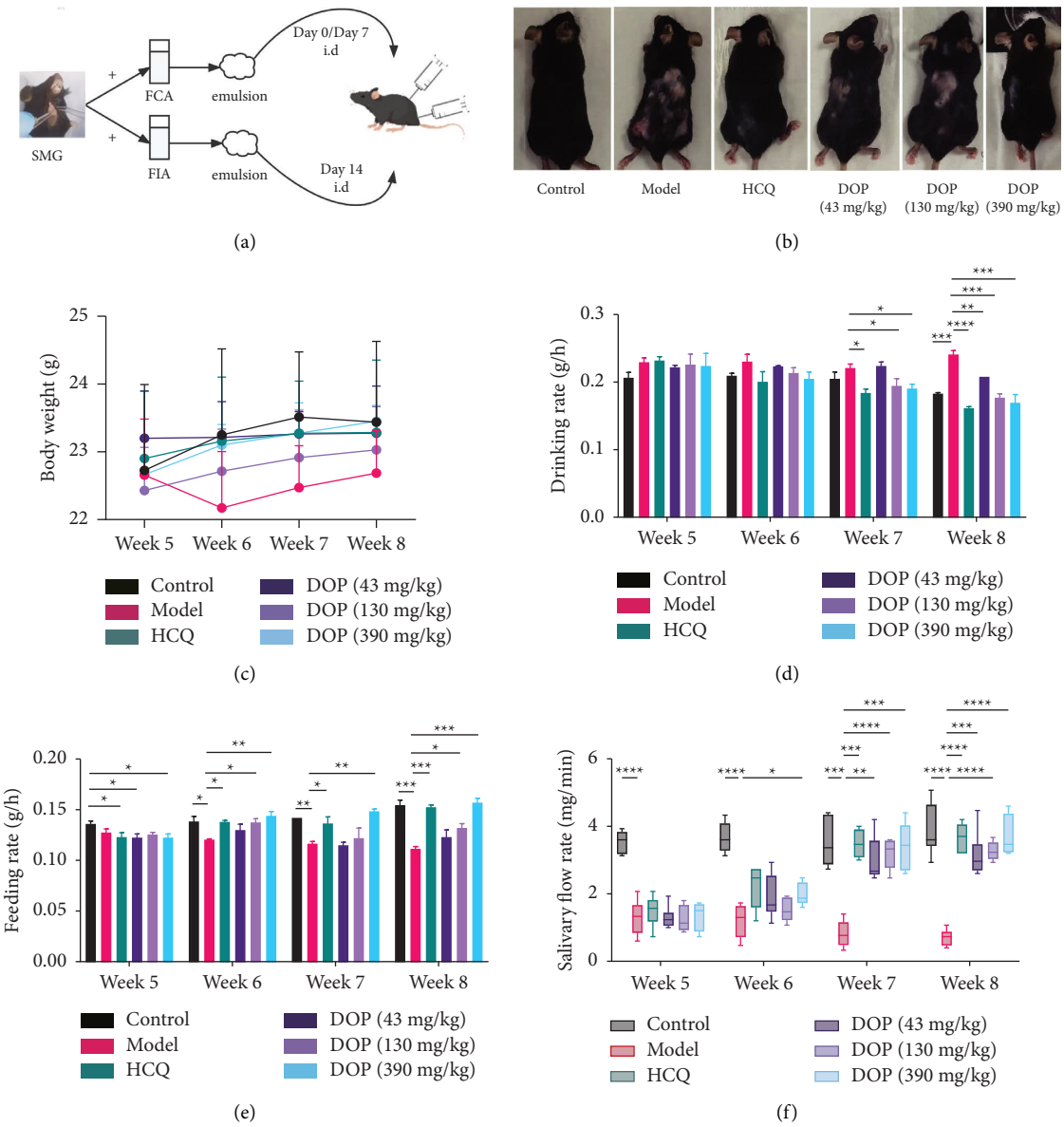

Figure 2: DOP improves the general condition of ESS mice. (a) Establishment of ESS mouse model (by Figdraw). (b) Hair loss of mice at week eight. (c) Weekly fluctuations in the mice body weight. (d) Weekly changes in drinking rate. (e) Weekly changes in feeding rate. (f) Weekly changes in salivary flow rate. A value of $p < 0.05$ was considered statistically significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ vs. mice in the model group.

their dry mouth and thirst symptoms. The efficacy of DOP (390 mg/kg) is comparable to that of HCQ.

3.3. DOP Alleviates Organ Damage in ESS Mice. The SMG has the most pronounced SS symptoms and pathologically shows a large lymphocytic infiltration with cellular necrosis. To a certain degree, the thymus and spleen indices can indicate the strength of the body's immune function. Our experimental findings indicated that the SMG index and thymus index decreased significantly ($p < 0.0001$), while the spleen index increased significantly ($p < 0.0001$) in the model group of mice than in the control group. After administering the respective medications, the SMG index and thymus index increased significantly, while the spleen index decreased significantly in all treatment groups than in the model group. Notably, the HCQ, DOP (130 mg/kg), and DOP (390 mg/kg) groups had the best effect ($p < 0.0001$, Figures 3(a)–3(c)). Except for the control group, histological examination revealed that the SMG tissues of all groups of mice had varying degrees of lymphocyte infiltration, with the infiltrated lymphocytes mostly distributed around the blood vessels and ducts. We utilized two histopathological assessment techniques (histopathological score and lesion count) to assess the extent of lymphocyte infiltration in the SMG. The findings indicated that the model group exhibited a significant severity of infiltration, primarily characterized by increased lymphocyte infiltration foci and area of

