



## *In vitro* study of sodium butyrate on soyasaponin challenged intestinal epithelial cells of turbot (*Scophthalmus maximus* L.) refer to inflammation, apoptosis and antioxidant enzymes

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### ARTICLE INFO

#### Keywords:

Sodium butyrate  
Soyasaponins  
Inflammation  
Apoptosis  
MAPKs  
Turbot

### ABSTRACT

The study is aimed to investigate the protective effect and potential mechanisms of sodium butyrate (NaBT) on soyasaponins (SA) induced intestinal epithelial cells (IECs) injury *in vitro*. The primary IECs of turbot were developed and treated with 0.4, 1 and 4 mM NaBT in the presence of 0.4 mg/mL SA for 6 h to explore the protective effects of NaBT. The results showed that the addition of NaBT significantly down-regulated gene expression of inflammatory cytokine TNF- $\alpha$ , IL-1 $\beta$  and IL-8, pro-apoptosis relevant gene BAX, caspase-3, caspase-7 and caspase-9 induced by SA, while up-regulated anti-apoptosis gene Bcl-2. SA stimulation did not induce reactive oxygen species production, but elevated gene expression of antioxidant enzyme heme oxygenase-1 and superoxide dismutase. Moreover, the gene expression of those antioxidant enzyme was further up-regulated in NaBT groups. Furthermore, NaBT supplementation decreased the acid phosphatase and alkaline phosphatase activities and suppressed phosphorylation of p38 and c-Jun N-terminal kinase (JNK). In conclusion, NaBT could mitigate SA-induced inflammation and apoptosis and elevate gene expression of antioxidant enzymes on IECs of turbot and p38 and JNK signaling pathway participated in those processes.

### 1. Introduction

Soyasaponins (SA) is one of the main thermostable antinutritional factors (ANFs) present in soybean meal (SBM), ranging from 0.43% to 0.67% [1]. Previous studies have demonstrated that the inclusion of SA in diet could increase the intestinal epithelial permeability and induce intestinal inflammation response in the distal intestine of Atlantic salmon in combination with other plant components present in legumes [2–4]. Kroghdahl, Gajardo [5] revealed that SA caused increased severity of inflammation independent of basal diet in a dose-dependent manner. Moreover, study carried out on turbot showed that saponins could also increase intestinal epithelial cells (IECs) apoptosis and decrease antioxidant enzyme activity *in vivo* [6]. These studies manifested that SA could be the causal agent in SBM.

Sodium butyrate (NaBT), the sodium salt of butyric acid, has many beneficial effects and has been considered as a promising functional feed additive in aquafeeds. Previous *in vivo* studies have found that dietary supplementation of NaBT could decrease the overexpression of pro-

inflammatory cytokines, inhibit cellular apoptosis and promote the antioxidative capacity of fish [7–11]. In terms of mechanism, studies on mammals have shown that NaBT can inhibit inflammation, apoptosis and oxidative damage by regulating mitogen-activated protein kinases (MAPKs) signaling pathway. MAPKs are a group of protein serine/threonine kinases that play an important role in response to extracellular stimuli, mainly consisting of p38, extracellular signal-regulated kinase (ERK), and c-Jun N-terminal kinase (JNK) [12–16]. Previously, studies on fish showed that NaBT supplementation protected turbot from SBM-induced intestinal impairment [17] and enhanced intestinal barrier function of young grass carp by MAPKs signaling pathway [9, 11]. Whereas, these studies on fish are limited to *in vivo*, and researches on signal pathway only stay at the level of gene expression.

IECs are the most important cells in the intestinal tract in contact with the external environment and are also important multifunctional cells in the intestinal tract, which are essential for maintaining the normal function of the intestinal mucosal barrier. Accordingly, the present study aims to investigate the protective effect and potential

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<https://doi.org/10.1016/j.fsirep.2021.100031>

Received 31 August 2021; Received in revised form 27 September 2021; Accepted 23 October 2021

Available online 25 October 2021

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**Table 1**  
Primers used for qRT-PCR analysis.

Target genes	Forward primer	Reverse primer	Accession number
TNF- $\alpha$	GGACAGGGTGGTACAACAC	TTCAATTAGTGGCCAGCACAAGAG	AJ276709.1
IL-1 $\beta$	CGCTTCCCAACTGGTACAT	ACCTTCCACTTTGGGTCGTC	AJ295836.2
IFN- $\gamma$	GCTTCCCGATCATCTTCTG	GGTTTCCAGATCCCATTC	DQ400686.1
IL-8	GGCAGACCCCTTGAAGAATA	TGGTGAACCCTTCCCATAT	[6]
TGF- $\beta$ 1	TCAGCATCCAGATGTAGGTG	GGAGAGTGGCTTCAGTTTTTC	KU238187.1
Bcl-2	TTCCTCAACTCTCAAAGCACAATTC	ATTACTCTCGCTGGCCATTCC	MN782168
BAX	AGCATCTTTGCTGACGGGAT	GCGCTCTCTGATGACCTGAA	MN782169
Caspase-3	TTCTGCCATTGTCTCTGTGC	GCCCTGCAACATAAAGCAAC	JU391554.1
Caspase-7	TCTGCAATGCTCAACGAG	TTGCGACCATGTAGTTGACC	JU373310.1
Caspase-9	CCCAGGACATGATCGACGAG	ACAATGGGAAGGCTCGACTG	KY979512.1
Prdx-6	ACTGCCCGCTGTGTTTGTG	CGGCGTGGCAACCTTCTTCTG	ADJ57694.1
HO-1	ACGAGGGTCTGTCTCTTTGCC	CCTGGAGCGCTTTACTGGTTTA	JX453446.1
SOD	AAACAATCTGCCAAACCTCTG	CAGGAGAACAGTAAAGCATGG	MG253620.1
$\beta$ -actin	CGTGCGTGACATCAAGGAG	AGGAAGGAAGGCTGGAAGAG	AY008305.1

Abbreviations: TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-1 $\beta$ , interleukin-1 $\beta$ ; IFN- $\gamma$ , interferon- $\gamma$ ; IL-8, interleukin-8; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; Prdx-6, Peroxiredoxin-6; HO-1, heme oxygenase-1; SOD, superoxide dismutase;

mechanisms of NaBT on SA-caused IECs injury of turbot *in vitro*.

