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Promotion of Extrinsic Apoptosis Pathway in HCT-116 Human Colorectal Cancer Cell Line by Sodium Butyrate as Histone Deacetylase Inhibitor

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Abstract

Background: Colorectal cancer (CRC) has already been considered the fourth leading cause of mortality worldwide as the genes involved in apoptotic pathways and alterations of reversible epigenetic have an important role in the progression of CRC.

Objectives: The current study aimed to evaluate the effect of sodium butyrate as a histone deacetylase inhibitor on the alterations of the gene expression of *FAS*, *Fas ligand* (*FASL*), *Death receptor 4* (*DR4*), *Death receptor 5* (*DR5*), and *tumor necrosis factor-related apoptosis-inducing ligand* (*TRAIL*) in HCT-116 CRC cell line.

Methods: HCT-116 cell line was cultured in Dulbecco's Modified Eagle Medium. The cytotoxicity effect of sodium butyrate on HCT-116 was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide assay for three incubation times (i.e., 24, 48, and 72 h). The half-maximal inhibitory concentration (IC_{50}) values were determined. The optimum concentration was within the range of 6.25-200 mM. The cellular ribonucleic acid was extracted, and complementary deoxyribonucleic acid was synthesized. Finally, the alterations of the gene expression of *FAS*, *FASL*, *DR4*, *DR5*, and *TRAIL* were assessed by real-time polymerase chain reaction (PCR).

Results: The IC_{50} levels for three incubation times were 50, 12.5, and 6.25 mM, respectively. The obtained results of real-time PCR demonstrated a significant increase in the gene expression of *TRAIL*, *DR5*, and *FAS* in comparison to that of the untreated cells as the control group at the three incubation times. The *DR4* gene expression significantly increased in comparison to that reported for the control group at 48 and 72 h of incubation. In addition, *FASL* gene expression remarkably decreased at the three incubation times.

Conclusion: Sodium butyrate could show cytotoxicity effect on CRC cell lines through the induction of death receptors in the extrinsic apoptotic pathway. The obtained results of this study revealed that the optimum effect of sodium butyrate is an incubation time-dependent and concentration-dependent manner.

Keywords: Apoptosis, Colorectal cancer, HCT-116, Sodium butyrate

1. Background

Colorectal cancer (CRC) is a multifactorial disease and the fourth leading cause of cancer-related mortality in the world (1). Performed studies on various agents involved in the incidence of CRC demonstrated that diet, obesity, body mass index, smoking, aging, and alcohol and excessive meat consumption could have an important role in the development of cancer. The CRC is often observed in individuals over the age of 75 years (2). Recently, the rising incidence of CRC has been reported that could be due to lifestyle, nutrition, and reduction of physical activities (3).

In this regard, apoptosis is a form of programmed cell death occurring in various organs, such as the liver, and spleen, and gastrointestinal system (4). Apoptosis impairment is one of the key mechanisms in cancer occurrence (5). In addition, apoptosis processes consist of two pathways in which releasing cytochrome C from mitochondria to the cytoplasm is related to intrinsic pathways. On the other hand, some of the molecules, such as *FAS, Fas ligand (FASL), tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), Death receptor 4 (DR4), and Death receptor 5 (DR5),* have important roles in the extrinsic pathway (6, 7). The genes of these molecules are considered tumor inhibitor genes (8).

Apoptosis could be triggered by the engagement of *FAS*L with its specific receptor *FAS*. The binding of the homotrimer ligand of *FAS* to *FAS* leads to the accumulation of the receptors, and the activated receptors recruit the adaptor molecule (i.e., Fas-associated protein with death domain [FADD]) through the interaction with the death domain. The FADD with the recruiting of procaspase-8 causes to form the death-inducing signaling complex (DISC), a multi-protein complex from death receptors; finally, activated caspase-8 is separated from the DISC, and caspase-3 leads to death (9).

The *FAS* is expressed in activated T cells and lymphoid cells, such as B, T, and natural killer (NK)

cells. The *FAS/FASL* cause to induce apoptosis in cancer cells and maintain immune hemostasis (10). The *FAS/FASL* expression by cancer cells indicates that these cells are resistant to apoptosis through *CD95L* mediation (11, 12). Furthermore, some studies showed that *FAS* expression is downregulated in CRC cells resulting in resistance to apoptosis.