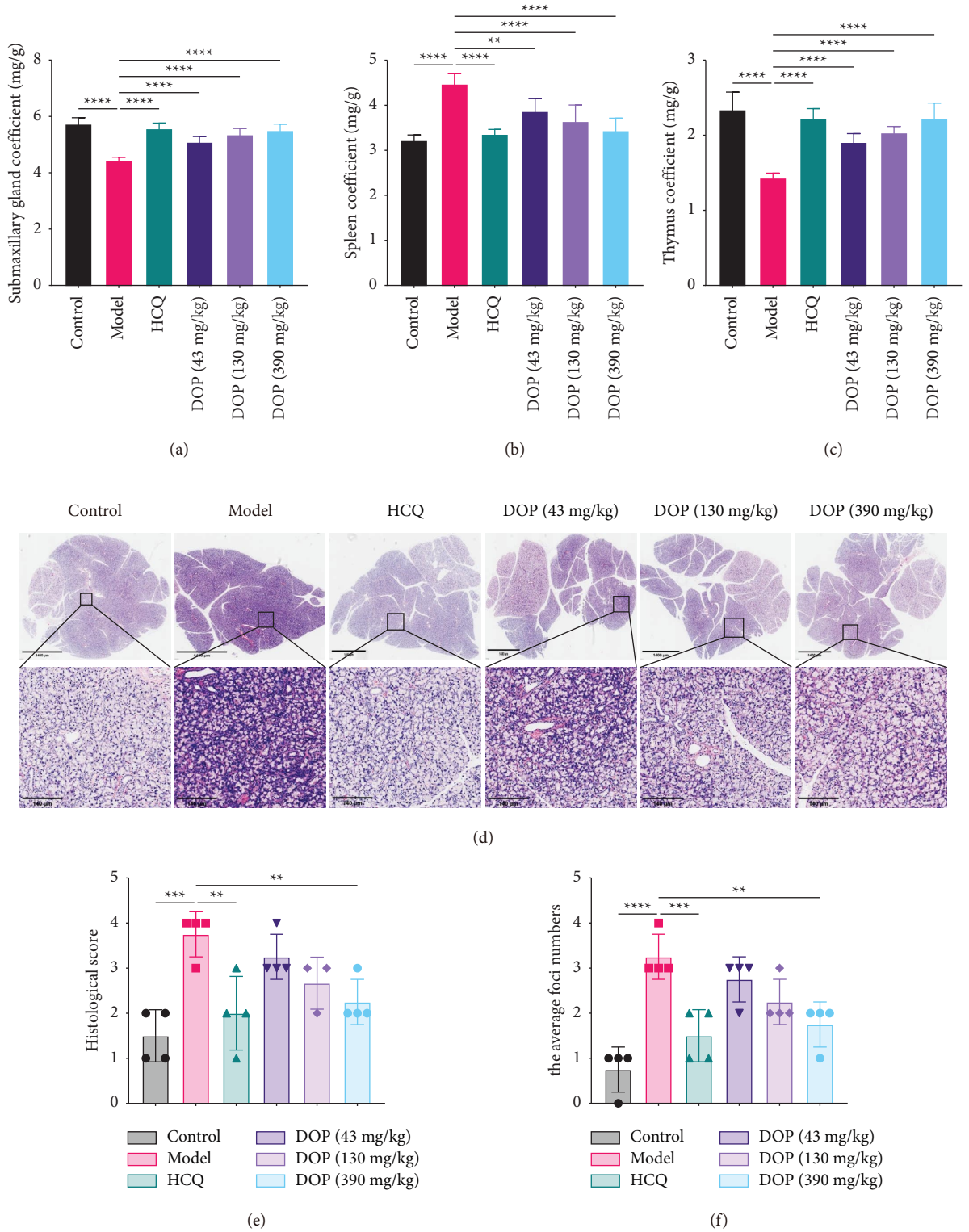

Figure 3: DOP alleviates organ damage in ESS mice. (a–c) SMG, spleen, and thymus indices (organ mass (mg)/animal body mass (g)). (d) Representative images depicting H&E-stained sections of SMG in each group of mice. The top panel displays the image under 10x objective, while the bottom panel showcases the image under 20x objective in the black rectangular box in the upper panel. (e, f) Two methods of histological analysis (histological score and number of lesions). A value of $p < 0.05$ was considered statistically significant. $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ vs. mice in the model group.

infiltrating foci, compared to the control group ($p < 0.0001$). Additionally, there were instances of blood vessels and duct dilation, varying glandular vesicle sizes, and a minor number of gland atrophy. However, following the application of medication, there was a notable decline in histological scores, lymphocyte infiltration, and reduction of foci in all groups receiving treatment, particularly in the HCQ ($p < 0.01$, $p < 0.001$) and DOP ($p < 0.01$, 390 mg/kg) groups (Figures 3(d)–3(f)). The above results demonstrated that DOP could protect organs, such as SMG, thymus, and spleen of ESS mice, from tissue damage due to autoantigen immune-induced generation.

3.4. DOP Regulates the Inflammatory and Apoptotic Factor Expression in the SMG of ESS Mice. The SMG is a non-lymphoid organ with minimal immune cell infiltration under normal conditions. In our experiments, the model group exhibited a significant influx of lymphocytes in the SMG. Imbalanced cell proliferation and the emergence of diverse autoimmune disorders can arise from aberrant control of apoptosis [21]. Therefore, we examined the effect of DOP on inflammatory factor gene expression in the SMG of ESS mice using qRT-PCR. The results revealed that DOP curbed the proinflammatory factor (TNF-α, IL-1β, and IL-17) mRNA expression levels in the SMG of ESS mice after three weeks of continuous administration of DOP. The greatest changes in TNF-α were observed following DOP (43 mg/kg) and DOP (390 mg/kg) stimulation, 1.48- and 3.14-fold, respectively (Figure 4(a)). DOP (390 mg/kg) significantly downregulated IL-1β gene transcription ($p < 0.05$, Figure 4(b)). DOP (43 mg/kg and 390 mg/kg) downregulated IL-17 by 1.21- and 1.38-fold, respectively (Figure 4(c)).

Next, we detected autoimmune-induced apoptosis. We examined the apoptotic factor expressions (Bax, Caspase-3, and Bcl-2) in SMG using qRT-PCR and WB. The results demonstrate that DOP effectively reduced the Bax and Caspase-3 mRNA and protein levels in the SMG of ESS mice. Furthermore, DOP (390 mg/kg) had a greater inhibitory effect than DOP (43 mg/kg) (Figures 4(d) and 4(f)–4(h)). It is noteworthy that DOP (390 mg/kg) significantly increased the protein expression of Bcl-2 ($p < 0.01$), but there was no significant change in Bcl-2 mRNA expression (Figures 4(e) and 4(h)). As exposed above, large lymphocyte infiltration in the SMG of ESS mice severely contributes to an inflammatory environment and apoptotic cell death, while DOP can effectively regulate proinflammatory factors and apoptosis in the SMG of ESS mice.

3.5. DOP Downregulates BAFF/BAFFR Expression in ESS Mice. BAFF is an endogenous protein with a crucial function in the B lymphocyte differentiation and proliferation. The binding of BAFF to its receptor BAFFR stimulates the anti-apoptotic gene transcription from the Bcl-2 family, thereby extending B cell survival, inducing inflammatory factor production, and causing autoimmune disorder progression [3]. We assessed the BAFF levels in the mice serum and analyzed the BAFF/BAFFR gene and protein expression in SMG using qRT-PCR and immunohistochemistry to examine the impact of DOP on B cells in ESS mice. The findings indicated that the BAFF concentrations in the mice blood were considerably elevated in the experimental group than in the control group ($p < 0.0001$). Following DOP administration, the BAFF levels in the serum of ESS mice decreased dose dependently, with DOP (390 mg/kg) significantly reducing the BAFF levels in ESS mice ($p < 0.001$) (Figure 5(a)). Meanwhile, DOP downregulated BAFF gene expression in SMG of ESS mice, whereas DOP (43 mg/kg and 390 mg/kg) downregulated BAFF gene by 1.24- and 1.96-fold, respectively (Figure 5(b)). After three weeks of DOP administration, the immunohistochemical analysis revealed a significant decrease in BAFF and BAFFR protein expression in the SMG of each DOP dose group. The DOP (390 mg/kg) group exhibited the most notable reduction ($p < 0.0001$, $p < 0.001$, Figures 5(c)–5(f)). The aforementioned results indicate that BAFF is produced in the serum and SMG of ESS mice, causing B cell recruitment and activation, amplifying the inflammatory response, and destroying the SMG. DOP can downregulate the BAFF and BAFFR expressions.