## 2. Materials and methods

### 2.1. Fish

Turbot (100 g  $\pm$  5 g) obtained from Yellow Sea Fishery Research Institute (Yantai, China) were fed with commercial feed to visual satiation twice daily at 8:00 and 18:00 in a flow-through system at Fisheries College of Ocean University of China. The water temperature ranged from 20 to 22°C, dissolved oxygen > 7.0 mg/mL, salinity 30 to 33‰ and pH 7.7–7.9. Animal care and handling procedures in present study were approved by the Animal Care Committee of Ocean University of China.

### 2.2. Isolation and culture of primary IECs

Fish were anesthetized with eugenol (1:10,000) (purity 99%, Shanghai Reagent Corp, China) after fasting for 24 h prior to sampling. The intestine was cut out longitudinally and rinsed with solution of hanks' balanced salt solution/penicillin-streptomycin (Thermo Fisher Scientific, USA) repeatedly. Then minced tissue about 1 mm<sup>3</sup> were obtained and digested with collagenase and dispase (Thermo Fisher Scientific, USA) for 15 to 20 min. Pipette the enzyme solution mildly after digestion, leave the contents to sediment, collect the supernatant and centrifuge for 5 min at 1000 rpm, repeat this procedure several times with L5520 medium (Sigma-Aldrich, USA). Collect the sediment and rinsed with culture medium for several times, then the cell were seeded into 6-well plates at a density of  $\sim 1.0 \times 10^6$  cells/mL with L5520 medium (5 % fetal bovine serum (Biological Industries, China), 10 ng/mL epidermal growth factor (Sigma-Aldrich, USA), 0.2 % insulin-transferrin-selenium-sodium pyruvate (Thermo Fisher Scientific, USA), 100 IU/mL penicillin and 100  $\mu$ g/mL streptomycin), and incubated in a 23°C incubator in ambient air.

### 2.3. SA challenge and NaBT treatments

The cells were seeded into 6-well plates at a density of  $\sim 1.0 \times 10^6$  cells/mL. After the cells adhered to the well for 24 h, fresh medium was introduced. Firstly, the cells were challenged with SA at 0 (control), 0.2, 0.4, 0.6, 0.8, and 1 mg/mL concentration for 6 h and the optimal SA concentration was determined according to the gene expression of inflammatory cytokines. Then, the cells were treated with 0, 0.4, 1 and 4 mM NaBT (Sigma-Aldrich, USA) together with 0.4 mg/mL SA for 6 h. All treatments were designed in triplicate wells.

### 2.4. Quantitative real-time PCR

Total RNA from the cells was isolated using RNAiso Plus (9108; Takara, Japan). The integrity of RNA was evaluated by running on a 1.2% agarose gel, and concentration determination was followed with NanoDrop®ND-1000 (Nano-Drop Technologies, USA). cDNA was obtained by following the manufacturer's instructions of PrimeScript® RT reagent Kit with gDNA Eraser (Takara, Japan).

Specific primers for the genes were generated by TSINGKE (China) and presented in Table 1.  $\beta$ -actin was used as the reference gene to normalize cDNA loading. The detailed methods were described in our previous work [18], with some modifications. In brief, the qPCR assays were carried out in a final volume of 20  $\mu$ L containing 10  $\mu$ L 2  $\times$  SYBR Green Real-time PCR Master Mix [SYBR® Premix Ex Taq™ (Tli RNaseH Plus)] (TaKaRa, Japan), 0.8  $\mu$ L (10  $\mu$ M) each forward and reverse primer, 1  $\mu$ L 100 ng/ $\mu$ L complementary DNA template, and 7.4  $\mu$ L dH<sub>2</sub>O.

### 2.5. Reactive oxygen species (ROS) production

The ROS level was detected by ROS kit (Beyotime Biotechnology Co. Ltd., cat. no. S0033S), which used fluorescent probe 2' - 7' dichloro-fluorescein diacetate (DCFH-DA). DCFH-DA itself has no fluorescence and can freely pass through the cell membrane, and can be hydrolyzed by esterase in the cell to produce fluorescent DCFH. Before the experiment, DCFH-DA was diluted with serum-free medium according to 1:1000. Remove the cell culture medium, add 1 mL diluted DCFH-DA to each well and incubate in the cell incubator for 30 min. Then, the cells were washed with L-15 medium for three times and observed under fluorescence microscope (Leica, Germany). The quantitative analysis were performed by Image J software.

### 2.6. Detection of acid phosphatase (ACP) and alkaline phosphatase (AKP) activity

ACP (cat. no. p0326) and AKP (cat. no. p0321s) kits were purchased from Beyotime Biotechnology Co. Ltd. (China). Cell lysates were obtained by cell lysis buffer without inhibitors (Beyotime Biotechnology Co. Ltd., cat. no. P0013J), the specific steps were performed according to the instructions, and the absorbance was measured at 405 nm by microplate reader. The protein concentration of the lysate was determined using the BCA kit (Beyotime Biotechnology Co. Ltd., cat. no. P0012).