The *TRAIL* is another protein acting as a ligand to kill the cells through programmed cell death. This protein can induce apoptosis in many types of tumor cells and deformed cells (13, 14). However, the *TRAIL* gene is expressed in various immune cells, such as T cells and NK cells, that are capable of preventing the metastasis of tumor cells; nevertheless, it does not have a performance in normal cells. Therefore, it could be a useful option for the treatment of some cancers (15, 16).

The *TRAIL* with binding to two closely related receptors that are *DR4* and *DR5* facilitates the induction of apoptosis in malignant cells. However, several studies indicated that some tumor cells are resistant to the apoptotic effects of *TRAIL*. The deleterious mutations arising in *DR4* and *DR5* can also cause such resistance (17, 18). On the other hand, histone deacetylase inhibitors (HDACi) are regarded as novel chemotherapeutic drugs inducing apoptosis via the promotion of two apoptotic pathways and upregulation of *DR5* gene expression. Furthermore, HDACi are capable of sensitizing colon cancer cells to *TRAIL*- and *FAS*- mediated apoptosis (19, 20).

Sodium butyrate (NaB) is a short-chain saturated fatty acid existing in the human intestinal wall as a food metabolite. NaB could exhibit an anticancer effect as an HDACi, and several studies indicated that NaB is a general inducer of growth arrest and apoptosis in colon cancer cell lines (21). Nonetheless, NaB is an effective metabolite in gene expression, transcription activators, and histone acetylation in cancer cells, with little effect on normal cells (22). According to the above-mentioned findings, NaB is considered a drug for the elimination of tumor cells through activating apoptosis mechanisms (23). In addition, the utilization of HDACi in cancer treatment has led to obtaining beneficial results in recent years.

2. Objectives

One of the most primary goals of drug design in cancer therapy is the effect on the vital mechanisms of cancerous cells. Apoptosis is the most important biological mechanism with a critical role in the balance between the expressions of genes involved in the cell hemostasis. The achievement of the molecular effects of drugs on cells is very important because they exert various effects on different types of cancers which have multiple effects on gene expression. Moreover, the effect varies according to the response rate to the drug leading to different gene expressions. Therefore, the present study aimed to evaluate the effect of NaB on extrinsic apoptosis pathways genes, *FAS* receptor, and *TRAIL* receptor simultaneously in colorectal carcinoma HCT-116 cells.

3. Methods

3.1. Cell culture

As the human CRC cell line, HTC-116 was used and prepared by Pasteur Institute of Iran. The HTC-116 cell lines were cultured in Dulbecco's Modified Eagle Medium (Gibco, US) with 10% fetal bovine serum (Gibco, US) and antibiotics, including streptomycin (100 µg/ml) and penicillin (1,000 units/ml^{-1}). They were incubated in a 5% CO_2 humidified incubator at 37˚C. When the confluency of the cells reached 80%, trypsin (25%; Gibco, US) and ethylenediaminetetraacetic acid (0.02%; Gibco, US) were used for the separation of the cells.

3.2. Sodium butyrate treatment

Sodium butyrate was dissolved in sterile water. The harvested cells were seeded into 96-well plates. The different concentrations of NaB (i.e., 6.25, 12.5, 25, 50, 100, 150, and 200 mM) were used for the treatment of the cells. They were incubated in a 5% CO_2 humidified incubator at 37˚C. After 24, 48, and 72 h of incubation, the cell viability was evaluated using 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide (MTT) assay.

3.3. MTT Assay

The MTT assay was used to evaluate the effect of NaB on cell proliferation, and the half-maximal inhibitory concentration (IC_{50}) values were also calculated. Briefly, the density values of 80,000, 50,000, and 20,000 cells/well were plated onto 96-well plates (Sigma-Aldrich, US) and treated with different concentrations of NaB (range: 6.25 -200 mM) and incubated for 24, 48, and 72 h. The untreated cells were used as the control group. Then, 100 µl of MTT solution (0.5 mg/ml) was added to each well and incubated at 37°C with 5% CO_2 for 3 h. Subsequently, 50 µl of dimethyl sulfoxide solution was dispensed to each well, and the plate was gently shacked. Finally, an enzyme-linked immunosorbent assay reader was applied for the measurement of optical density (OD) at a wavelength of 546/630. Cell viability percentage was calculated using the following formula:

(%) = (ODexp) / (ODcon) * 100

Where *ODexp* and *ODcon* stand for the ODs of the treated and untreated cells, respectively.