3.6. DOP Downregulates Fas/Fas-L mRNA and Protein Expression in the SMG of ESS Mice. Fas and its ligand Fas-L are important apoptosis regulators and members of the regulatory induction family, closely related to autoimmune diseases [7]. qRT-PCR and immunohistochemistry techniques were employed to assess the Fas and Fas-L gene and protein expressions in the SMG and the impact of DOP on Fas and its ligand Fas-L in the SMG of ESS mice. The findings indicated that the Fas and its ligand Fas-L mRNA levels were considerably elevated in the SMG of the model group than in the control group ($p < 0.0001$). However, DOP significantly reduced Fas and Fas-L gene expression in the SMG of ESS mice, with the most notable reduction observed in the DOP (390 mg/kg) group, presenting a 3.02-fold decrease for Fas gene and a 2.38-fold decrease for Fas-L gene. Additionally, DOP (43 mg/kg) downregulated the Fas gene by 0.98-fold and the Fas-L gene by 1.55-fold (Figures 6(a) and 6(b)). The immunohistochemical results revealed that the Fas and Fas-L protein expressions were significantly reduced in SMG of each DOP dose group after three weeks of DOP administration. The reduction was most pronounced in the DOP (390 mg/kg) group ($p < 0.0001$, $p < 0.01$). Additionally, the immunohistochemical findings agreed with the qRT-PCR results (Figures 6(c)–6(f)). The aforementioned findings indicate that DOP can downregulate Fas and Fas-L expressions in the SMG of ESS mice, possibly playing a regulatory role in apoptosis.

4. Discussion

The pathogenesis of SS is still unclear, and herbal medicine has great therapeutic potential for SS. DOP is one of the primary substance bases extracted from the stem of *D. officinale*, and it is relatively nontoxic and has few side effects [13, 14, 22, 23]. Extracting active ingredients has

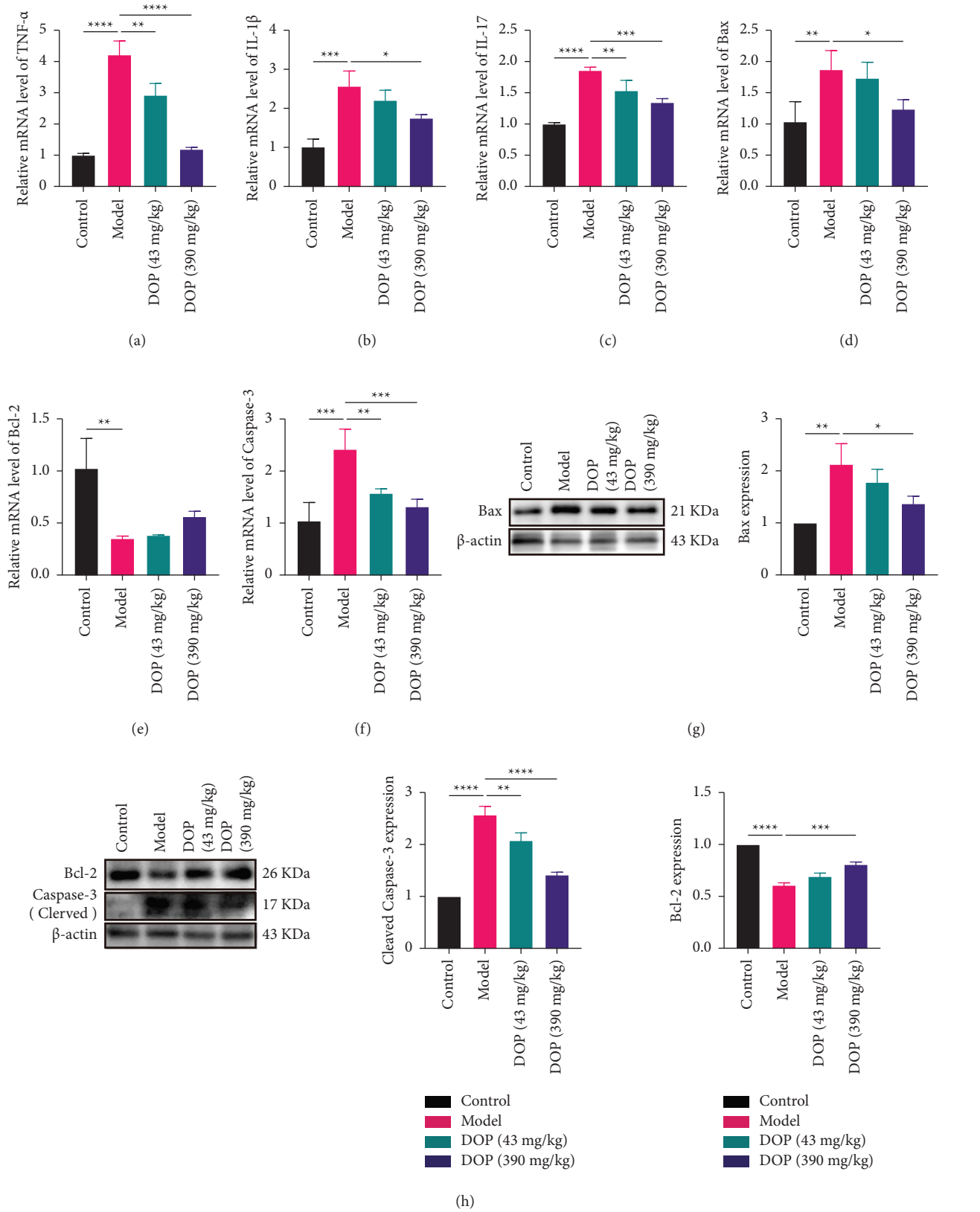

Figure 4: DOP regulates the inflammatory and apoptotic factor expressions in the SMG of ESS mice. (a–c) qRT-PCR to detect the TNF-α, IL-1β, and IL-17 expressions in SMG. (d–f) qRT-PCR to detect the Bax, Bcl-2, and Caspase-3 expressions in SMG. (g, h) Bax, Bcl-2, and Cleaved Caspase-3 protein expression levels in SMG were evaluated using western blot. A value of $p < 0.05$ was considered statistically significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ vs. mice in the model group.