### 2.7. Western blot analysis

Total protein extract of the cells was harvested in RIPA lysis buffer (Beijing Solarbio Science & Technology Co., Ltd., China) with inhibitors

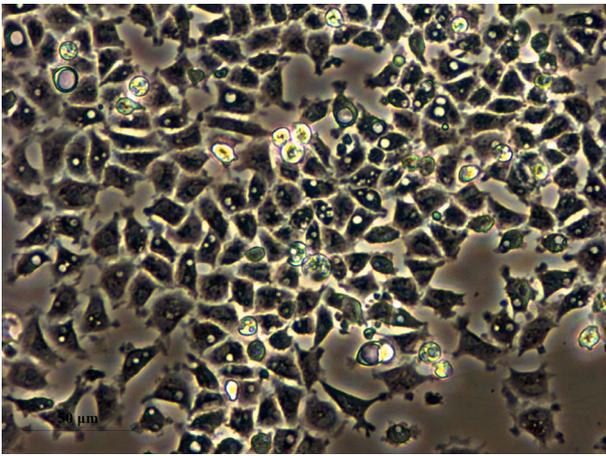


Fig. 1.. Morphology of primary culture intestinal epithelial cells of turbot.

of protease and phosphatase. After centrifugation at 12,000 g for 15 min at 4°C, the supernatant was collected and the protein concentration was determined using the BCA kit (Beyotime Biotechnology Co. Ltd., cat. no. P0012). Equal amounts of proteins of each sample were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis, followed by transfer to polyvinylidene difluoride membranes (PVDF) (Millipore, USA). After blocking with 5% non-fat milk in tris-buffered saline with tween-20 (TBST) buffer for 2 h, the PVDF membrane was

incubated with primary antibody at 4°C for 12 h. Then, the membrane was cleaned three times with TBST for 5 min each time. The membranes were then incubated with the secondary antibodies with HRP-conjugated horseradish peroxidase for 1 h at room temperature. After washing, the immunoreactivity was visualized with BeyoECL Star (Beyotime Biotechnology Co. Ltd., cat. no. P0018AM). The total gray values of each protein band were digitized by Image J software. Mouse p-p38 (cat. no. sc-166182), JNK (cat. no. sc-7345), p-JNK (cat. no. sc-6254), ERK (cat. no. sc-514302), p-ERK (cat. no. sc-7383) were obtained from Santa Cruz Biotechnology Inc. (USA). p38 (cat. no. AM065) and secondary antibodies (goat anti-mouse IgG-HRP, cat. no. A0216; goat anti-rabbit IgG-HRP, cat. no. A0208) were purchased from Beyotime Biotechnology Co. Ltd. (China). GAPDH (cat. no. 309154) were obtained from Golden Bridge Biotechnology (China).

## 2.8. Statistical analysis

The experimental data were analyzed by T-Test or One-way analysis of variance ANOVA with SPSS 22.0. Difference among different groups were analyzed by Tukey's multiple comparisons test.  $P$ -value < 0.05 was considered statistically significant, data were expressed as means  $\pm$  S.E. (standard error).