3.4. RNA extraction and quantitative real-time PCR

Total cellular ribonucleic acid (RNA) was extracted from both groups, including the group treated with NaB and the control group (i.e.,

untreated cells), using RNX reagent (Sinaclon, Iran) according to the manufacturer' instructions. The quality of the extracted RNA was evaluated by agarose electrophoresis gel. In addition, 2000 ng of RNA was reverse-transcribed to complementary deoxyribonucleic acid (cDNA). The cDNA was synthesized using a cDNA synthesis kit (Yekta-Tajhiz, Iran) according to the manufacturer's instruction. The *TRAIL, DR4, DR5, CD95*, and *CD95L* messenger RNA (mRNA) levels were determined by quantitative real-time PCR. *Glyceraldehyde 3-phosphate dehydrogenase* (*GAPDH*) primer was used as an internal control. All the experiments were performed in duplicate. Specific primers were designed that are brought as follows:

GAPDH forward (F): 5'-GAAGGTGAAGGTCGGAGTC-3', reverse (R): 3'-GAAGATGGTGATGGGATTTC-5'

CD95 forward (F): 5'-TGCCAAGAAGGGAAGGAGAA-3', reverse (R): 3'-CGGGTGCAGTTTATTTCCAC-5'

CD95L forward (F): 5'-AGCAAATAGGCCACCCCAGTCC-3', reverse (R): 5'-TGGCTCAGGGGCAGGTTGTTG-3'.

DR4 forward (F): 5'- TCCAGCAAATGGTGCTGAC-3', reverse (R): 3'-GAGTCAAAGGGCACGATGTT-5'

DR5 forward (F): 5'-CCAGCAAATGAAGGTGATCC-3', reverse (R): 3'-GCACCAAGTCTGCAAAGTCA-5'

TRAIL forward (F): 5'-GAAGGTGAAGGTCGGAGTC-3', reverse (R): 5'-GACCAGTTCACCATTCCTC-3'

Real-time PCR was carried out with the conditions of 95°C for 180 sec, 40 cycles of 5 sec at 95°C and 30 sec at 60°C, 95°C for 30 sec, and 55°C for 30 sec. Melting curve analysis was used to verify the single PCR product amplification of each primer.

3.5. Statistical analysis

The obtained results were presented as the mean standard error of the mean. One-way analysis of variance statistical method was utilized to analyze the data. Statistical analysis was also carried out using Graph Pad Prism software (version 6). Regression analysis was used for the calculation of IC_{50} values. A p-value of less than 0.05 was considered statistically significant. Relative expression software tool 2009 ($REST_{2009}$) software was utilized to analyze target gene expression.

4. Results

4.1. Cytotoxicity assay

In the present study, 80000, 50000, and 20000 HCT-116 cells were seeded into 96-well plates for 24, 48, and 72 h (unpublished), respectively, and treated with different concentrations of NaB (range: 6.25-200 mM). The cytotoxicity effect was evaluated by the MTT assay. The cell growth rate was inhibited in a dose-dependent manner. The IC_{50} values of NaB on HCT-116 cells were determined (figures 1A, 1B, and 1C). The IC_{50} value after 24 h of incubation was