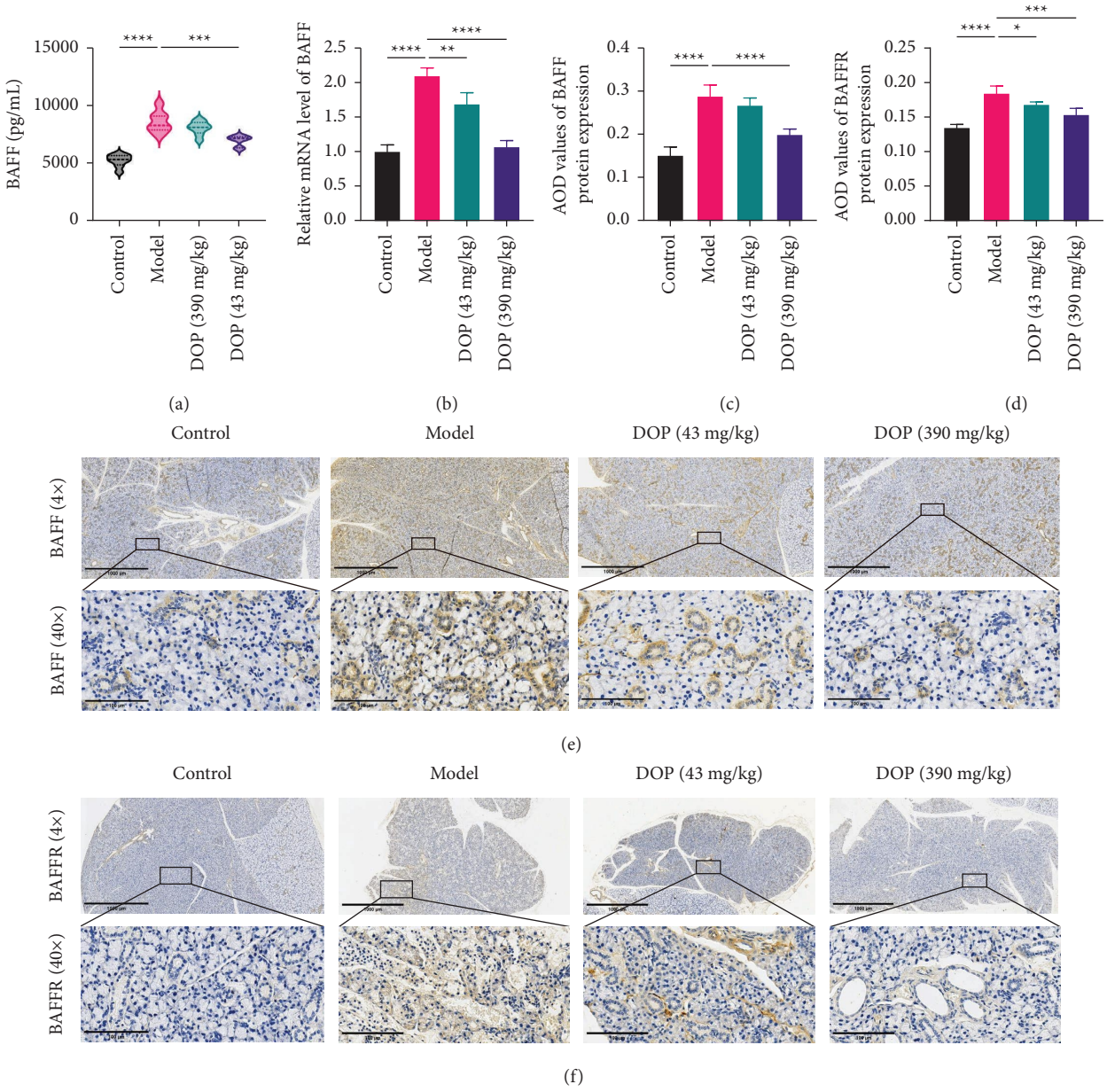

Figure 5: DOP downregulates BAFF/BAFFR expression in ESS mice. (a) ELISA to detect BAFF content in mouse serum. (b) qRT-PCR to detect the BAFF expression in SMG. (c, d) Mean optical density values of BAFF and BAFFR protein expression in SMG of each group of mice. Immunohistochemical methods to detect the changes of BAFF (e) and BAFFR (f) protein expression in SMG of each group of mice. The top panel displays the image under a 4x objective lens, while the bottom panel showcases the image under a 40x objective lens in the black rectangular box in the upper panel. A value of $p < 0.05$ was considered statistically significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ vs. mice in the model group.

always been challenging in applying traditional Chinese medicine. Extracting as many active components, such as polysaccharides, from *D. officinale* can help improve the application of *D. officinale*. Therefore, we investigated whether there was a difference in polysaccharide extraction from *D. officinale* using different pretreatment methods (fresh or dehydrated). The findings indicated that the dehydrated product's yield was 3.43 times higher than the fresh product's yield. It was suggested that polysaccharide yield could be significantly improved by pretreatment of fresh *D. officinale* by drying, crushing, and passing the powder through No. 4 sieve while maintaining all other extraction conditions. Liu et al. [24] identified the polysaccharide extracted by the water extraction-alcohol precipitation method as D-Glcp and D-Manp with a ratio of 1.00 : 4.41. Li et al. [16] used Fourier transform infrared spectrophotometer analysis and high-performance liquid chromatography to identify the polysaccharides extracted by the water extraction-alcohol precipitation method. The final DOP identification consisted of mannose, glucose, and