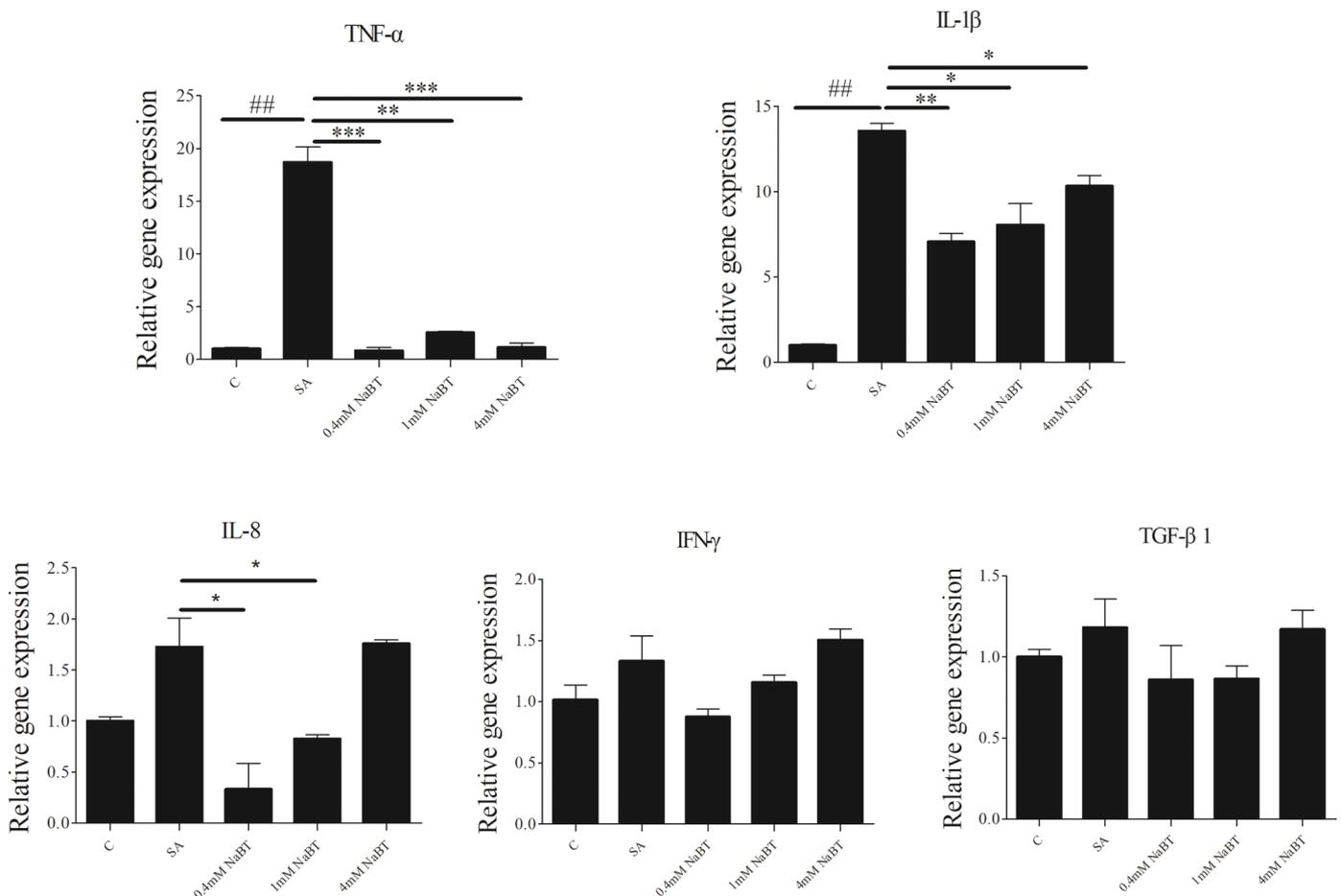
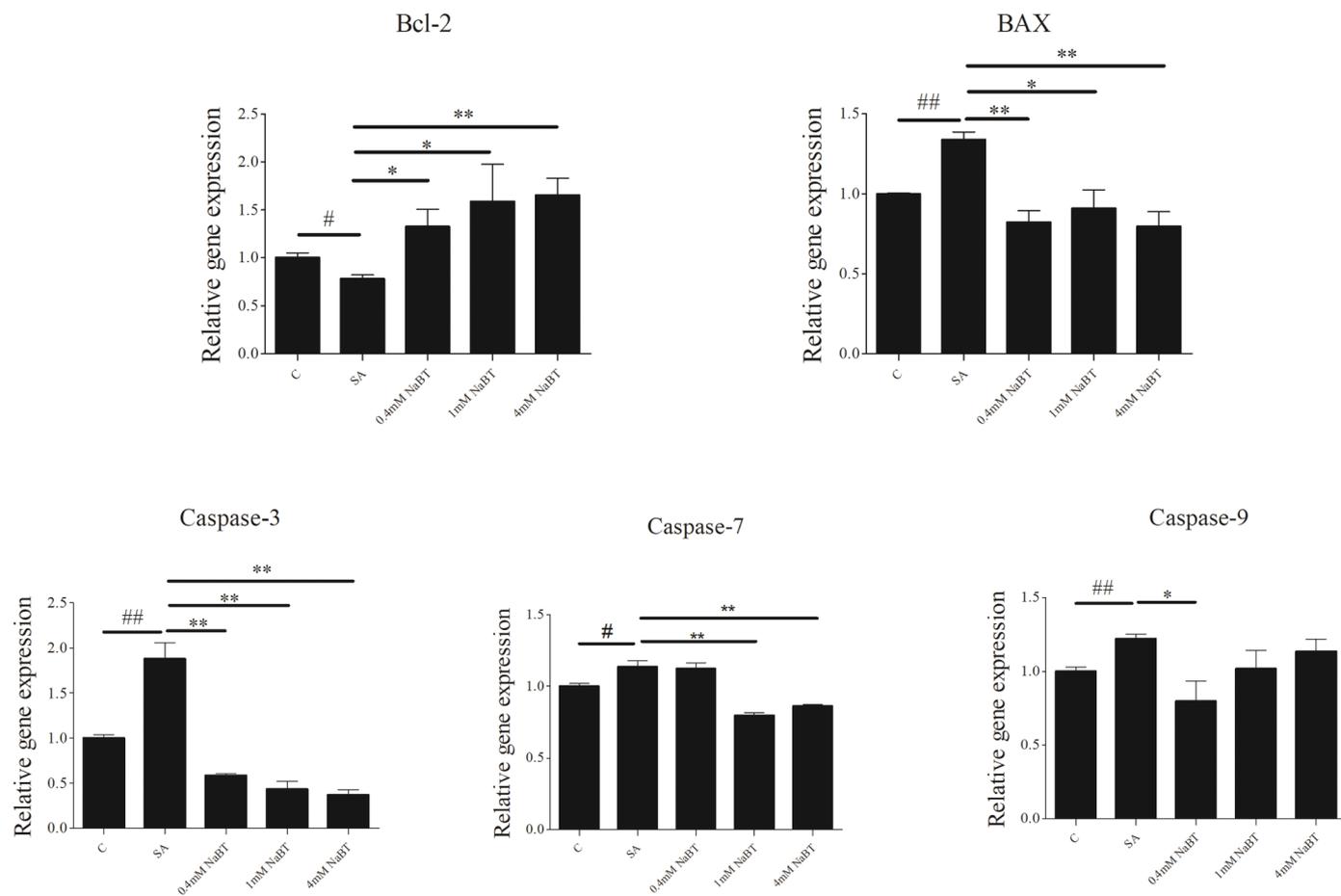


Fig. 2.. The effect of NaBT on genes expression of inflammatory cytokine in SA-stimulated primary intestinal epithelial cells. The cells were treated with SA or co-treated with NaBT (0.4 mM, 1 mM, 4 mM) and SA for 6 h. Values are expressed as means  $\pm$  SE (n=3). ##  $P$ <0.01, as compared with control; \*  $P$ <0.05, \*\*  $P$ <0.01, \*\*\*  $P$ <0.001, as compared with SA. TNF- $\alpha$ , tumor necrosis factor- $\alpha$ ; IL-1 $\beta$ , interleukin-1 $\beta$ ; IFN- $\gamma$ , interferon- $\gamma$ ; IL-8, interleukin-8; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; SA, soyasaponins.