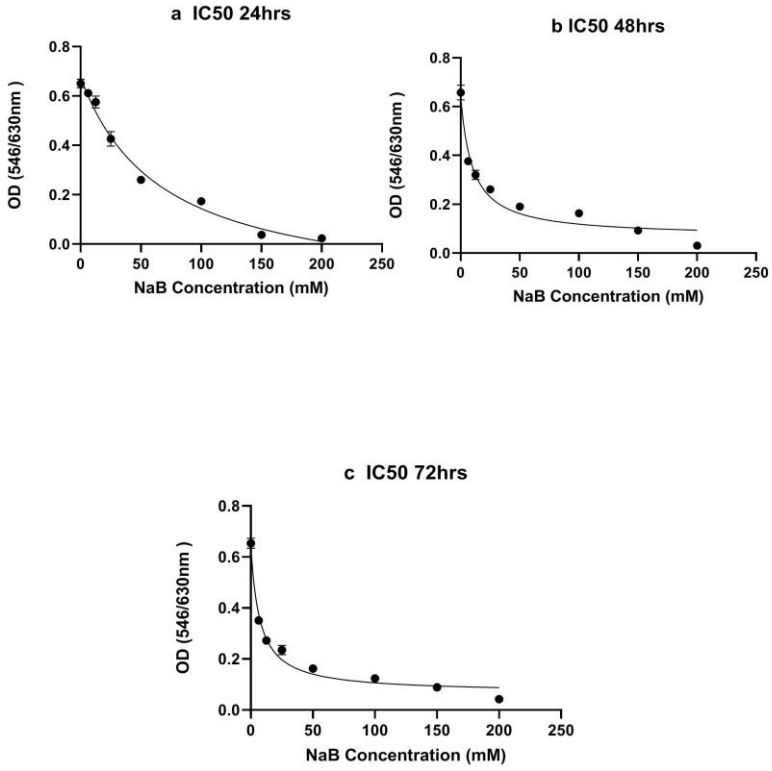

Figure 1. Cytotoxic effect of NaB on HCT-116 cells measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5- diphenyltetrazolium bromide assay; half-maximal inhibitory concentration values at 24, 48, and 72 h reported as 50, 12.5, and 6.25 mM, respectively

reported as 50 mM. The IC_{50} concentration after 48 h of incubation was less than 12.5 mM (within the range of 6.25-12.5 mM), and the IC_{50} concentration after 72 h of incubation was almost 6.25 mM. The confidence interval was reported as 95%.

4.2. Gene expression

The effect of NaB on the mRNA levels of *FAS, FasL, DR4, DR5,* and *TRAIL* was statistically significant.

4.2.1. FASL mRNA expression

HCT-116 cells were incubated with IC_{50} concentration of NaB and concentration after and before IC_{50} concentrations (i.e., 25, 50, and 100 mM for 24 h of incubation; 6.25, 12.5, and 25 mM for 48 and 72 h of incubation). After 24 h of treatment with NaB, the mRNA level of *FASL* significantly reduced about 21 and 9.84 folds at 25 and 50 mM concentrations, respectively, compared to that reported for the untreated group ($p<0.05$, Figure 2A). After 48 h of treatment with NaB, *FASL* expression significantly decreased about 14.77 and 12.38 folds at concentrations of 12.5 and 6.25 mM, respectively, compared to that of the untreated group ($P<0.05$; Figure 2B). After 72 h of treatment with NaB, the gene expression of *FASL* significantly reduced about 1.86, 16.33, and 30.27 folds at concentrations of 6.25, 12.5, and 25 mM, compared