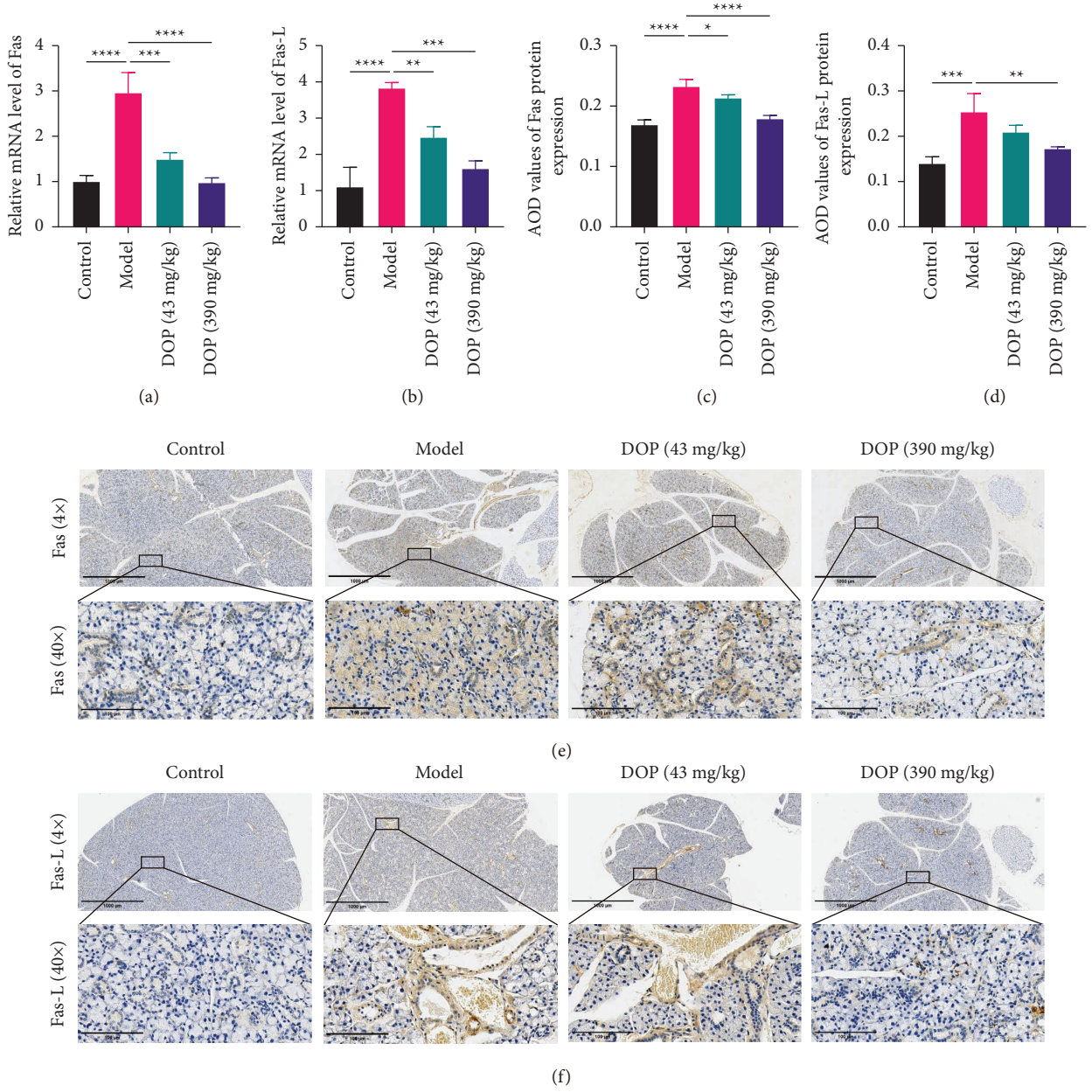

Figure 6: DOP downregulates Fas/Fas-L mRNA and protein expression in the SMG of ESS mice. (a, b) qRT-PCR to detect Fas and Fas-L expressions in SMG. (c, d) The mean optical density values of Fas and Fas-L protein expressions in SMG of each group of mice. Immunohistochemical methods to detect the Fas (e) and Fas-L (f) protein expression changes in the SMG of each group of mice. The top panel displays the image under 4x objective, while the bottom panel showcases the image under 20x objective in the black rectangular box in the upper panel. A value of $p < 0.05$ was considered statistically significant. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, and $^{****}p < 0.0001$ vs. mice in the model group.

galacturonic acid, comprising 61.28%, 31.87%, and 2.53% of the total mass, respectively. Thus, DOP is primarily composed of monosaccharides, such as glucose, mannose, galacturonic acid, glucuronic acid, and galactose, in various molar ratios via specific structures. Many studies have demonstrated the multiple therapeutic effects of DOP in various disease models, such as immunomodulatory, anti-inflammatory, antioxidant, and hypoglycemic [25]. However, few studies focused on DOP for treating SS. Therefore, this study extended the potential mechanism through which DOP protects the SMG from damage in a mouse model of ESS.

Ideal experimental animal models are important to explore disease pathogenesis. The study of human SS pathogenesis has extensively utilized two primary categories of animal models: spontaneous and induced. Spontaneous animal models frequently produce SS symptoms secondary to another disease, and the commonly used species include NOD/Ltj mice, MRL/lpr mice, and TGF-β1 knockout mice [26]. Induced animal models are frequently induced using

peptide antigens, protein antigens, and carbonic anhydrase II to mimic the manifestations of human SS. Commonly used species include CD-1 mice, C57BL/6 mice, and BALB/c mice. The morphological structure and functional metabolism of the induced mouse model are altered, with impaired exocrine function of salivary glands, lymphocyte infiltration in tissues, dilated blood vessels and ducts, atrophy of glandular vesicles, and autoantibody production [27]. This study used an antigen-induced method to construct an animal model of ESS, facilitating the investigation of the effect of DOP on SS. Previous studies have demonstrated that SS model mice produce significantly less saliva than normal mice. Therefore, salivary secretion levels can be used to determine whether SS occurs in mice [28]. This study discovered that mice with antigen-induced SS model had severe hair loss, increased water intake, and significantly reduced food intake and salivary flow rate compared to control mice. Pathological examination revealed lymphocyte infiltration in SMG and lymphatic foci formation. This suggests that the antigen-induced SS model mice possess the pathological characteristics and laboratory diagnostic indices of SS, indicating the successful construction of the ESS mouse model.

This study demonstrated that DOP had a notable alleviating and therapeutic effect on disease progression in ESS mice. DOP treatment reduced hair loss and drinking rate and increased feeding rate and salivary flow in ESS mice. The SMG index can be used as an objective evaluation index of the functional status of the SMG in SS mice. The thymus and spleen are the sites of immune cell formation and cytokine production in mammals, constituting the body's defense system. Following antigenic stimulation, the immune response is initiated in ESS mice. In the model group of mice, the SMG index and thymus index decreased significantly, while the spleen index increased significantly. Compared to the control group, the SMG and thymus indicators notably increased, while the spleen indicator significantly decreased in mice subjected to DOP treatment. Histological examination, histological score, and number of lesions in the SMG of mice revealed a decrease in histological score, lymphocyte infiltration, and lesions in each treatment group, and SMG tissue destruction was improved in ESS mice, especially in the DOP (390 mg/kg) group. This suggests that DOP treatment can improve the symptoms of dry mouth in ESS mice and protect organs, such as SMG, thymus, and spleen, from tissue damage due to autoantigen immune induction.

pSS is highlighted as a systemic immune inflammatory disease caused by lymphocyte infiltration, reflecting its highly hyperactive B lymphocyte function and abnormal T lymphocyte function [1]. The epithelial cells of the exocrine gland tissue recruit and activate B and T lymphocytes by secreting cytokines. Numerous lymphocytes are observed to accumulate into foci in the interstitium of the exocrine gland tissue, disrupting exocrine gland function [29]. TNF-α is an important proinflammatory factor that promotes phagocytosis of neutrophils, resists infection, induces cell proliferation and differentiation, and significantly impacts immune disorders [30]. IL-1β is an important mediator of inflammatory responses, such as the reciprocal interaction between IL-1β and TNF-α frequently at inflammation sites [31]. T-helper 17 (Th17) cells are key players in mucosal diseases and inflammatory diseases and secrete the characteristic cytokine IL-17 [32], which has been observed to be present at higher levels in the peripheral blood of individuals with SS [33]. Yamano et al. [34] reported that the mRNA levels for inflammatory cytokines, including TNF-α and IL-1β, were markedly elevated in SMG tissues of NOD mice than in the normal mice. Hwang et al. [35] discovered that retinoic acid could alleviate SS symptoms by reducing IL-17 levels. Li et al. [2] indicated that treating NOD mice with total peony glycosides could reduce TNF-α and IL-1β levels in SMG and downregulate IL-17 mRNA expression in SMG, which had a therapeutic effect on SS. In this study, the proinflammatory marker expressions, including TNF-α, IL-1β, and IL-17, were markedly elevated in the SMG of mice in the model group than in the control group. However, after administering DOP, these levels were significantly reduced, with the most pronounced effect observed at a dosage of DOP (390 mg/kg). The findings indicate that DOP can protect SMG by inhibiting the release of inflammatory factors. However, additional research is required to explore the precise mechanism.