**Fig. 3.** The effect of NaBT on apoptosis-relevant gene expression in SA-stimulated primary intestinal epithelial cells. The cells were treated with SA or co-treated with NaBT (0.4 mM, 1 mM, 4 mM) and SA for 6 h. Values are expressed as means  $\pm$  SE (n=3). #  $P < 0.05$ , ##  $P < 0.01$ , as compared with control; \*  $P < 0.05$ , \*\*  $P < 0.01$ , as compared with SA. SA, soyasaponins; NaBT, sodium butyrate.

### 3. Results

#### 3.1. Cell morphology of primary culture of IECs

After seeding, the primary IECs could adhere well within 24 h, and showed flagstone-like appearance which is the typical features of epithelial-like cells (Fig. 1).

#### 3.2. Effects of NaBT on SA-induced inflammatory cytokine gene expression

SA at 0.4 mg/mL was selected for the following experiments as the gene expression of TNF- $\alpha$ , IL-1 $\beta$  and IL-8 was significantly up-regulated in response to 0.4 mg/mL SA treatment (Supplementary Fig. S1). Under this condition, NaBT was added to explore the anti-inflammatory effects. The results showed that the addition of 0.4 – 4 mM NaBT significantly down-regulated the gene expression of TNF- $\alpha$ , IL-1 $\beta$  and IL-8 (except 4 mM NaBT group) compared with SA group (Fig. 2).

#### 3.3. The effect of NaBT on apoptosis relevant gene expression

SA treatment significantly up-regulated the gene expression of BAX, caspase-3, caspase-7 and caspase-9, while down-regulated anti-apoptotic protein Bcl-2 gene expression (Fig. 3) compared with control group. Meanwhile, NaBT (0.4 - 4 mM) supplement significantly reversed the change of BAX, caspase-3, and Bcl-2 expression caused by SA. Moreover, the gene expression of caspase-7 was significantly down-regulated by 1 and 4 mM NaBT. Besides, the caspase-9 expression was

also down-regulated in 0.4 mM NaBT treatment.

#### 3.4. Effects of NaBT on the level of ROS

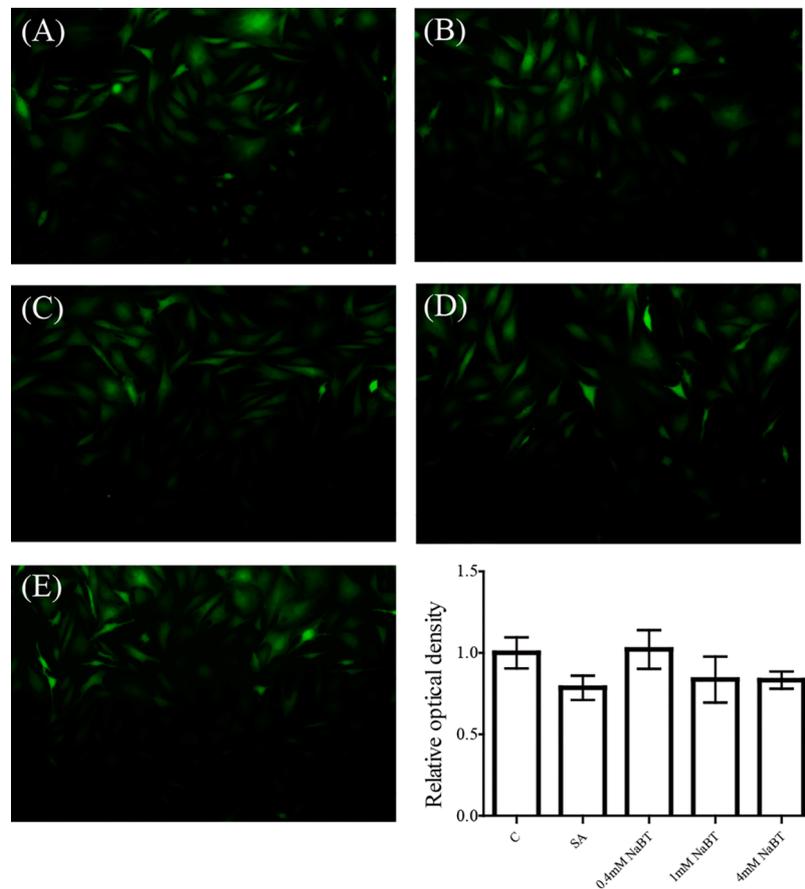
As showed in Fig. 4, SA treatment showed a slightly decreased ROS level, but there is no significant difference compared with control group. The addition of NaBT had no influence on ROS level compared to SA.

