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Research Article

Dendrobium officinale Polysaccharides Inhibit CDCA-Induced Gastric Intestinal Metaplasia through Activating NRF2/HO-1 and Modulating HNF4α/CDX2 Signaling Pathway

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Bile reflux (BR) was considered to be an independent risk factor for the development of precancerous gastric lesions and GC. *Dendrobium officinale* polysaccharides (DOP) show a novel potential in preventing the progress of gastric cancer. However, the specific mechanism of DOP that causes such activities remains a mystery. This study aimed to investigate the effects of DOP on chenodeoxycholic acid (CDCA)-induced gastric intestinal metaplasia and explore the underlying mechanisms. Different concentrations of DOP had no significant damage to normal GSE-1 cells and gastric intestinal metaplasia model cells by CCK-8 assay. After DOP treatment, the mRNA and protein expression of CDX2 ($p < 0.01$) and HNF4α ($p < 0.01$) were decreased, and HO-1 ($p < 0.05$) and TFF2 ($p < 0.01$) were increased. The NRF2 protein expression was slightly upregulated ($p < 0.05$), and H-DOP further promoted NRF2 protein expression in the nucleus ($p < 0.05$). Hence, our findings reveal that DOP could be used as a potential anti-inflammation supplement by activating NRF2/HO-1 and modulating the HNF4α/CDX2 signaling pathway to inhibit the progress of CDCA-induced gastric intestinal metaplasia.

1. Introduction

Gastric adenocarcinoma (GAC) is one of the most common digestive tract cancers in China, and its morbidity and mortality rates exceeded 10 and 13%, respectively, in 2018 [1]. Gastric intestinal metaplasia (GIM) is related to premalignant conditions in which areas of human stomach epithelium express mixed gastric and intestinal features [2]. Substantial epidemiologic evidence has indicated that GIM is the result of the comprehensive effects of multiple environmental factors, such as *Helicobacter pylori* (Hp) infection, bile reflux, aging, race, and lifestyle [3, 4]. Many epidemiologic studies established a close relationship between long-term bile reflux and GIM risk [4], and bile reflux was considered to be an independent risk factor for the development of precancerous gastric lesions and gastric cancer (GC) [5]. Although the antibiotics can eliminate *Helicobacter pylori* in clinical treatment, such as amoxicillin or clarithromycin, many of them cause undesirable subsequent health complications, including hypersensitivity, arrhythmias, hematopoietic disorders [6, 7], and gynecomastia [8]. Therefore, the IM stage can be an important broad time window to block the progression from gastritis to GC. Currently, there is no effective progress in the treatment or prevention of IM [9]. Importantly, some natural compounds and small molecules that could be used for the treatment have been discovered.

Bile reflux, such as chenodeoxycholic acid (CDCA) and deoxycholic acid (DCA)-induced GIM model in vivo and in vitro, has been widely applied [10, 11]. Nuclear factor erythroid-related factor 2 (NRF2), a central transcriptional regulatory factor, can be activated by oxidative stress to achieve self-protection and increase antioxidase expression [12]. Upregulated NRF2/HO-1 shows an important

protective function in various inflammatory diseases [13]. After chenodeoxycholic acid (CDCA) treatment, the activity of various transcription factors in gastric epithelial GES-1 cells was changed, and the result showed that NRF2 was increased [14]. Several studies showed that the plenty of polysaccharides from herbal medicines induce NRF2-mediated antioxidant/detoxifying enzymes to improve antioxidant capacity and reduce oxidative stress in cancer, diabetes, and Alzheimer's and inflammation disease [15–18].

Caudal type homeobox 2 (CDX2) is an essential intestinal marker in the development and maintenance of intestinal tissue in adult mammals [19]. Hepatocyte nuclear factor 4 alpha (HNF4 α) in the gastric epithelium and subsequent progression of IM contribute significantly to the expression of bile acid-induced columnar genes via regulating KLF4 and CDX2 [20]. Trefoil family factor 2 (TFF2) is a mucin-associated peptide expressed in gastrointestinal epithelial cells where it serves to maintain mucosal integrity and promote epithelial repair [21]. Meanwhile, TFF2-promoted epithelial restitution does not require cyclooxygenase activation [22]. Indomethacin and aspirin upregulated TFF2 by the activation of PPAR gamma to reduce gastric mucosal injury since TFF peptides play an important role in gastric mucosal protection [23, 24]. Therefore, TFF2 will enhance the gastric mucosal protection, and HNF4a/CDX2 will directly reduce damage from CDCA.

Alternative and complementary therapies have brought favorite results in precancerous lesions of gastric cancer (PLGC) which is based on traditional Chinese medicine (TCM) theory [25]. *Dendrobium officinale Kimura et Migo* (called Tie Pi Shi Hu in Chinese, usually used as stems) is listed in the pharmacopoeia of the People's Republic of China which belongs to the orchid genera. It has been reported to have anticancer, anti-inflammation, and immunomodulatory properties [26–29]. *Dendrobium officinale* (DO), its dried leaves, and flowers have been permitted for use as food resources in Guizhou, Zhejiang, Yunnan, and Fujian provinces [30]. Modern research confirmed that DO had protected gastric mucosal injury, modulated enteric dysbacteriosis, improved gut barrier, and promoted digestion [31–33]. We found that DOP from stems reduced intestinal metaplasia and made the atypical hyperplasia be kept at the moderate or mild degree in a N-methyl-N′-nitro-N-nitrosoguanidine (MNNG)-induced PLGC rat model [34, 35]. However, it is unclear whether DOP has an inhibitory effect on bile reflux-induced GIM *in vitro*.

Based on the above facts, we hypothesized that DOP inhibits the intestinal metaplasia process on the bile acid-induced gastric intestinal metaplasia model by activating NRF2/HO-1 and regulating the HNF4α/CDX2 signal pathway. Therefore, we could better confirm and understand the effect and the mechanism of *Dendrobium officinale* polysaccharides on precancerous lesions of gastric cancer.

2. Materials and Methods

2.1. Chemicals and Reagents. DOP was isolated and purified from *D. officinale*, its purity was more than 80%, and the molecular weight was detected by high-performance gel permeation chromatography (HPGPC) [35]. Generally speaking, 50 g water extraction was dissolved in 2.5 L water two times and then mixed with 95% alcohol at a ratio of 1 : 5. After 24 hours, the mixture was collected by centrifuging at 4000 rpm for 15 min, then deproteinized by savage reagent, and dried to form crude DOP. Crude DOP is dissolved in water at a concentration of 0.1 g/mL as the mother liquor, and then it is diluted to the final concentration (800 μg/mL, 400 μg/mL, and so on). Human gastric epithelial GES-1 cell lines were purchased from the Institute of Biochemistry and Cell Biology at the Chinese Academy of Sciences (Shanghai, China). Fetal bovine serum (FBS) and 1640 medium were purchased from Solarbio (Shanghai, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) and ferric ion-reducing antioxidant power (FRAP) kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Cell counting kit-8 (CCK-8) assay kit was received from MCE (New Jersey, USA), total protein extraction kit (Beyotime, Shanghai, China), SYBR Premix Ex Taq II (Takara, Japan), Lowry protein assay, and nuclear protein extraction kit (Thermo Fisher Scientific, MA, USA). HNF4a, HO-1, and TFF2 antibodies were purchased from Abcam (Cambridge, MA, USA). CDX2 and β-actin antibodies were obtained from Cell Signaling Technology (Danvers, MA, USA) and SuperSignal™ West Pico PLUS Chemiluminescent Substrate (Pierce Biotechnology, Rockford, USA). Secondary antibodies were purchased from Beijing Zhongshan Biotechnology Company (Beijing, China).