SMG epithelial cells produce BAFF, recruit and activate B cells, amplify the inflammatory response, and disrupt the exocrine glands [36]. BAFF, a recent addition to the tumor necrosis factor group, plays a crucial role in developing B cells and prolonging B cell survival [37]. BAFFR is a specific receptor for BAFF, and BAFF promotes the mouse B cell maturation, prolongs the mature human B cell lifespan, and promotes paracrine T cell activation primarily via BAFFR. BAFF binds to BAFFR to promote the anti-apoptotic gene transcription of the Bcl-2 family, preventing the transition of autoreactive B cells from immature to mature process deleted, thereby prolonging the B cell survival cycle and leading to autoimmune disease development [3]. Therefore, BAFF and BAFFR are crucial in SS pathogenesis [38]. Domestic and international studies demonstrated a notable increase in BAFF levels in the peripheral blood and tissues of individuals diagnosed with SS [39, 40]. Nevertheless, there are some conflicting data regarding BAFFR expression [41–44]. Li et al. [2] identified that total peony glucoside treatment of NOD mice downregulated BAFF mRNA expression in SMG and positively impacted SS. Our research indicated that the BAFF serum levels were considerably elevated in the model group mice than those of the control group. Additionally, BAFF's gene and protein expression and its receptor BAFFR increased significantly in the SMG. DOP could decrease the BAFF and its receptor levels in ESS mice. This suggests that DOP can prevent pathological autoimmunity by decreasing the BAFF and BAFFR levels, eliminating self-reactive B cells at various stages.

The normal emission of apoptotic signals is necessary to maintain immune cell homeostasis in vivo and to sustain a normal immune response [45]. Increased levels of apoptosis in SG epithelial cells can activate auto-reactive lymphocytes and induce autoimmune diseases similar to pSS [6]. According to suggestions, the abnormal immune response in SS patients is closely related to their abnormal lymphocyte apoptosis [46]. Apoptosis has two major signaling pathways. When Fas

specifically binds to the Fas ligand (Fas-L), it induces Fas-expressing cells to initiate the apoptotic program [47]. Caspase-3 is an important downstream effector protease of Fas/Fas-L. When Fas and Fas-L specifically bind, Caspase-3 will be activated, and direct action by Caspase-3 on functional proteins of cells causes their cleavage and ultimately contributes to apoptosis [48]. The self-reactive B cell elimination was observed to involve the Fas system in immunodeficient mice [49]. Bcl-2 and Bax are key protein substances that regulate apoptosis. Bcl-2 acts as an anti-apoptotic protein and inhibits apoptotic protein activation. Bcl-2 overexpression enhances cell viability. Bax, the most prominent member of apoptosis-promoting proteins, has an antagonistic effect on Bcl-2. Overexpressed Bcl-2 binds to Bax to form a dimer, inhibiting altered mitochondrial permeability and Bax activity, thereby impeding apoptosis [50]. A study revealed reduced Bcl-2 protein expression and increased Bax and Caspase-3 protein expression in NOD/Ltj mice [51]. Bcl-2, Fas, and Fas-L plasma levels in individuals diagnosed with pSS appear to be associated with the inflammation severity and present a notable increase compared to those without the condition [7]. This experiment identified that Fas, Fas-L, and Bax mRNA content, as well as Caspase-3 gene and protein expressions, were elevated in the SMG of mice in the model group, and the Bcl-2 gene and protein expressions were decreased. After DOP intervention, Fas, Fas-L, and Bax mRNA content, the Caspase-3 gene and protein expression in the SMG of ESS mice were decreased, whereas Bcl-2 protein expression was increased, indicating that DOP could regulate Bax, Bcl-2, and Caspase-3 expressions probably via the inhibition of the Fas system. Furthermore, the inconsistent Bcl-2 mRNA and protein expression in the SMG of ESS mice after DOP administration may be influenced by BAFF and its receptor BAFFR and Fas and its receptor Fas-L. Further studies can be conducted on the interaction between the BAFF/BAFFR and Fas/Fas-L signaling pathways and SS in the future.

DOP, the primary active component of *D. officinale*, is widely recognized as an immune-boosting medication due to its traditional tonic properties, supported by a plethora of literature. However, according to this experiment results, DOP not only has immune-enhancing effects [9, 52] but also inhibits proinflammatory factors and apoptosis via different pathways in a mouse model of hyperimmune ESS, which provides a rationale for the role of DOP with bidirectional immunomodulatory functions. Additionally, our laboratory will further investigate the BAFF/BAFFR and Fas/Fas-L signaling pathways, as well as the interaction between these two pathways, in the context of the ESS mice experiment in which DOP treatment reduces inflammation and apoptosis. Future research should focus on more potential mechanisms of DOP treatment of SS symptoms.

5. Conclusions

Based on the aforementioned experiments, our findings suggest that DOP therapy reduces SS symptoms in ESS mice, potentially by inhibiting proinflammatory factors and apoptosis. The mechanism may decrease the BAFF/BAFFR and Fas/Fas-L expression in serum or SMG, thus producing a therapeutic effect on SS mice. The present research offers evidence and understanding regarding the pharmacodynamic impacts of DOP in treating SS, establishing the groundwork for its potential clinical utilization.

Data Availability

The data used to support the findings of this study are available from the corresponding authors upon request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

This work was financially supported by the Zhejiang Provincial Department of Science and Technology (no. LGF21H280008), National Natural Science Foundation of China (no. 82174050), Educational Commission of Zhejiang Province (nos. Y202045371 and Y202045418), Hangzhou Medical College (no. YS2021015), Health Commission of Zhejiang Province (no. 2021KY130), and Administration of Traditional Chinese Medicine of Zhejiang Province (no. 2022ZB221).

References

[1] K. Rui, Y. Hong, Q. Zhu et al., "Olfactory ecto-mesenchymal stem cell-derived exosomes ameliorate murine Sjögren's syndrome by modulating the function of myeloid-derived suppressor cells," *Cellular and Molecular Immunology*, vol. 18, no. 2, pp. 440–451, 2021.

[2] B. Li, G. Liu, R. Liu et al., "Total glucosides of paeony (TGP) alleviates Sjogren's syndrome through inhibiting inflammatory responses in mice," *Phytomedicine*, vol. 71, Article ID 153203, 2020.