#### 3.5. Effects of NaBT on antioxidant enzyme relevant gene expression

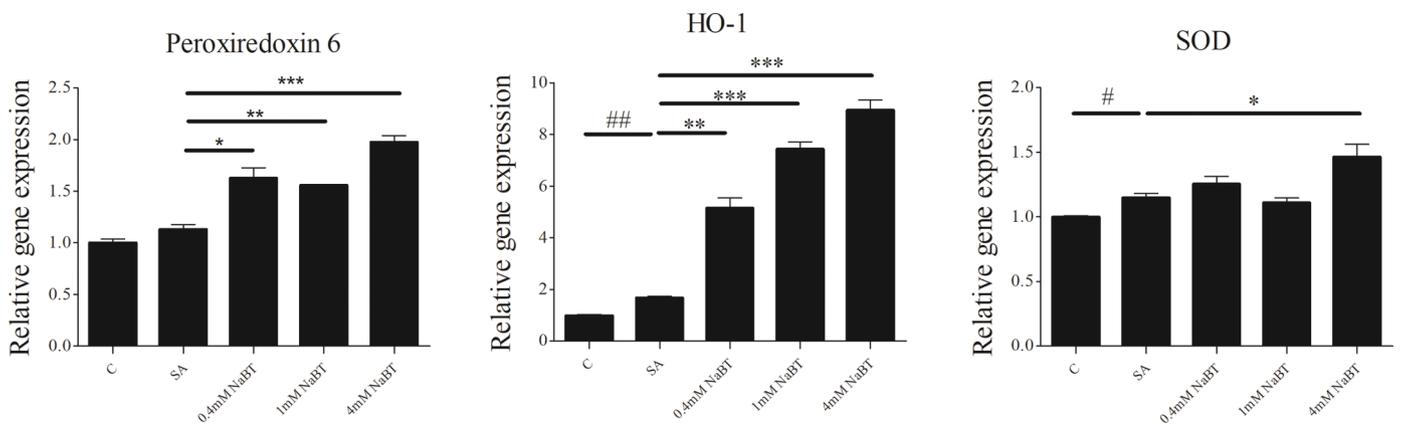
The cells exposed to SA significantly up-regulated heme oxygenase-1 (HO-1) and superoxide dismutase (SOD) gene expression, and had no significant effect on the expression of peroxiredoxin-6 (Prdx-6) (Fig. 5). Compared with SA group, the addition of NaBT remarkable elevated Prdx-6, HO-1 and SOD (only in 4 mM NaBT group) expression. The mRNA levels were induced by 5–9-fold of NaBT treatments compared to controls.

#### 3.6. Effects of NaBT on ACP and AKP activities

As shown in Fig. 6, compared with the control group, SA treatment had no significant effect on the activities of ACP and AKP. The addition of 0.4 mM NaBT significantly decreased ACP and AKP activities, and the decreased AKP activity also observed in 4 mM NaBT group compared with SA group.



**Fig. 4.** The effect of NaBT on ROS production. The cells were treated with SA or co-treated with NaBT (0.4 mM, 1 mM, 4 mM) and SA for 6 h. (A): C; (B): SA; (C): 0.4 mM NaBT; (D): 1 mM NaBT; (E): 4 mM NaBT.



**Fig. 5.** The effect of NaBT on antioxidant-relevant gene expression in SA-stimulated primary intestinal epithelial cells. The cells were treated with SA or co-treated with NaBT (0.4 mM, 1 mM, 4 mM) and SA for 6 h. Values are expressed as means  $\pm$  SE (n=3). #  $P < 0.05$ , ##  $P < 0.01$ , as compared with control; \*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.01$ , as compared with SA. HO-1, Heme Oxygenase-1; SOD, superoxide dismutase; SA, soyasaponins; NaBT, sodium butyrate.

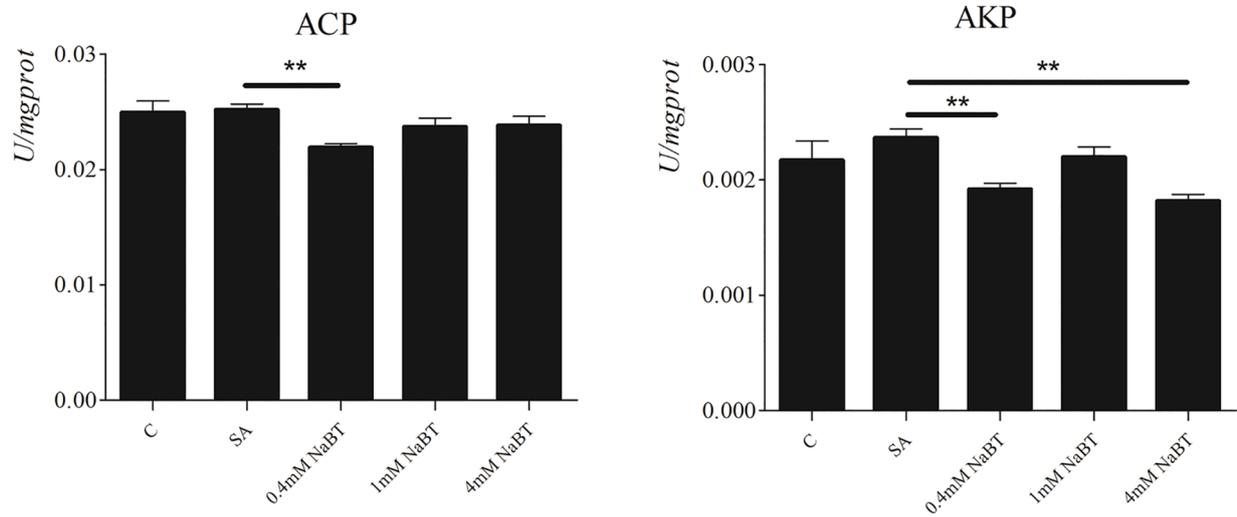
### 3.7. Effects of NaBT on MAPKs signaling pathway

As shown in Fig 7, compared with the control group, the phosphorylation levels of p38, JNK and ERK did not show any difference in SA-stimulated cell. The addition of 1 and 4 mM NaBT significantly suppressed the phosphorylation of p38 and JNK, and 0.4 mM NaBT supplement decreased the phosphorylation of JNK compared with SA group.