2.2. Antioxidant Activities of Dendrobium officinale Polysaccharides by DPPH Assay. The DPPH assay is the most popular method for measuring antioxidant activity because they are reliable and economical [36]. First, DOP was diluted 10-fold, and 45 μL of the diluted sample was added to a 96-well plate. Then, 100 μL of 0.2 mM DPPH solution (in ethanol) was added, mixed well, and placed in the dark for 30 min at room temperature. The absorbance A1 at 516 nm was measured by a spectrophotometer, and 45 μL of anhydrous ethanol was used to replace the sample as blank absorbance A0. 0.05 μg/mL ascorbic acid was selected as the positive control.

The unit of the DPPH·scavenging rate is recorded as "%". DPPH·scavenging rate (%) = (A0 − A1)/A0 × 100 where A0 is the absorbance of the blank sample and A1 is the absorbance of the DOP. All experiments were performed at least 3 times.

2.3. Antioxidant Activities of Dendrobium officinale Polysaccharides by FRAP Assay. The ferric reducing/antioxidant power (FRAP) assay was used to determine the total antioxidant capacity [37]. FRAP assay was carried out according to the kit instructions [38]. 180 μL of FRAP working solution was added to the 96-well plate, and 5 μL of sample (400 μg/mL) or ultrapure water was added to the working solution and mixed. The absorbance was recorded at 593 nm by a spectrophotometer after incubation at 37°C for 3 min. The standard curve was determined with FeSO4 as the standard product. The results were expressed as Trolox equivalents. The unit of the total antioxidant capacity of the

sample is expressed in "mM FeSO4 equivalent." All experiments were performed at least 3 times.

2.4. Establishment of CDCA-Induced Intestinal Metaplasia Model on GES-1 Cells by Western Blot (WB). According to [39], GES-1 cells were seeded in 60 mm dishes at a density of 8×10^4 cells/well, were cultured in an environment of 37°C and 5% CO_2 for 24 hours to allow cells to adhere, and then were serum-deprived for 12 hours prior to treatment with 50, 100, and 200 μm CDCA. After being treated with CDCA for 24 hours, harvested cells were lysed with RIPA cell lysis buffer supplemented with 1% PMSF to extract total protein. Protein concentration was evaluated by Lowry protein assay [40]. The protein sample was separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene fluoride (PVDF) membranes by the wet blotting method. Membranes were blocked with 5% dried skimmed milk powder in Tris-buffered saline Tween-20 (TBST). Membranes were incubated with primary antibodies to CDX2 (1 : 1000) and β-actin (1 : 1000). After washing in TBST, secondary antibodies (1 : 10000) conjugated with horseradish peroxidase (HRP) were incubated on the membranes for 1 hour. ECL, the enhanced chemiluminescence detection reagent, was used to visualize the protein bands after the incubation. All experiments were performed at least 3 times.

2.5. Establishment of CDCA-Induced Intestinal Metaplasia by RT-PCR (Reverse Transcription-Polymerase Chain Reaction). These genes CDX2, MUC2, HNF4α, KLF4, VILLIN1, SOX2, and TFF2 were key intestinal metaplasia genes. After choosing the appropriate concentration to establish CDCA-induced intestinal metaplasia, the expression of key intestinal metaplasia genes was investigated by RT-PCR. The cell was collected, and its total RNA was extracted by the Eastep™ Super Total RNA Extraction Kit. The integrity of the RNA sample was measured by the Agilent Bioanalyzer 2100 system. 1 μg of total RNA was used to synthesize first-stand cDNA by the PrimeScript RT reagent kit with gDNA Eraser following the manufacturer's instructions. Primers and templates were mixed with TB Green Premix Ex Taq II. The primers are listed in Table 1. RT-PCR was conducted using the SYBR method on a CFX96™ Real-Time PCR Detection system (Bio-Rad Laboratories, Inc., Hercules, CA, USA). The $2^{-\Delta\Delta CT}$ method was used to quantify the relative mRNA expression levels [41]. All experiments were performed at least 3 times.

2.6. The Inhibitory Effect of DOP at Different Concentrations on Normal GES-1 Cell and CDCA-Induced Intestinal Metaplasia Model [42]. A 96-well plate at a density of 4×10^3 cells/well was tested for the inhibitory effect of DOP at final concentrations of 800, 400, 200, 100, and 50 μg/mL on normal GES-1 cells. In the CDCA-induced intestinal metaplasia model, DOP were at final concentrations of 400, 200, 100, and 50 μg/mL, which combined with 100 and 200 μm CDCA. The control group was cultured in a medium supplemented with 10% FBS. The model groups were induced by 100 and 200 μm CDCA. After twenty-four hours, 10 μL of CCK-8 solutions was added to 100 μL medium and incubated for 4 hours according to the CCK-8 kit. Absorbance was measured at 450 nm after shaking and in the dark for 5 min. The cell survival rate was calculated as follows: cell survival rate (%) = (OD value in sample group/OD value in normal group) × 100%. All experiments were performed at least 3 times.

2.7. Effect of DOP on CDCA-Induced Intestinal Metaplasia by RT-PCR. GES-1 cells were seeded in 60 mm dishes for 24 hours to adhere and then were serum-deprived 12 hours later, and 100 μm CDCA was used to induce intestinal metaplasia. High, medium, and low concentrations of DOP (400, 200, and 100 μg/mL) were added lasting for 24 hours. The cells were collected for RT-PCR. The procedure of RT-PCR was the same as above. The genes were several intestinal markers (CDX2, HNF4α, KLF4, MUC2, VILLIN1, and TFF2), NRF2, HO-1, and NQO-1. All experiments were performed at least 3 times.

2.8. Nuclear Translocation of NRF2 on CDCA-Induced Intestinal Metaplasia Treated with DOP. The nuclear translocation of NRF2 was assessed by WB. Nuclear and cytoplasmic proteins were extracted using the Nuclear Protein Extraction Kit. Histone H3 (1 : 1000) and β-actin (1 : 1000) were used as internal reference antibodies and subsequently incubated with the horseradish peroxidase-conjugated goat antirabbit secondary antibody (dilution 1 : 5000) for 1 h at room temperature. Protein bands were then detected using enhanced chemiluminescence Western blot detection reagents (Thermo Fisher Scientific, MA, USA). All experiments were performed at least 3 times.

2.9. Effect of DOP on CDCA-Induced Intestinal Metaplasia by WB. The method of collecting and dealing with the cell was the same as above, the primary antibodies to HNF4a (1 : 1000), HO-1 (1 : 2000), NQO-1 (1 : 1000), and TFF2 (1 : 2000) at 4°C for 12 hours. β-Actin (1 : 1000) was used as an internal reference antibody after all experiments were performed at least 3 times.

2.10. Statistical Analysis. Data were analyzed using SPSS version 16.0 software (IBM, Chicago, IL, USA). Data were presented as the mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the differences between groups. P value <0.05 was considered to be statistically significant.

3. Results

3.1. Antioxidant Activities of Dendrobium officinale Polysaccharides by DPPH and FRAP Assay. DPPH value is expressed as ascorbic acid (GAE) having an equivalent antiradical capacity. The clearance rate of 50, 100, 200, 400, and 800 μg/mL DOP was from 18.5% to 50.8%. For FRAP

Table 1: RT-PCR primer information.