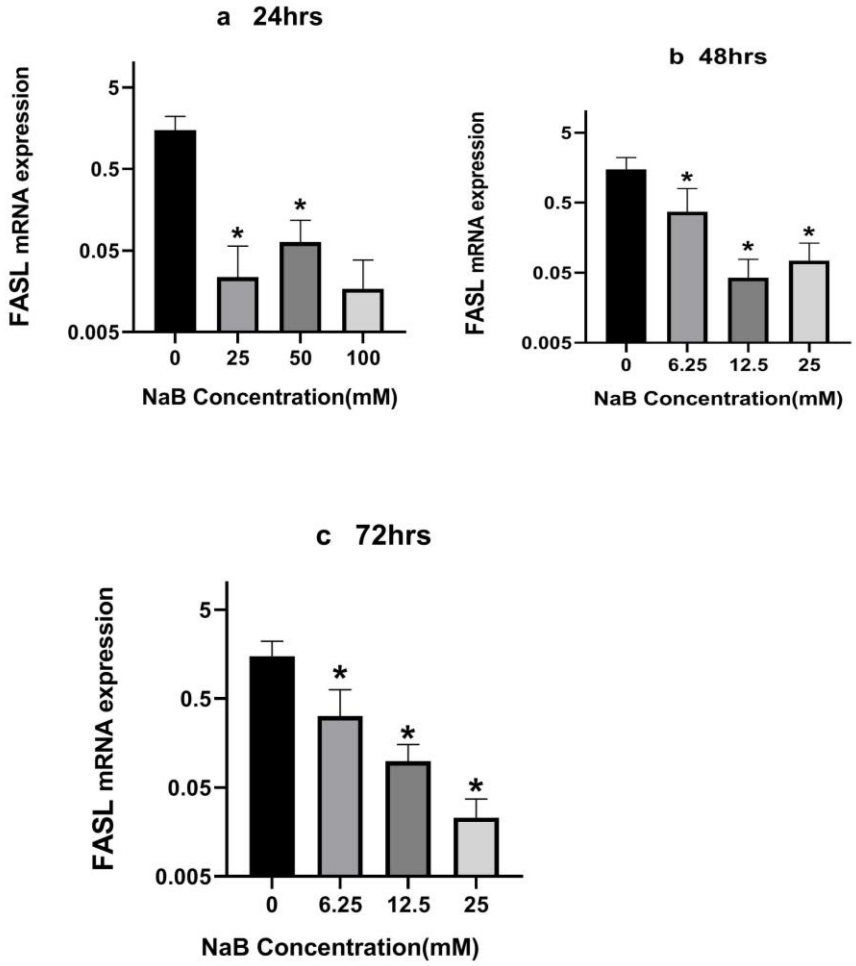

Figure 2. *Fas ligand* (*FASL*) gene expression; A) messenger cellular ribonucleic acid level of *FASL* after 24 h of treatment with NaB showing a significant reduction at concentrations of 25 and 50 mM; B), remarkable reduction of gene expression of *FASL* at concentrations of 6.25 and 12.2 mM after 48 h of treatment with NaB; C) significant reduction at concentrations of 6.25, 12.5, and 25 mM after 72 h of treatment with NaB; evaluation of *FASL* gene expression using quantitative real-time polymerase chain reaction; *P<0.05

to that reported for the untreated group (P<0.05; Figure 2C). The obtained results showed a time- and dose-dependent downregulation in the mRNA level of the *FASL* gene.

4.2.2. FAS mRNA expression

NaB-treated HCT-116 cells were incubated with IC_{50} concentrations (i.e., 25, 50, and 100 mM for 24 h of incubation; 6.25, 12.5, and 25 mM for 48 and 72 h of incubation). There was a significant increase in 2.31 and 1.12 folds at concentrations of 50 and 100 mM in the treated group with NaB, compared to that reported for the control group after 24 h of incubation (P=0.005; Figure 3A). However, a concentration of 25 mM caused a 1.33-fold remarkable reduction in comparison to that of the control group (P=0.001). After 48 h of treatment with NaB, the mRNA level of *FAS* significantly increased 6.23, 3.021, and 7.38 folds, compared to that reported for the control group (P=0.001; Figure 3B). In addition, the results after 72 h of treatment with NaB showed a significant increase of 10.41 and 4.79 folds, compared to that of the control group at concentrations of 6.25 and 12.5 mM (P=0.005; Figure 3C).