[3] Y. Saito, Y. Miyagawa, K. Onda et al., "B-cell-activating factor inhibits CD20-mediated and B-cell receptor-mediated apoptosis in human B cells," *Immunology*, vol. 125, no. 4, pp. 570–590, 2008.

[4] T. Zhan, B. Wang, J. Fu et al., "Artesunate inhibits Sjögren's syndrome-like autoimmune responses and BAFF-induced B cell hyperactivation via TRAF6-mediated NF-κB signaling," *Phytomedicine*, vol. 80, Article ID 153381, 2021.

[5] Z. Tan, L. Wang, and X. Li, "Composition and regulation of the immune microenvironment of salivary gland in Sjögren's syndrome," *Frontiers in Immunology*, vol. 13, Article ID 967304, 2022.

[6] E. K. Kapsogeorgou, H. M. Moutsopoulos, and M. N. Manoussakis, "Functional expression of a costimulatory B7.2 (CD86) protein on human salivary gland epithelial cells that interacts with the CD28 receptor, but has reduced binding to CTLA4," *The Journal of Immunology*, vol. 166, no. 5, pp. 3107–3113, 2001.

[7] S. Benchabane, A. Slimani-Kaddouri, D. Acheli, T. Bendimerad-Iratene, R. Mesbah, and C. Touil-Boukoffa, "Association between increased bcl-2, Fas and FasL levels and inflammation extent in labial salivary glands during primary Sjögren's syndrome," *Endocrine, Metabolic & Immune Disorders- Drug Targets*, vol. 22, no. 3, pp. 328–338, 2022.

[8] W. Song, J. Luo, H. Shen, and D. Li, "Research progress on the distribution of Chinese medical evidence and diagnostic criteria for primary Sjögren's syndrome," *Chinese Journal of Traditional Chinese Medicine*, vol. 35, no. 1, pp. 380–382, 2020.

[9] X. Wei, W. Sun, P. Zhu et al., "Refined polysaccharide from *Dendrobium devonianum* resists H1N1 influenza viral infection in mice by activating immunity through the TLR4/MyD88/NF-κB pathway," *Frontiers in Immunology*, vol. 13, Article ID 999945, 2022.

[10] Q. Zeng, C.-H. Ko, W.-S. Siu et al., "Polysaccharides of *Dendrobium officinale* Kimura & Migo protect gastric mucosal cell against oxidative damage-induced apoptosis in vitro and in vivo," *Journal of Ethnopharmacology*, vol. 208, pp. 214–224, 2017.

[11] National Pharmacopoeia Committee, *Pharmacopoeia of the People's Republic of China*, China Pharmaceutical Science and Technology Press, China, 2020.

[12] Y. Wu, Z. Zhao, B. Wu et al., "A dendrobium chewing gum with tonicity and stomach benefit and its preparation method and application," *Center for High-rate Nanomanufacturing Patent*, vol. 5, 2021.

[13] K. Wang, X. Yang, Z. Wu et al., "*Dendrobium officinale* polysaccharide protected CCl4-induced liver fibrosis through intestinal homeostasis and the LPS-TLR4-NF-κb signaling pathway," *Frontiers in Pharmacology*, vol. 11, p. 240, 2020.

[14] W. Jiang, W. Ruan, and Z. Wang, "*Dendrobium officinale* polysaccharide inhibits vascular calcification via anti-inflammatory and anti-apoptotic effects in chronic kidney disease," *The Federation of American Societies of Experimental Biology Journal*, vol. 36, no. 9, Article ID e22504, 2022.

[15] Y. Xiao, L. Luo, J. Yang, and X. Liang, "Study on the therapeutic effect of *Dendrobium officinale* on patients with v Sjögren's syndrome and its possible mechanism," *Laboratory Medicine and Clinical*, vol. 14, no. 23, pp. 3464–3466, 2017.

[16] B. Li, H.-Y. Wang, J.-H. Huang et al., "Polysaccharide, the active component of *Dendrobium officinale*, ameliorates metabolic hypertension in rats via regulating intestinal flora-SCFAs-vascular Axis," *Frontiers in Pharmacology*, vol. 13, Article ID 935714, 2022.

[17] G. Guggino, X. Lin, A. Rizzo et al., "Interleukin-25 Axis is involved in the pathogenesis of human primary and experimental murine Sjögren's syndrome," *Arthritis and Rheumatology*, vol. 70, no. 8, pp. 1265–1275, 2018.

[18] Y. Wang, J. Xiao, Y. Duan et al., "Lycium barbarum polysaccharide ameliorates Sjögren's syndrome in a murine model," *Molecular Nutrition and Food Research*, vol. 65, no. 11, Article ID e2001118, 2021.

[19] B. Wu, Y. Wu, Z. Zhao, and H. Liu, "Research progress on immunomodulatory effects and related active ingredient polysaccharides of *Dendrobium officinale*," *Chinese Herbal Medicine*, vol. 50, no. 21, pp. 5373–5379, 2019.

[20] X. Xing, S. W. Cui, S. Nie, G. O. Phillips, H. D. Goff, and Q. Wang, "Study on *Dendrobium officinale* O-acetyl-glucomannan (Dendronan®): Part II. Fine structures of O-acetylated residues," *Carbohydrate Polymers*, vol. 117, pp. 422–433, 2015.

[21] Q. Zhang, J. Liu, M. Zhang et al., "Apoptosis induction of fibroblast-like synoviocytes is an important molecular-mechanism for herbal medicine along with its active components in treating rheumatoid arthritis," *Biomolecules*, vol. 9, no. 12, p. 795, 2019.

[22] S.-J. Sun, P. Deng, C.-E. Peng, H.-Y. Ji, L.-F. Mao, and L.-Z. Peng, "Extraction, structure and immunoregulatory activity of low molecular weight polysaccharide from *Dendrobium officinale*," *Polymers*, vol. 14, no. 14, p. 2899, 2022.

[23] X. Lin, P.-C. Shaw, S. C.-W. Sze, Y. Tong, and Y. Zhang, "*Dendrobium officinale* polysaccharides ameliorate the abnormality of aquaporin 5, pro-inflammatory cytokines and inhibit apoptosis in the experimental Sjögren's syndrome mice," *International Immunopharmacology*, vol. 11, no. 12, pp. 2025–2032, 2011.

[24] Y. Liu, L. Yang, Y. Zhang et al., "*Dendrobium officinale* polysaccharide ameliorates diabetic hepatic glucose metabolism via glucagon-mediated signaling pathways and modifying liver-glycogen structure," *Journal of Ethnopharmacology*, vol. 248, Article ID 112308, 2020.

[25] W.-H. Chen, J.-J. Wu, X.-F. Li et al., "Isolation, structural properties, bioactivities of polysaccharides from *Dendrobium officinale* Kimura et. Migo: a review," *International Journal of Biological Macromolecules*, vol. 184, pp. 1000–1013, 2021.