Gene name	Sense ($5' - 3'$)	Antisense ($5' - 3'$)
NRF2	TCAGCGACGGAAAGAGTATGA	CCACTGGTTTCTGACTGGATGT
HO-1	AAACTTCAGAGGGGGCGAAG	GACAGCTGCCACATTAGGGT
NQO-1	AAGAGCACTGATCGTACTGG	CTTCAGTTTACCTGTGATGTCC
CDX2	GCTATAAATGCCAGAGCCAACC	CACAGACCAACAACCCAAACAG
MUC2	AGTCCATCCTGCTGACCATC	GGTGTAGGCATCGCTCTTCTC
KLF4	AAGAGTTCCCATCTCAAGGCAA	GGGCGAATTTCCATCCACAG
Villin1	GCTTGGCAACTCTAGGGACTGG	TGAGGTTGCTGTTAGCATTGACAC
TFF2	CCAAAGCAAGAGTCGGATCAG	CAGTCTTCCACAGACTTCGGG
HNF4α	GTTCAAGGACGTGCTGCTCCTA	AGGCATACTCATTGTCATCGATCTG
SOX2	GGAGTTGTCAAGGCAGAGAAG	CGCCGCCGATGATTGTTAT
β-Actin	GAAACTACCTCAACTCCATC	CGAGGCCAGGAGGAGCCGCC

assay, FRAP value is expressed as the millimolar concentration of Fe^{2+}, obtained from a dilution of $FeSO_4$ having an equivalent antioxidant capacity. The FRAP value of 50–800 μg/mL DOP was 1.75–2.45 mmol Fe^{2+}/g (Figure 1).

3.2. Establishment of CDCA-Induced Intestinal Metaplasia Model on GES-1 Cells by WB. As shown in Figures 2(a) and 2(b), treated with 50 μm, 100 μm, and 200 μm CDCA, the expression of CDX2 protein was all increased, especially 100 μm and 200 μm CDCA had statistical significance ($p < 0.05$; $p < 0.05$). Accordingly, 100 μm CDCA was chosen as a follow-up experimental study.

3.3. Establishment of CDCA-Induced Intestinal Metaplasia by RT-PCR. As shown in Figure 3, the 100 μm CDCA model group showed significantly higher gene expression of CDX2, MUC2, HNF4α, KLF4, VILLIN 1, and SOX2 and a lower level of TFF2 when compared to the control group [43], and the result was consistent with the report, which means 100 μm CDCA could induce a successful model of intestinal metaplasia.

3.4. The Inhibitory Effect of DOP at Different Concentrations on Normal GES-1 Cell and on CDCA-Induced Intestinal Metaplasia Model. In order to evaluate the inhibitory effect of DOP, different concentrations of DOP were treated on normal GES-1 cell and CDCA-induced cell models. When treated with 800 μg/mL DOP, the cell viability was about 85% and induced a little reduction when compared with the control group ($p < 0.05$). Treated with these lower 50–400 μg/mL DOP, the cell viability was about 95–100%. Therefore, 50–400 μg/mL DOP was used for follow-up pharmacodynamic evaluation (Figure 4(a)).

Depending on the inhibitory effect of DOP on GES-1 cells, we tested the inhibitory effect of DOP on CDCA-induced gastric intestinal metaplasia at different concentrations. The cell inhibitory rate was, respectively, $11 \pm 2.1\%$ and $20 \pm 1.2\%$ when induced by 100 μM and 200 μM CDCA. When 200 μM CDCA was combined with 50–800 μg/mL DOP, the cell inhibitory was significantly higher at 800 μg/mL DOP when compared with the control group ($p < 0.05$). However, the cell inhibitory has no change when treated with 200 μM CDCA alone or combined with 50–400 μg/mL DOP (Figure 4(b)). When 100 μM CDCA is combined with 50–800 μg/mL DOP, the cell inhibitory has no differences with 100 μM CDCA or DOP alone (Figure 4(c)).

3.5. Effect of DOP on CDCA-Induced Intestinal Metaplasia by RT-PCR. The mRNA expression of CDX2, HNF4α, KLF4, MUC2, and VILLIN1 was significantly increased, and TFF2 was decreased by stimulation of CDCA in the model group by RT-PCR. The mRNA expression of NRF2, HO-1, and NQO-1 was obviously increased. After DOP treatment, the mRNA expression of CDX2, HNF4α, and VILLIN1 was found to be significantly decreased. Although there was a trend of lower KLF4mRNA expression treated with H-DOP and M-DOP compared with the model group, they did not have statistical significance. MUC2 has not been affected. Interestingly, the mRNA expression of NRF2 and HO-1 was further found to be increased by DOP, but NQO-1 was decreased. TFF2 mRNA was downregulated in CDCA-treated GSE-1 cells and was upregulated by DOP in the cell model. Collectively, these results suggest that DOP increases NRF2, HO-1, and TFF2 expression but decreases CDX2, NQO-1, and HNF4α expression (Figure 5).

3.6. The Protein Expression of NRF2 on CDCA-Induced Intestinal Metaplasia Treated with DOP in Nucleus and Cytoplasm. The different protein NRF2 expressions were determined by Western blot after separating the nucleus and cytoplasm. The NRF2 protein expression in the nucleus was upregulated in the CDCA-induced intestinal metaplasia model. The high dose of DOP promoted NRF2 protein expression which means NRF2 enters the nucleus ($p < 0.05$) (Figures 6(a) and 6(b)). At the same time, the protein expression of NRF2 in the cytoplasm was decreased by CDCA and increased by high and medium doses of DOP ($p < 0.05$) (Figures 6(a) and 6(c)).

3.7. The Protein Expression of CDX2, HNF4a, HO-1, NQO-1, and TFF2 on CDCA-Induced Intestinal Metaplasia Treated with DOP. The results showed that CDCA treatment increased the protein expression of CDX2 ($p < 0.01$), HNF4α ($p < 0.01$), and HO-1 and decreased the protein expression of TFF2 ($p < 0.05$). Treated with different concentrations of DOP, the protein expression of CDX2 ($p < 0.01$) and HNF4α

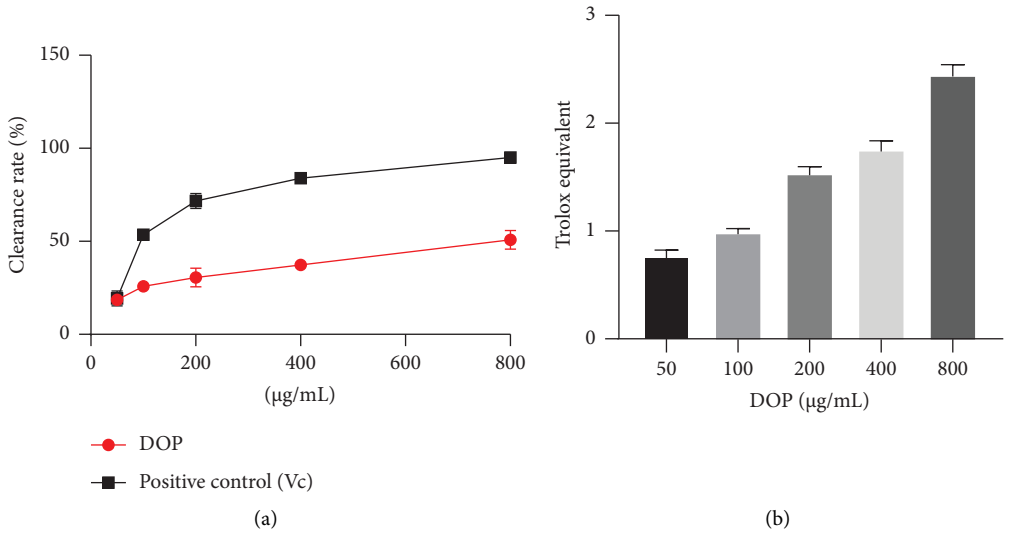

Figure 1: The antioxidant capacity of DOP by DPPH and FARP assay. (a) DPPH free radical-scavenging ability and (b) antioxidant capacity by FRAP assay. $n = 6$ and repeated three times.