4.2.3. DR4 gene expression

NaB-treated HCT-116 cells were incubated with

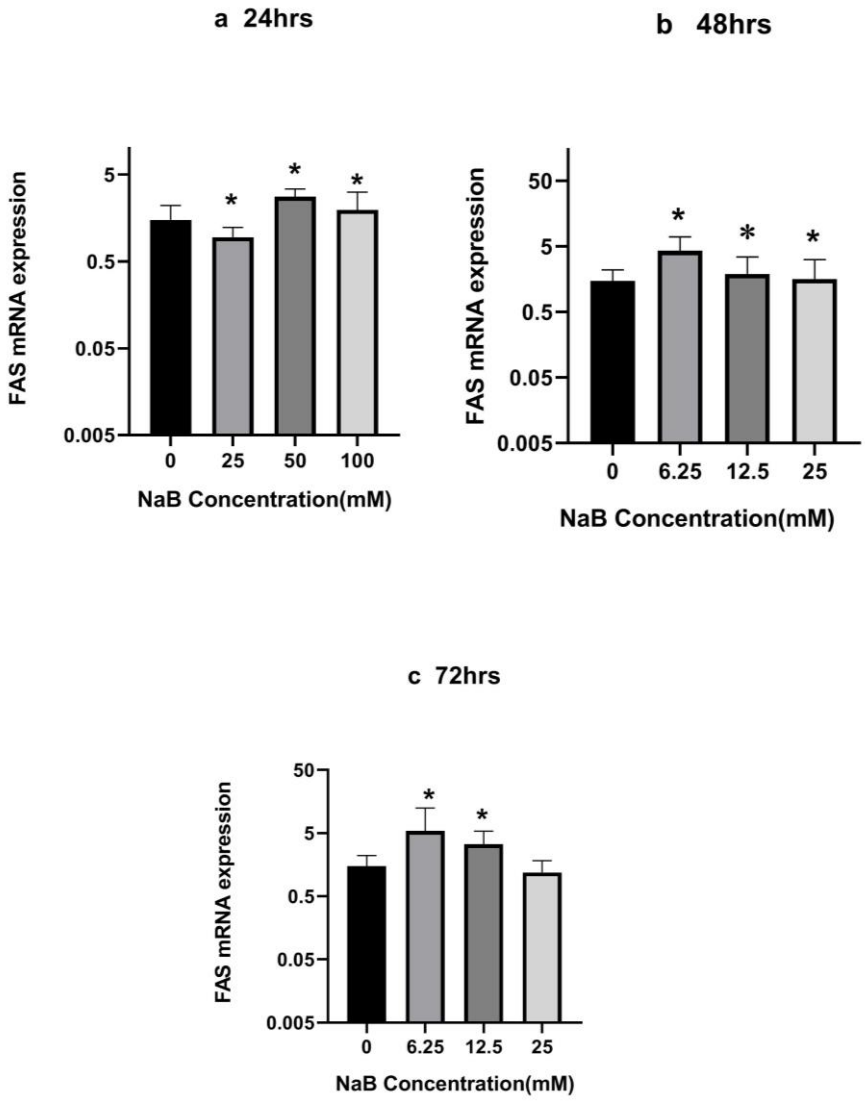

Figure 3. *FAS* gene expression; A), significant increase of *FAS* gene expression at concentrations of 50 and 100 mM after 24 h of treatment with NaB, compared to that reported for the untreated group; B) significant increase at concentrations of 6.25, 12.5, and 25 mM caused by *FAS* gene expression after 48 h of treatment with NaB, compared to that reported the untreated group; C) *FAS* gene expression after 72 h of treatment with NaB demonstrating a significant upregulation at concentrations of 6.25 and 12.5 Mm, compared to that reported for the untreated control group; evaluation of *FAS* gene expression using quantitative real-time polymerase chain reaction; *P<0.05

IC_{50} concentrations (i.e., 25, 50, and 100 mM for 24 h of incubation; 6.25, 12.5, and 25 mM for 48 and 72 h of incubation). The results obtained from the *DR4* gene expression demonstrated a 5.83-fold remarkable reduction at a concentration of 25 mM, compared to that reported for the control group after 24 h of treatment with NaB (P=0.005; Figure 4A). At concentrations of 50 and 100 mM, there was a reduction in *DR4* gene expression. However, this reduction was not remarkable in comparison to that of the control group. After 48 h of treatment with NaB, *DR4* gene expression showed a 21.85- and 88.95-fold significant increase at concentrations of 6.25 and 25 mM respectively, compared to that reported for the control group (P=0.005; Figure 4B). Furthermore, there was a 69.79-fold significant increase at a concentration of 6.25 mM in comparison to that of the control group after 72 h of treatment with NaB (P=0.005; Figure 4C).

4.2.4. DR5 gene expression

NaB-treated HCT-116 cells were incubated with IC_{50} concentrations of 25, 50, and 100 mM for 24 h of incubation in addition to 6.25, 12.5, and 25 mM for 48 and 72 h of incubation. The results of the *DR5* gene expression showed that 1.67-, 1.81-, and 1.59-fold significant increases were observed at concentrations of 25, 50, and 100 mM, respectively, compared to those reported for the control group after 24 h (P=0.005; Figure 5A). The mRNA level of the *DR5* gene remarkably increased about 6.86 and 74 folds at concentrations of 6.25 and 25 mM, respectively, compared to that of the control group after 48 h of incubation (P=0.005). Nevertheless, at a concentration of 12.5 mM, there was a significant decrease of about 3.08 folds in *DR5* gene expression in comparison to that of the control group after 48 h of treatment with NaB (P=0.005; Figure 5B). Additionally, *DR5* gene expression in NaB-treated