[26] Y. Tian, H. Yang, N. Liu, Y. Li, and J. Chen, "Advances in pathogenesis of Sjögren's syndrome," *Journal of Immunology Research*, vol. 2021, Article ID 5928232, 8 pages, 2021.

[27] Y. Gao, Y. Chen, Z. Zhang, X. Yu, and J. Zheng, "Recent advances in mouse models of Sjögren's syndrome," *Frontiers in Immunology*, vol. 11, p. 1158, 2020.

[28] P. L. Cohen and A. McCulloch, "Fingolimod reduces salivary infiltrates and increases salivary secretion in a murine Sjögren's model," *Journal of Autoimmunity*, vol. 115, Article ID 102549, 2020.

[29] E. Rivière, J. Pascaud, N. Tchitchek et al., "Salivary gland epithelial cells from patients with Sjögren's syndrome induce B-lymphocyte survival and activation," *Annals of the Rheumatic Diseases*, vol. 79, no. 11, pp. 1468–1477, 2020.

[30] Ö. Özçaka, E. Alpöz, A. Nalbantsoy, G. Karabulut, and Y. Kabasakal, "Clinical periodontal status and inflammatory cytokines in primary Sjögren syndrome and rheumatoid arthritis," *Journal of Periodontology*, vol. 89, no. 8, pp. 959–965, 2018.

[31] C. Garlanda, C. A. Dinarello, and A. Mantovani, "The interleukin-1 family: back to the future," *Immunity*, vol. 39, no. 6, pp. 1003–1018, 2013.

[32] Y. Chen, S. K. Chauhan, C. Shao, M. Omoto, T. Inomata, and R. Dana, "IFN-γ-Expressing Th17 cells are required for development of severe ocular surface autoimmunity," *The Journal of Immunology*, vol. 199, no. 3, pp. 1163–1169, 2017.

[33] G. M. Verstappen, O. B. J. Corneth, H. Bootsma, and F. G. M. Kroese, "Th17 cells in primary Sjögren's syndrome: pathogenicity and plasticity," *Journal of Autoimmunity*, vol. 87, pp. 16–25, 2018.

[34] S. Yamano, J. C. Atkinson, B. J. Baum, and P. C. Fox, "Salivary gland cytokine expression in NOD and normal BALB/c mice," *Clinical Immunology*, vol. 92, no. 3, pp. 265–275, 1999.

[35] S.-H. Hwang, J. S. Woo, J. Moon et al., "IL-17 and CCR9$^{+}\alpha 4\beta 7^{-}$ Th17 cells promote salivary gland inflammation, dysfunction, and cell death in sjögren's syndrome," *Frontiers in Immunology*, vol. 12, Article ID 721453, 2021.

[36] M. Ittah, C. Miceli-Richard, J. Eric Gottenberg et al., "B cell-activating factor of the tumor necrosis factor family (BAFF) is expressed under stimulation by interferon in salivary gland epithelial cells in primary Sjögren's syndrome," *Arthritis Research and Therapy*, vol. 8, no. 2, p. R51, 2006.

[37] J. S. Thompson, S. A. Bixler, F. Qian et al., "BAFF-R, a newly identified TNF receptor that specifically interacts with BAFF," *Science*, vol. 293, no. 5537, pp. 2108–2111, 2001.

[38] A. Papageorgiou, C. P. Mavragani, A. Nezos et al., "A BAFF receptor His159Tyr mutation in Sjögren's syndrome-related

lymphoproliferation," *Arthritis and Rheumatology*, vol. 67, no. 10, pp. 2732–2741, 2015.

[39] H. Li, J. A. Ice, C. J. Lessard, and K. L. Sivils, "Interferons in Sjögren's syndrome: genes, mechanisms, and effects," *Frontiers in Immunology*, vol. 4, p. 290, 2013.

[40] X. Mariette, S. Roux, J. Zhang et al., "The level of BLyS (BAFF) correlates with the titre of autoantibodies in human Sjögren's syndrome," *Annals of the Rheumatic Diseases*, vol. 62, no. 2, pp. 168–171, 2003.

[41] J. Groom, S. L. Kalled, A. H. Cutler et al., "Association of BAFF/BLyS overexpression and altered B cell differentiation with Sjögren's syndrome," *Journal of Clinical Investigation*, vol. 109, no. 1, pp. 59–68, 2002.

[42] P. Youinou and J.-O. Pers, "Disturbance of cytokine networks in Sjögren's syndrome," *Arthritis Research and Therapy*, vol. 13, no. 4, p. 227, 2011.

[43] F. J. Carrillo-Ballesteros, C. A. Palafox-Sánchez, R. A. Franco-Topete et al., "Expression of BAFF and BAFF receptors in primary Sjögren's syndrome patients with ectopic germinal center-like structures," *Clinical and Experimental Medicine*, vol. 20, no. 4, pp. 615–626, 2020.

[44] J. Krejsek, M. Koláčková, I. Lindrová, R. Slezák, and C. Andrýs, "Increase of intracellular BAFF in B cells of Sjögren's patients is not affected by decrease of BAFFR," *Assam College Teachers' Association Medica*, vol. 58, no. 1, pp. 25–31, 2015.

[45] S. Nagata and M. Tanaka, "Programmed cell death and the immune system," *Nature Reviews Immunology*, vol. 17, no. 5, pp. 333–340, 2017.

[46] P. Manganelli and P. Fietta, "Apoptosis and sjögren syndrome," *Seminars in Arthritis and Rheumatism*, vol. 33, no. 1, pp. 49–65, 2003.

[47] A. M. Chinnaiyan, K. O'Rourke, M. Tewari, and V. M. Dixit, "FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis," *Cell*, vol. 81, no. 4, pp. 505–512, 1995.

[48] M. Muzio, A. M. Chinnaiyan, F. C. Kischkel et al., "FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death--inducing signaling complex," *Cell*, vol. 85, no. 6, pp. 817–827, 1996.

[49] J. C. Rathmell, M. P. Cooke, W. Y. Ho et al., "CD95 (Fas)-dependent elimination of self-reactive B cells upon interaction with CD4+ T cells," *Nature*, vol. 376, no. 6536, pp. 181–184, 1995.

[50] Z. N. Oltval, C. L. Milliman, and S. J. Korsmeyer, "Bcl-2 heterodimerizes in vivo with a conserved homolog, Bax, that accelerates programed cell death," *Cell*, vol. 74, no. 4, pp. 609–619, 1993.

[51] L. Chang, A. Kong, Y. Guo et al., "Quercetin ameliorates salivary gland apoptosis and inflammation in primary Sjögren's syndrome through regulation of the leptin/OB-R signaling," *Drug Development Research*, vol. 83, no. 6, pp. 1351–1361, 2022.

[52] Y. Wang, Y. Tong, O. I. Adejobi, Y. Wang, and A. Liu, "Research advances in multi-omics on the traditional Chinese herb *Dendrobium officinale*," *Frontiers of Plant Science*, vol. 12, Article ID 808228, 2021.