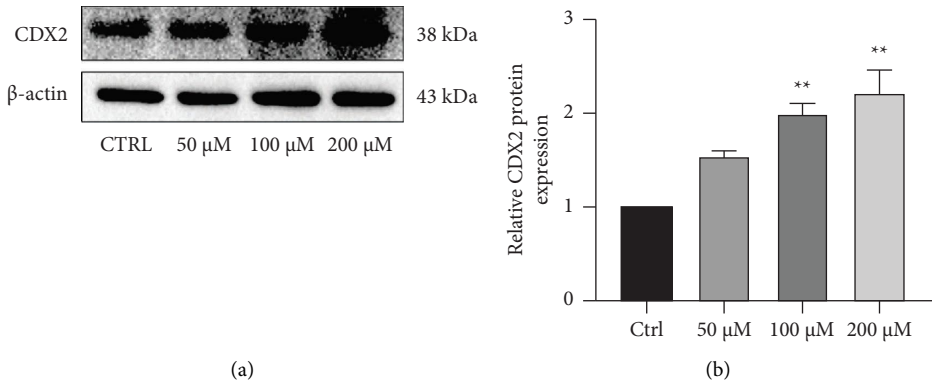

Figure 2: Establishment of intestinal metaplasia model with CDCA detected by WB. (a) Gray band of each protein. (b) Analysis of gray value. Compared with the controlgroup: $^{*}p < 0.05$ and $^{**}p < 0.01$. $n = 3$.

($p < 0.01$) was remarkably decreased. The protein expression of HO-1 and TFF2 was increased by three concentrations of DOP ($p < 0.05$; $p < 0.01$). The protein expression of NQO-1 was downregulated by DOP ($p < 0.05$) (Figure 7).

4. Discussion

Dendrobium plants are famous traditional medicines and are mainly distributed in South Asia and Oceania. There are 1547 accepted Dendrobium species worldwide, more than 80 Dendrobium species have been found in China with a large annual production [44]. DO has a strong effect on nourishing the stomach Yin, playing an important role in treating gastrointestinal diseases, and modern pharmacology research has shown that DO protects against gastrointestinal injury and aids in digestion [45, 46]. GIM is considered an important precursor to gastric cancer. In this study, DOP inhibited CDCA-induced gastric intestinal metaplasia in the cell model, and furthermore, it improved the NRF2/HO-1 and HNF4α/CDX2 signal pathway, which was a further extension of our preliminary work in vitro. Our previous studies had proved that the DOP prevented MNNG-induced PLGC along with subsequent liver and kidney damage, and the mechanism is associated with the reduction of 8-OHdG levels and the activation of the NRF2 pathway and its related antioxidant enzymes HO-1 and NQO-1 [35].

For DOP, many studies highlighted advances in antioxidant properties from in vivo and in vitro by decreasing free radicals, enhancing the antioxidant system, inhibiting nuclear factor-kappa B, and downregulating inflammatory response [47–49]. In this study, we evaluated the antioxidant activities of *Dendrobium officinale* polysaccharides by DPPH and FRAP assay. The result showed that the clearance rate of 50, 100, 200, 400, and 800 μg/mL DOP was from 18.5% to 50.8%. Xing et al. found when the concentrations of DOP

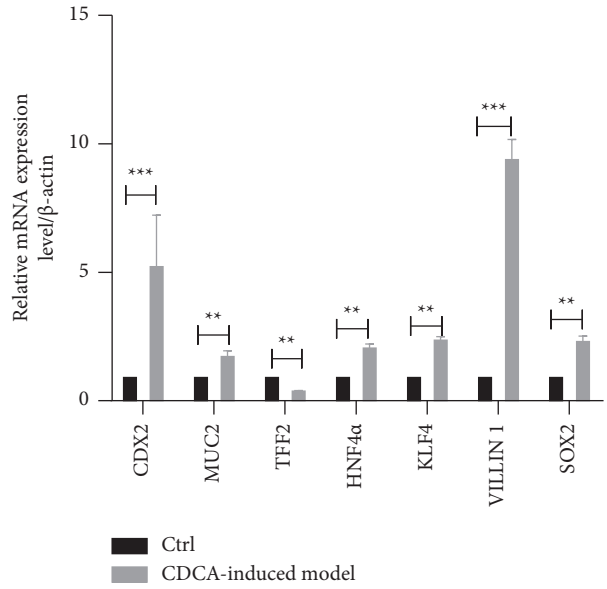

Figure 3: Establishment of intestinal metaplasia model with CDCA detected by RT-PCR. Compared with the control group: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. $n = 3$ and repeated three times.

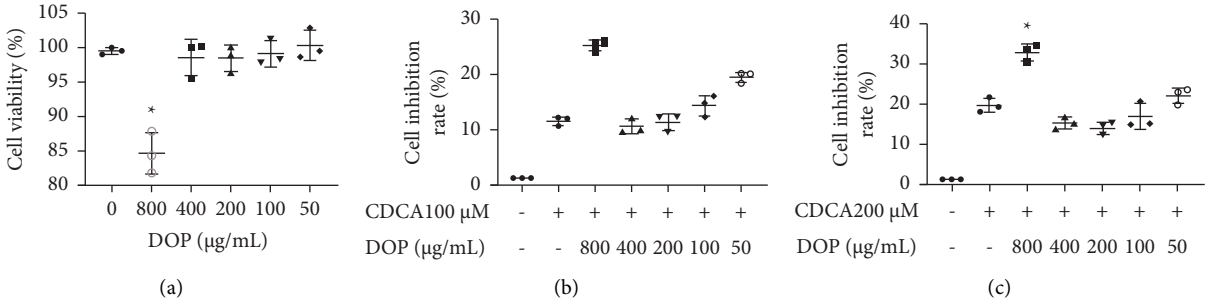

Figure 4: The inhibitory effect of different concentrations of DOP on GES-1 cells and CDCA-induced intestinal metaplasia model. Compared with the CDCA group: $^{*}p < 0.05$. $n = 3$ and repeated three times.

were 0.5–5.0 mg/mL, the DPPH radical-scavenging rates were between 40 and 50% [50], which was consistent with our experimental results.