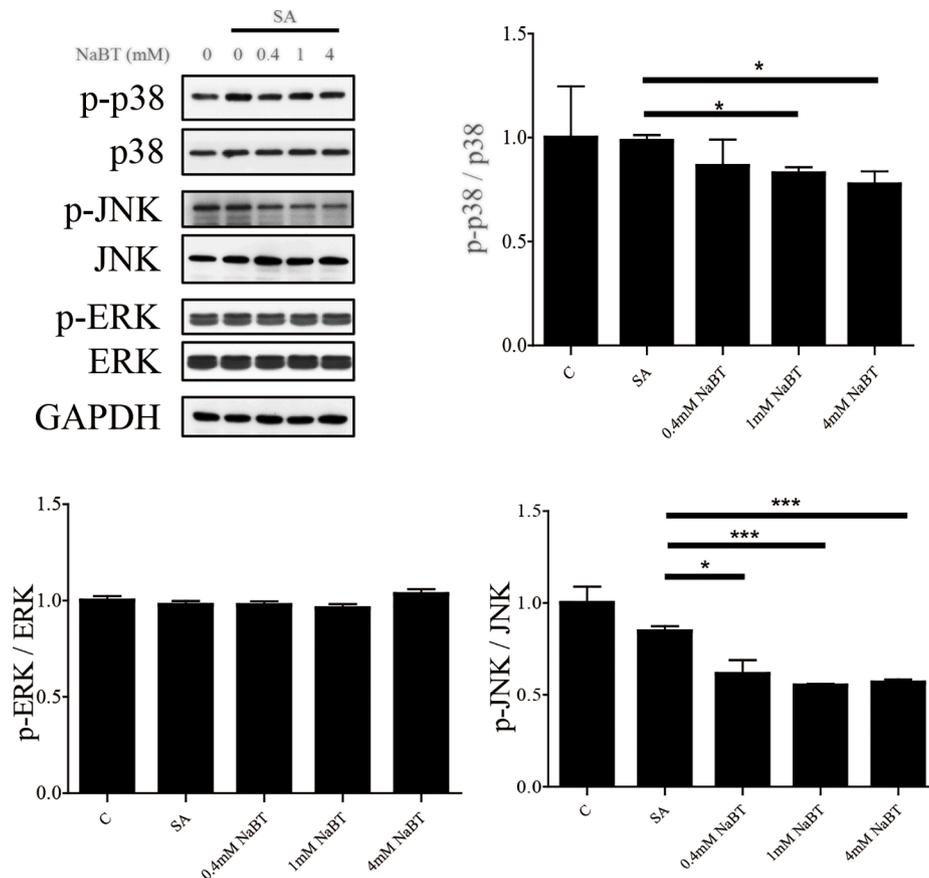
## 4. Discussion

*In vitro* culture of IECs is of great significance for studying the absorption and metabolism of nutrients and intestinal pathological responses induced by various pathogenic factors. In this study, the IECs of the turbot were developed to explore the protective effects of NaBT in response to SA induced cell injury of turbot *in vitro*.

SA is a causal agent in SBM-induced enteritis. In this study, the IECs challenged with 0.4 mg/mL SA significantly up-regulated the gene expression of TNF- $\alpha$  and IL-1 $\beta$ , which were dramatically suppressed by



**Fig. 6.** The effect of NaBT on ACP and AKP production in SA-stimulated primary intestinal epithelial cells. The cells were treated with SA or co-treated with NaBT (0.4 mM, 1 mM, 4 mM) and SA for 6 h. Values are expressed as means  $\pm$  SE (n=3). \*\*  $P < 0.01$ , as compared with SA. ACP, acid phosphatase; AKP, alkaline phosphatase; SA, soyasaponins; NaBT, sodium butyrate.



**Fig. 7.** The effect of NaBT on MAPK signaling in SA-stimulated primary intestinal epithelial cells. The cells were treated with SA or co-treated with NaBT (0.4 mM, 1 mM, 4 mM) and SA for 6 h. Values are expressed as means  $\pm$  SE (n=3). \*  $P < 0.05$ , \*\*\*  $P < 0.001$ , as compared with SA. SA, soyasaponins; NaBT, sodium butyrate.

the addition of NaBT. In fish, TNF- $\alpha$  and IL-1 $\beta$  are early pro-inflammatory cytokines at early stage of infection and play a key role in leading to inflammation by inducing a cascade of reaction and mediating the expression of other cytokines and chemokines [19,20]. Moreover, the expression of TNF- $\alpha$  and IL-1 $\beta$  is usually used to indicate the inflammation of fish *in vivo* and *in vitro* [21–25]. In present study, the significantly increased gene expression of TNF- $\alpha$  and IL-1 $\beta$  suggested SA

successfully triggered inflammatory response on IECs of turbot. Generally speaking, acute inflammatory response is essential for tissue homeostasis, however, if the inflammation cannot be resolved, it will develop chronic inflammation, which gives rise to many chronic inflammatory diseases [26–28]. NaBT, the sodium salt of butyrate, is a kind of potential anti-inflammatory substance. Previous studies on fish show that dietary NaBT could improve intestinal immunity and

ameliorate SBM-induced intestinal inflammation [9,17]. *In vitro* research on RAW246.7 macrophages found that pre-treatment with NaBT (5 mM) could inhibit LPS-induced inflammation by down-regulating the expression of TNF- $\alpha$  and IL-6 [29]. Similarly, in present study, the gene expression of inflammatory cytokines TNF- $\alpha$ , IL-1 $\beta$  and IL-8 was significantly down-regulated following NaBT treatment under SA stimulated. These results confirmed the anti-inflammatory effects of NaBT on SA-stimulated IECs.