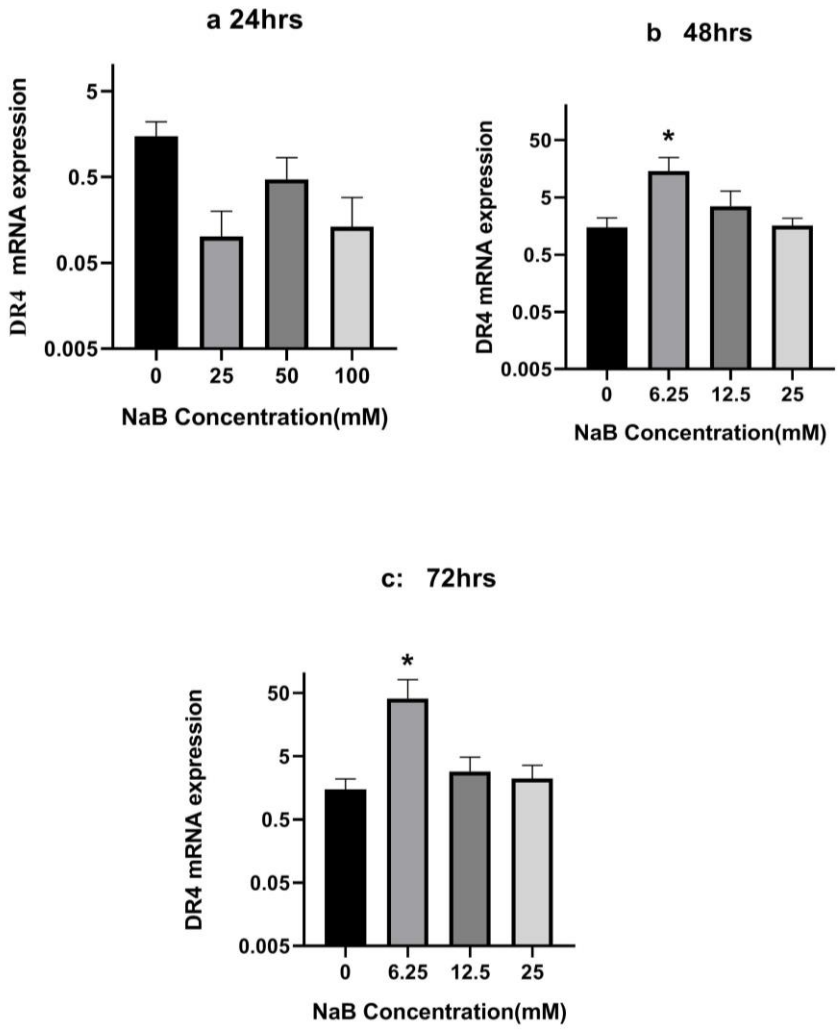

Figure 4. *Death receptor 4 (DR4)* gene expression; A) significant reduction of *DR4* gene expression at concentration of 25 mM after 24 h of treatment with NaB; B) *DR4* gene expression after 48 h of treatment with NaB showing significant increase at concentrations of 6.25 and 25 mM, compared to that reported for the control group; C) *DR4* gene expression demonstrating significant increase at concentration of 6.25 mM after 72 h of treatment with NaB, compared to that reported for the control group; evaluation of *DR4* gene expression using quantitative real-time polymerase chain reaction; *P<0.05