NRF2 is a key transcription factor regulating antioxidant enzymes and, therefore, protecting against antioxidant stress and anti-inflammation [51–53]. After CDCA treatment, the activity of various transcription factors in GES-1 cells was changed, and the result showed that NFE2 was increased [14]. HO-1 as a downstream gene of NRF2 has a cytoprotective effect in normal tissues via restraining oxidative dysregulation, inappropriate immune response, and related disorders, especially in cancer carcinogenesis [52, 54]. Much data report and in vivo studies revealed the increased expression of the HO-1 enzyme in tumors compared with the surrounding normal tissues [55]. Our previous research demonstrated the ability of DOP to induce NRF2 upregulation and to increase HO-1 in the PLGC rat model [35]. In this study, we also found that CDCA enables NRF2 to translocate into the nucleus and upregulate redox-regulated genes HO-1. After treatment with DOP, it could promote the entry of NRF2 into the nucleus and upregulate the protein expression of NRF2, especially the high concentration of DOP. At the same time, the protein expression of NRF2 in the cytoplasm was decreased by CDCA and increased by high and medium doses of DOP. Meanwhile, the HO-1 was upregulated after being treated with DOP on the CDCA-induced intestinal metaplasia model. These data indicated that DOP had the potential capacity to reduce the damage from intestinal metaplasia through the NRF2/HO-1 pathway in vitro. The data have similarities with published reports that *D. nobile* extract protects retinal pigment epithelial cells from UV and oxidative stress damage via NRF2/HO-1 and MAPK pathways [56]. The bile acid-induced gastric intestinal metaplasia model was widely used [57, 58], especially chenodeoxycholic acid (CDCA), which could increase caudal type homeobox 2 (CDX2) and induce following intestine-specific markers in GES-1 cells [39]. CDX2 is an essential intestinal marker in the development and maintenance of intestinal tissue in adult mammals [19]. HNF4α, one of the TFs (Transcription factors), activates ectopic CDX2 expression through a shadow 3′enhancer [59]. HNF4α, in gastric epithelium and subsequent progression of

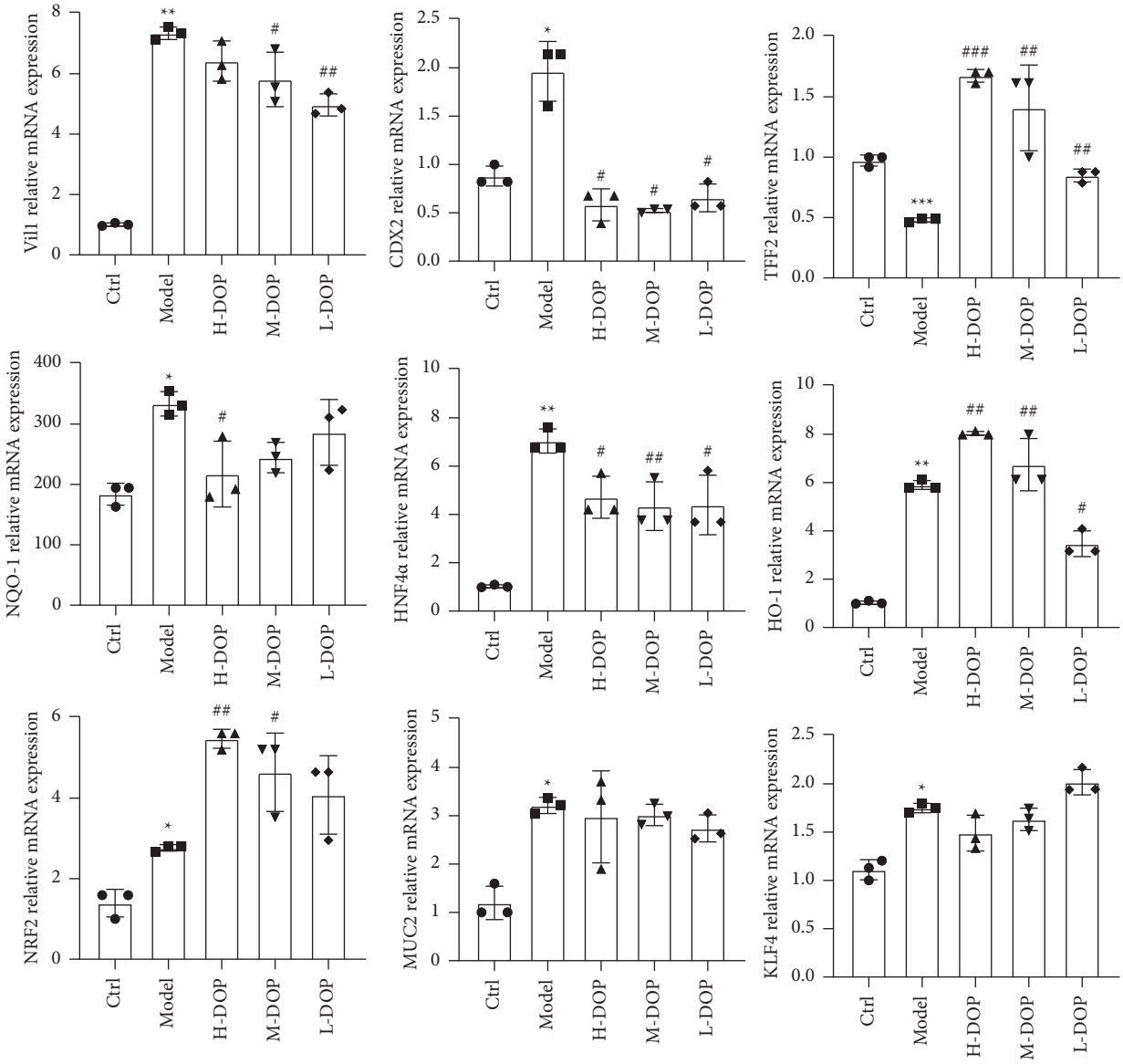

Figure 5: The inhibition effect of DOP on CDCA-induced intestinal metaplasia model by RT-PCR. High, medium, and low concentration of DOP was 400, 200, and 100 μg/mL. The relative level was calculated with target genes/β-actin. Compared with the control group: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Compared with the CDCA model group: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$. $n = 3$ and repeated three times.

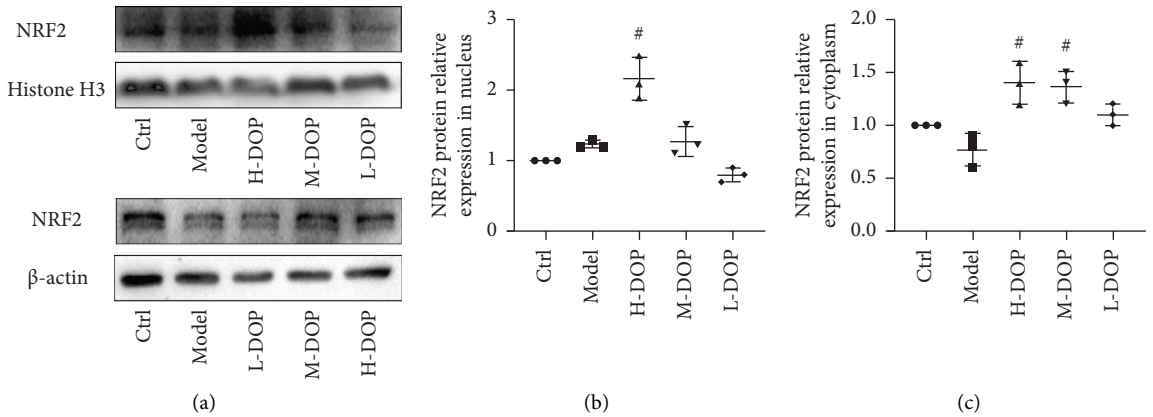

Figure 6: The protein expression of NRF2 on CDCA-induced intestinal metaplasia treated with DOP in the nucleus and cytoplasm. High, medium, and low concentration of DOP was 400, 200, and 100 μg/mL. (a, b) NRF2 expression in the nucleus; (a, c) NRF2 expression in cytoplasm. Compared with the CDCA model group: $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$. $n = 3$ and repeated three times.