Intestinal epithelium is the key barrier to insulate the internal environment of the body from external harmful substances. The damage or excessive apoptosis of IECs can destroy barrier integrity and lead to inflammation [30]. Apoptosis usually inevitably happened in SBM-induced enteritis of turbot [6,31,32]. Previous *in vivo* study on turbot found that dietary saponin induced the IECs apoptosis in a dose-dependent manner by TdT-mediated dUTP nick end labeling [6]. Similarly, the cells apoptosis caused by SA was also observed in the current study, which was manifested as the increase of BAX, caspase-3, caspase-7 and caspase-9 and decrease of Bcl-2. Bcl-2 family, composed of anti-apoptotic and pro-apoptotic members, is the most characteristic protein family involved in the regulation of apoptotic cell death [33]. Bcl-2 and Bax are representative proteins of anti-apoptotic and pro-apoptotic protein families, respectively. The Bcl-2/Bax ratio determines the direction of apoptosis [34]. The caspase-cascade system plays an important role in the induction, transduction and amplification of intracellular apoptotic signals. At the beginning of apoptosis, initiator caspases (caspase-8 and -9) cleave and activate downstream effector caspases (caspase-3, -6, and -7), which directly cause apoptosis. It has been shown that NaBT could manipulate the expression of caspase-1, caspase-3, BAX, and Bcl-2 to ameliorate TNF- $\alpha$  induced apoptosis in SH-SY5Y cells [35]. Another experiment on juvenile grass carp showed that inclusion of NaBT in the diet down-regulated the gene expression of caspase-2, -3, -7, -8 -9 and BAX [11]. Here we found that administration with NaBT remarkably reversed those apoptosis-relevant gene expression in SA stimulated cells, exhibiting a potential apoptotic property.

Oxidative stress plays a pathogenic role in chronic inflammatory diseases. Previously, imbalance of redox homeostasis and impairment of intestinal antioxidant system were observed in SA induced intestinal inflammation of turbot [6]. Oxidative stress refers to the overproduced intracellular ROS and is caused by the imbalance between antioxidants and free radicals. However, in present study, SA stimulation did not cause oxidative stress in IECs of turbot as the level of ROS did not show any difference in all groups. Even so, the gene expression of antioxidant enzyme HO-1 and SOD were significantly up-regulated after SA treatment, and these gene expression were further increased after NaBT treatment. The gene expression and activity of antioxidant enzymes are regulated by multiple factors, such as oxidative status and inflammation [36]. In recent years, a large number of studies have found that antioxidant enzymes such as Prdx-6, HO-1 and SOD can not only scavenge free radicals and maintain redox balance, but also have immunomodulatory function [37–39]. As mentioned in the literature review of Sebastian, Salazar [40], HO-1 activation could reduce intestinal inflammation in several animal models of inflammatory bowel disease by inducing anti-inflammatory cytokine pathway. Overexpression of SOD3 suppressed the expression of inflammatory cytokines through modulating many signaling pathway [38]. Based on these studies, we speculated that the significantly up-regulated expression of antioxidant enzymes in this study might play an important anti-inflammatory role in NaBT group. A possible explanation for the up-regulated expression of HO-1 and SOD in SA group was that the increased expression of these enzyme was insufficient to counteract the inflammation. ACP and AKP are lysosomal enzymes, participate in the body's immune response and are regarded as a biomarker for toxicological evaluation of external toxicants. The increased ACP and AKP activity usually indicated a damage of lysosomal membranes and immunological toxicity [41]. In current study, although the stimulation of SA did not affect the activities of ACP and AKP, their activities were significantly suppressed after

NaBT treatment, which indicated that the addition of NaBT had a cytoprotective effect on SA-treated cells.

MAPKs cascades are key signaling pathway mediating the transduction of extracellular signals to the intracellular environment. It has been reported that NaBT could mediate MAPKs signaling pathway. Wang, Wu [42] reported that dietary supplementation with NaBT could inhibit the phosphorylation of JNK in jejunal mucosa of weaned pigs accompanied with improved intestinal barrier and decreased inflammatory mediator production. Similarly, Wu, Tian [11] found that JNK signaling pathway involved in NaBT mediated inhibition of intestinal cell apoptosis, improvement of antioxidant level and intestinal structural integrity in grass carp. In present study, we found that the addition of NaBT significantly suppressed the relative protein levels of phosphorylated JNK and p38. The result was consistent with previous study, NaBT supplementation down-regulated p38 gene expression and improved intestinal immune function in young grass carp [9]. These data suggested that the addition of NaBT could protect IECs of turbot from SA-induced cell inflammation and apoptosis through mediating JNK and p38 signaling pathway.

In conclusion, the current study showed that NaBT treatment inhibited the gene expression of inflammatory cytokines and apoptosis mediators induced by SA, up-regulated the expression of prdx6, HO-1 and SOD and suppressed the activities of ACP and AKP. In addition, the JNK and p38 signaling pathways participated in the protective effects of NaBT on SA-stimulated IECs of turbot.

## Funding

This work was supported by National Key R&D Program of China [2019YFD0900104]; the National Natural Science Foundation of China [No. 31872577]; and China Agriculture Researches System [Grant No. CARS 47-G10].

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

We appreciate Zhichu Chen and Qiuchi Chen for their assistance in this study.

## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fsirep.2021.100031.

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