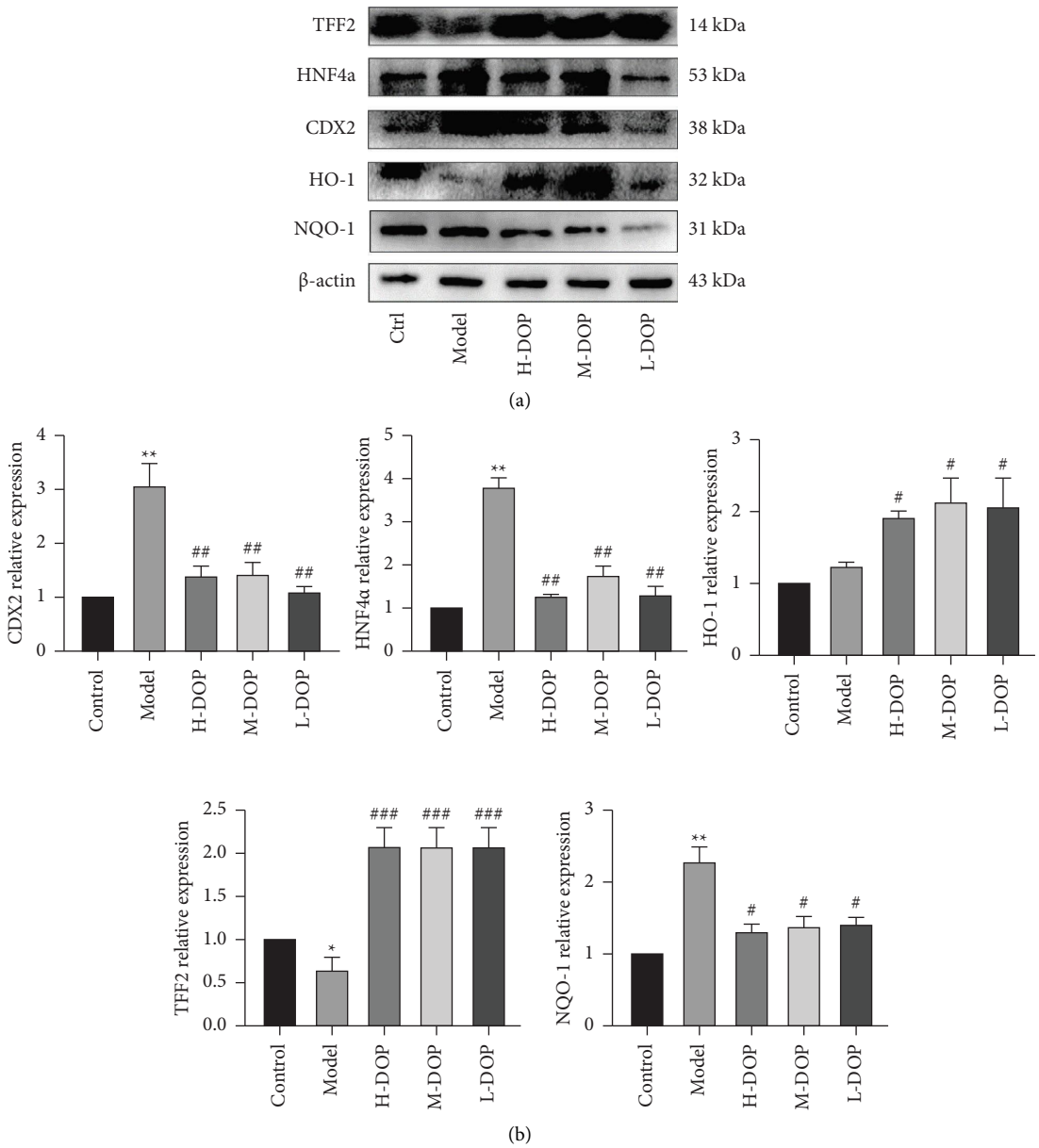

Figure 7: Western blot analysis of HNF4α, CDX2, TFF2, HO-1, and NQO-1 protein expression. High, medium, and low concentration of DOP was 400, 200, and 100 μg/mL. (a) The western blot bands and (b) the relative level of HNF4α, CDX2, TFF2, HO-1, and NQO-1. The relative level was calculated with target proteins/β-actin reference protein. Compared with the control group: $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$. Compared with the CDCA model group: $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, and $^{\#\#\#}p < 0.001$. $n = 3$ and repeated three times.

IM, contributes significantly to the expression of bile acid-induced columnar genes via regulating KLF4 and CDX2 [20]. TGR5-ERK1/2-HNF4α axis plays an important role in the bile acid-induced gastric intestinal metaplasia model [20, 60]. In our data, CDCA could increase CDX2 and HNF4α mRNA as well as protein expression, while DOP could decrease their expression. We demonstrate that DOP could inhibit CDCA-induced gastric intestinal metaplasia by downregulating the HNF4α/CDX-2 signal pathway. A relation between HO-1 and HNF4α has been previously proposed for both to share a common microRNA, and HNF4α regulates miR-377 leading to upregulation of HO-1 [61]. This will be a potential pathway for further investigation.

TFFs play a major role within the gastrointestinal mucosal barrier. It has been demonstrated to promote cell migratory and antiapoptotic activities so as to mediate mucosal repair in epithelial cell culture models [62]. TFF2 is illustrated with a homeostatic pattern during inflammatory processes, which results in an anti-inflammatory effect that would be conducive to create the necessary microenvironment for tissue repair [21]. TFF2 drives epithelial repair by activating CXCR4 in gastric organoids [63]. In the results, DOP could upregulate the mRNA and protein expression of

TFF2, indicating that DOP generates an anti-inflammatory effect which will help to defend against mucosal injury.

5. Conclusion

DOP inhibits the intestinal metaplasia process on the bile acid-induced gastric intestinal metaplasia model by activating NRF2/HO-1 and regulating the HNF4α/CDX2 signal pathway. These findings provide an experimental basis for further application in gastric precancerous lesions.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] R. M. Feng, Y. N. Zong, S. M. Cao, and R. H. Xu, "Current cancer situation in China: good or bad news from the 2018 Global Cancer Statistics?" *Cancer Communications*, vol. 39, no. 1, pp. 1–12, 2019.

[2] P. Jonaitis, L. Kupcinskas, and J. Kupcinskas, "Molecular alterations in gastric intestinal metaplasia," *International Journal of Molecular Sciences*, vol. 22, no. 11, p. 5758, 2021.

[3] G. Esposito and M. Dinis-Ribeiro, "Gastric intestinal metaplasia: can we abandon random biopsies," *Endoscopy International Open*, vol. 10, no. 4, pp. E280–E281, 2022.

[4] S. Du, Y. Yang, S. Fang et al., "Gastric cancer risk of intestinal metaplasia subtypes: a systematic review and meta-analysis of cohort studies," *Clinical and Translational Gastroenterology*, vol. 12, no. 10, Article ID e00402, 2021.

[5] X. Qu and Y. Shi, "Bile reflux and bile acids in the progression of gastric intestinal metaplasia," *Chinese Medical Journal*, vol. 135, no. 14, pp. 1664–1672, 2022.

[6] M. Inghammar, O. Nibell, B. Pasternak, M. Melbye, H. Svanström, and A. Hviid, "Long-term risk of cardiovascular death with use of clarithromycin and roxithromycin: a nationwide cohort study," *American Journal of Epidemiology*, vol. 187, no. 4, pp. 777–785, 2018.

[7] E. Berni, H. de Voogd, J. P. Halcox et al., "Risk of cardiovascular events, arrhythmia and all-cause mortality associated with clarithromycin versus alternative antibiotics prescribed for respiratory tract infections: a retrospective cohort study," *BMJ Open*, vol. 7, no. 1, Article ID e013398, 2017.

[8] D. G. Ran, "Drug-induced gynecomastia in children and adolescents," *Canadian family physician Medecin de famille canadien*, vol. 56, no. 4, pp. 344-345, 2010.

[9] N. Kim, "Chemoprevention of gastric cancer by *Helicobacter pylori* eradication and its underlying mechanism," *Journal of Gastroenterology and Hepatology*, vol. 34, no. 8, pp. 1287–1295, 2019.

[10] D. Jin, K. Huang, M. Xu et al., "Deoxycholic acid induces gastric intestinal metaplasia by activating STAT3 signaling and disturbing gastric bile acids metabolism and microbiota," *Gut Microbes*, vol. 14, no. 1, Article ID 2120744, 2022.

[11] Z. Xu, L. Xiao, S. Wang et al., "Alteration of gastric microbiota and transcriptome in a rat with gastric intestinal metaplasia induced by deoxycholic acid," *Frontiers in Microbiology*, vol. 14, Article ID 1160821, 2023.

[12] Y. Ayıkgöz, M. Salih Aydın, N. Kankılıç, and E. Temiz, "Nuclear factor erythroid 2-related factor 2 (Nrf2), tumor necrosis factor alpha protein (TNF-α), heme oxygenase-1 (HO-1) gene expressions during cardiopulmonary bypass," *Gene*, vol. 790, Article ID 145690, 2021.

[13] A. Loboda, M. Damulewicz, E. Pyza, A. Jozkowicz, and J. Dulak, "Role of Nrf2/HO-1 system in development, oxidative stress response and diseases: an evolutionarily conserved mechanism," *Cellular and Molecular Life Sciences*, vol. 73, no. 17, pp. 3221–3247, 2016.

[14] W. Q. Lu, Z. Ni, S. Q. Jiang et al., "Resveratrol inhibits bile acid-induced gastric intestinal metaplasia via the PI3K/AKT/p-FoxO4 signalling pathway," *Phytotherapy Research*, vol. 35, no. 3, pp. 1495–1507, 2020.

[15] Y. Y. Xing, Y. K. Zheng, S. Yang et al., "Artemisia ordosica polysaccharide alleviated lipopolysaccharide-induced oxidative stress of broilers via nrf2/keap1 and TLR4/NF-κB pathway," *Ecotoxicology and Environmental Safety*, vol. 223, no. 5, Article ID 112566, 2021.

[16] H. Pan, L. Niu, Y. Wu, L. Chen, X. Zhou, and Y. Zhao, "Lycium barbarum polysaccharide protects rats and cardiomyocytes against ischemia/reperfusion injury via Nrf2 activation through autophagy inhibition," *Molecular Medicine Reports*, vol. 24, no. 5, p. 778, 2021.

[17] Y. Hu, J. Huang, Y. Li et al., "Cistanche deserticola polysaccharide induces melanogenesis in melanocytes and reduces oxidative stress via activating NRF2/HO-1 pathway," *Journal of Cellular and Molecular Medicine*, vol. 24, no. 7, pp. 4023–4035, 2020.

[18] Z. Li, X. Chen, Y. Zhang et al., "Protective roles of Amanita caesarea polysaccharides against Alzheimer's disease via Nrf2 pathway," *International Journal of Biological Macromolecules*, vol. 121, pp. 29–37, 2019.

[19] D. G. Silberg, G. P. Swain, E. R. Suh, and P. G. Traber, "Cdx1 and cdx2 expression during intestinal development," *Gastroenterology*, vol. 119, no. 4, pp. 961–971, 2000.

[20] Z. Ni, Y. L. Min, C. Han et al., "TGR5-HNF4 alpha axis contributes to bile acid-induced gastric intestinal metaplasia markers expression," *Cell Death & Disease*, vol. 6, no. 1, p. 56, 2020.

[21] A. Ghanemi, M. Yoshioka, and J. St-Amand, "Trefoil factor family member 2 (TFF2) as an inflammatory-induced and anti-inflammatory tissue repair factor," *Animals*, vol. 10, no. 9, Article ID 1646, 2020.

[22] L. Xue, E. Aihara, D. K. Podolsky, T. C. Wang, and M. H. Montrose, "In vivo action of trefoil factor 2 (TFF2) to speed gastric repair is independent of cyclooxygenase," *Gut*, vol. 59, no. 9, pp. 1184–1191, 2010.

[23] T. Shimada, A. Koitabashi, Y. Fujii et al., "PPARγ mediates NSAIDs-induced upregulation of TFF2 expression in gastric epithelial cells," *FEBS Letters*, vol. 558, no. 1-3, pp. 33–38, 2004.

[24] A. Koitabashi, T. Shimada, Y. Fujii et al., "Indometacin upregulates TFF2 expression in gastric epithelial cells,"

Alimentary Pharmacology & Therapeutics, vol. 20, no. 1, pp. 171–176, 2004.

[25] L. Yang, J. Li, Z. Hu et al., "A systematic review of the mechanisms underlying treatment of gastric precancerous lesions by traditional Chinese medicine," *Evidence-based Complementary and Alternative Medicine*, vol. 2020, Article ID 9154738, 12 pages, 2020.

[26] W. Zhao, J. Li, C. Zhong et al., "Green synthesis of gold nanoparticles from Dendrobium officinale and its anticancer effect on liver cancer," *Drug Delivery*, vol. 28, no. 1, pp. 985–994, 2021.

[27] K. G. Wu, T. Li, C. J. Chen et al., "A pilot study evaluating the clinical and immunomodulatory effects of an orally administered extract of Dendrobium huoshanense in children with moderate to severe recalcitrant atopic dermatitis," *International Journal of Immunopathology & Pharmacology*, vol. 24, no. 2, pp. 367–375, 2011.

[28] S. Tao, Z. Ren, Z. Yang et al., "Effects of different molecular weight polysaccharides from Dendrobium officinale Kimura & Migo on human colorectal cancer and transcriptome analysis of differentially expressed genes," *Frontiers in Pharmacology*, vol. 12, Article ID 704486, 2021.

[29] Y. Liang, R. Du, R. Chen et al., "Therapeutic potential and mechanism of Dendrobium officinale polysaccharides on cigarette smoke-induced airway inflammation in rat," *Biomedicine & Pharmacotherapy*, vol. 143, Article ID 112101, 2021.

[30] Y. H. Wang, "Traditional uses, chemical constituents, pharmacological activities, and toxicological effects of Dendrobium leaves: a review," *Journal of Ethnopharmacology*, vol. 270, Article ID 113851, 2021.

[31] M. Yan, Q. Si, J. Su et al., "Effects of polysaccharides from Dendrobium officinale on intestinal barrier functions of rats induced by excessive consumption of high fat and sucrose diet," *The FASEB Journal*, vol. 33, no. S1, 2019.

[32] 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.

[33] Y. Chuan, L. Fan, D. Wu et al., "Screening of Active Sites in Dendrobium officinale Kimura et Migo on Aspirin-induced Gastric Mucosal Injury in Rats and Investigating Mechanism," *Natural Product Research and Development*, vol. 11, 2016.

[34] Y. Zhao, B. T. Li, G. Y. Wang et al., "Dendrobium officinale polysaccharides inhibit 1-methyl-2-nitro-1-nitrosoguanidine induced precancerous lesions of gastric cancer in rats through regulating wnt/β-catenin pathway and altering serum endogenous metabolites," *Molecules*, vol. 24, no. 14, p. 2660, 2019.

[35] Y. Zhao, Y. Z. Sun, G. Y. Wang, S. C. Ge, and H. N. Liu, "Dendrobium officinale polysaccharides protect against MNNG-induced PLGC in rats via activating the NRF2 and antioxidant enzymes HO-1 and NQO-1," *Oxidative Medicine and Cellular Longevity*, vol. 2019, Article ID 9310245, 11 pages, 2019.

[36] M. Blois, "Antioxidant determination by the sue of a stable free radical," *Nature*, vol. 181, 1958.

[37] I. Benzie and Y. T. Szeto, "Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay," *Journal of Agricultural and Food Chemistry*, vol. 47, no. 2, pp. 633–636, 1999.

[38] H. Y. Kwon, S. I. Choi, H. I. Park et al., "Comparative analysis of the nutritional components and antioxidant activities of different Brassica juncea cultivars," *Foods*, vol. 9, no. 6, p. 840, 2020.

[39] K. T. Huang and G. X. Zhang, "Bile acids promote gastric intestinal metaplasia via pou2f1-dependent upregulation of the expression of Cdx2," *Gastroenterology*, vol. 158, no. 6, p. S320, 2020.

[40] O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein measurement whit the folin phenol reagent," *Journal of Biological Chemistry*, vol. 193, no. 1, pp. 265–275, 1951.

[41] T. D. Schmittgen and B. A. Zakrajsek, "Effect of experimental treatment on housekeeping gene expression: validation by real-time, quantitative RT-PCR," *Journal of Biochemical and Biophysical Methods*, vol. 46, no. 1-2, pp. 69–81, 2000.

[42] A. P. Rolo, C. M. Palmeira, J. M. Holy, and K. B. Wallace, "Role of mitochondrial dysfunction in combined bile acid-induced cytotoxicity: the switch between apoptosis and necrosis," *Toxicological Sciences*, vol. 79, no. 1, pp. 196–204, 2004.

[43] T. Li, H. Guo, H. Li et al., "MicroRNA-92a-1-5p increases CDX2 by targeting FOXD1 in bile acids-induced gastric intestinal metaplasia," *Gut*, vol. 68, no. 10, pp. 1751–1763, 2019.

[44] P. Y. Li, L. Li, and Y. Z. Wang, "Traditional uses, chemical compositions and pharmacological activities of Dendrobium: a review," *Journal of Ethnopharmacology*, vol. 310, Article ID 116382, 2023.

[45] J. Liang, S. Chen, Y. Hu et al., "Protective roles and mechanisms of Dendrobium officinal polysaccharides on secondary liver injury in acute colitis," *International Journal of Biological Macromolecules*, vol. 107, pp. 2201–2210, 2018.

[46] S. Chen, Y. Li, Y. Wu, Z. Zhou, and L. Sun, "Effect of Dendrobium nobile Lindl. on gastric acid secretion, serum gastrin and plasma somatostatin concentration," *Zhongguo Zhongyao Zazhi*, vol. 20, no. 3, pp. 181–121, 1995.

[47] G. Yu, Q. Xie, W. Su et al., "Improvement of antioxidant activity and active ingredient of Dendrobium officinale via microbial fermentation," *Frontiers in Microbiology*, vol. 14, Article ID 1061970, 2023.

[48] Z. Luo, L. Liu, Q. Nie et al., "HPLC-based metabolomics of Dendrobium officinale revealing its antioxidant ability," *Frontiers of Plant Science*, vol. 14, Article ID 1060242, 2023.

[49] W. Zhang, X. Liu, X. Sun et al., "Comparison of the Antioxidant Activities and Polysaccharide Characterization of Fresh and Dry Dendrobium officinale Kimura et Migo," *Molecules*, vol. 27, no. 19, Article ID 6654, 2022.

[50] S. Xing, X. Zhang, H. Ke, J. Lin, Y. Huang, and G. Wei, "Physicochemical properties of polysaccharides from Dendrobium officinale by fractional precipitation and their preliminary antioxidant and anti-HepG2 cells activities in vitro," *Chemistry Central Journal*, vol. 12, no. 1, p. 100, 2018.

[51] H. Kahroba and Y. Davatgaran-Taghipour, "Exosomal Nrf2: from anti-oxidant and anti-inflammation response to wound healing and tissue regeneration in aged-related diseases," *Biochimie*, vol. 171, pp. 103–109, 2020.

[52] C. Kim, N. Kim, and J. S. Bae, "Anti-inflammatory and antioxidant functions of cornuside by regulating NF-κB, STAT-1, and nrf2-HO-1 pathways," *The American Journal of Chinese Medicine*, vol. 50, no. 5, pp. 1349–1360, 2022.

[53] M. Raish, M. Shahid, Y. A. Bin Jardan et al., "Gastroprotective effect of sinapic acid on ethanol-induced gastric ulcers in rats: involvement of Nrf2/HO-1 and NF-κB signaling and anti-apoptotic role," *Frontiers in Pharmacology*, vol. 12, no. 25, Article ID 622815, 2021.

[54] M. Gao, Z. Qi, M. Deng et al., "The deubiquitinase USP7 regulates oxidative stress through stabilization of HO-1," *Oncogene*, vol. 41, no. 33, pp. 4018–4027, 2022.

[55] L. L. Ma, L. Sun, Y. X. Wang, B. H. Sun, Y. F. Li, and Y. L. Jin, "Association between HO-1 gene promoter polymorphisms and diseases (Review)," *Molecular Medicine Reports*, vol. 25, no. 1, p. 29, 2021.

[56] W. H. Hsu, C. P. Chung, Y. Y. Wang et al., "Dendrobium nobile protects retinal cells from UV-induced oxidative stress damage via Nrf2/HO-1 and MAPK pathways," *Journal of Ethnopharmacology*, vol. 288, no. 24, Article ID 114886, 2022.

[57] J. H. Yu, J. B. Zheng, J. Qi et al., "Bile acids promote gastric intestinal metaplasia by upregulating CDX2 and MUC2 expression via the FXR/NF-κB signalling pathway," *International Journal of Oncology*, vol. 54, no. 3, pp. 879–892, 2019.

[58] M. Tatsugami, M. Ito, S. Tanaka et al., "Bile acid promotes intestinal metaplasia and gastric carcinogenesis," *Cancer Epidemiology, Biomarkers & Prevention*, vol. 21, no. 11, pp. 2101–2107, 2012.

[59] H. Singh, D. Seruggia, S. Madha et al., "Transcription factor-mediated intestinal metaplasia and the role of a shadow enhancer," *Genes & Development*, vol. 36, no. 1-2, pp. 38–52, 2022.

[60] Z. Ni, W. Q. Lu, W. Z. Zeng, and Y. Q. Shi, "Tgr5 is involved in bile acids induced gastric intestinal metaplasia through Hnf4a activation," *Gastroenterology*, vol. 158, no. 6, pp. S770–S771, 2020.

[61] S. Khole, S. Mittal, N. Jagadish et al., "Andrographolide enhances redox status of liver cells by regulating microRNA expression," *Free Radical Biology and Medicine*, vol. 130, pp. 397–407, 2019.

[62] E. Aihara, K. A. Engevik, and M. H. Montrose, "Trefoil factor peptides and gastrointestinal function," *Annual Review of Physiology*, vol. 79, no. 1, pp. 357–380, 2017.

[63] K. A. Engevik, H. Hanyu, A. L. Matthis et al., "Trefoil factor 2 activation of CXCR4 requires calcium mobilization to drive epithelial repair in gastric organoids," *The Journal of Physiology*, vol. 597, no. 10, pp. 2673–2690, 2019.