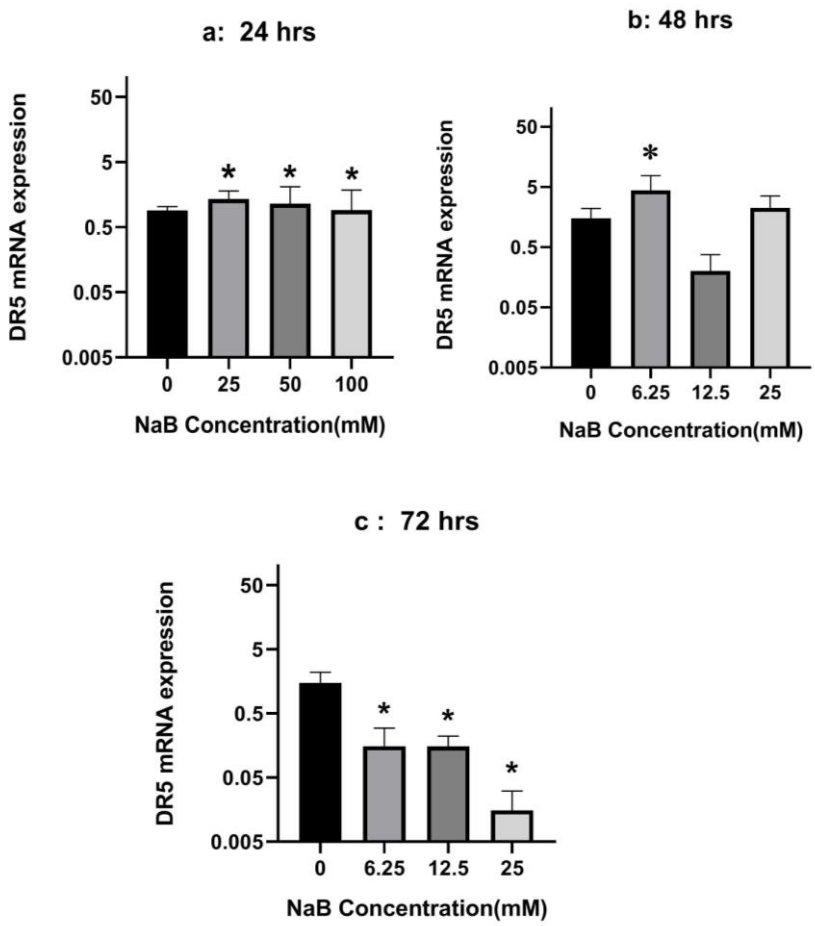

Figure 5. *Death receptor 5* (*DR5*) gene expression; A) *DR5* gene expression after 24 h of treatment with NaB showing a significant increase at three concentrations of 25, 50, and 100 mM, compared to that reported for the control group; B) *DR5* gene expression after 48 h of treatment with NaB causing significant increase at concentrations of 6.25 and 25 mM; significant reduction at concentration of 12.5 mM in NaB-treated HCT-116 cells, compared to that reported for the untreated control group; C) significant reduction of *DR5* gene expression at concentrations of 6.25, 12.5, and 25 mM after 72 h of treatment with NaB; evaluation of *DR5* gene expression using quantitative real-time polymerase chain reaction; *P<0.05

HCT-116 cells showed significant reductions of about 3.23, 4.07, and 2.92 folds at concentrations of 6.25, 12.5, and 25 mM, respectively, compared to those reported for the control group after 72 h of incubation (P=0.001; Figure 5C).

4.2.5. TRAIL gene expression

NaB-treated HCT-116 cells were incubated with IC_{50} concentrations of 25, 50, and 100 mM for 24 h of incubation in addition to 6.25, 12.5, and 25 mM for 48 and 72 h of incubation. After 24 h of treatment with NaB, the mRNA level of *TRAIL* significantly increased about 2.66, 17.87, and 5.18 mM folds at concentrations of 25, 12.5, and 25 mM, respectively, compared to that of the control group (P=0.005; Figure 6A). Furthermore, after 48 h of treatment with NaB, *TRAIL* gene expression remarkably increased at concentrations of 6.25 and 25 mM, respectively, compared to that reported for the untreated group (P<0.05; P=0.005; Figure 6B). The results obtained from the NaB treated HCT-116 cells demonstrated significant increases of about 4.33 and 4.45 folds at concentrations of 6.25 and 25 mM, compared to those of the control group after 72 h of incubation (P=0.005; Figure 6C).

5. Discussion

The CRC was caused by the excessive growth of abnormal colon cells in the part of the rectum or colon. Moreover, the apoptosis process removes damaged cells, and the hallmark index of cancer cells evade programmed cell death. The extrinsic pathway of apoptosis is usually triggered by binding *FASL* to *CD95* on the surface of the cell membrane. The lacking balance between cell growth and apoptosis is one of the important mechanisms for the induction of CRC (2, 24, 25). In addition, hyperacetylation is the mechanism through which the tumor cells disrupt gene expression. The gene expression of histone deacetylase increases in CRC performing toward the deacetylation of target gene expression. The HDACi

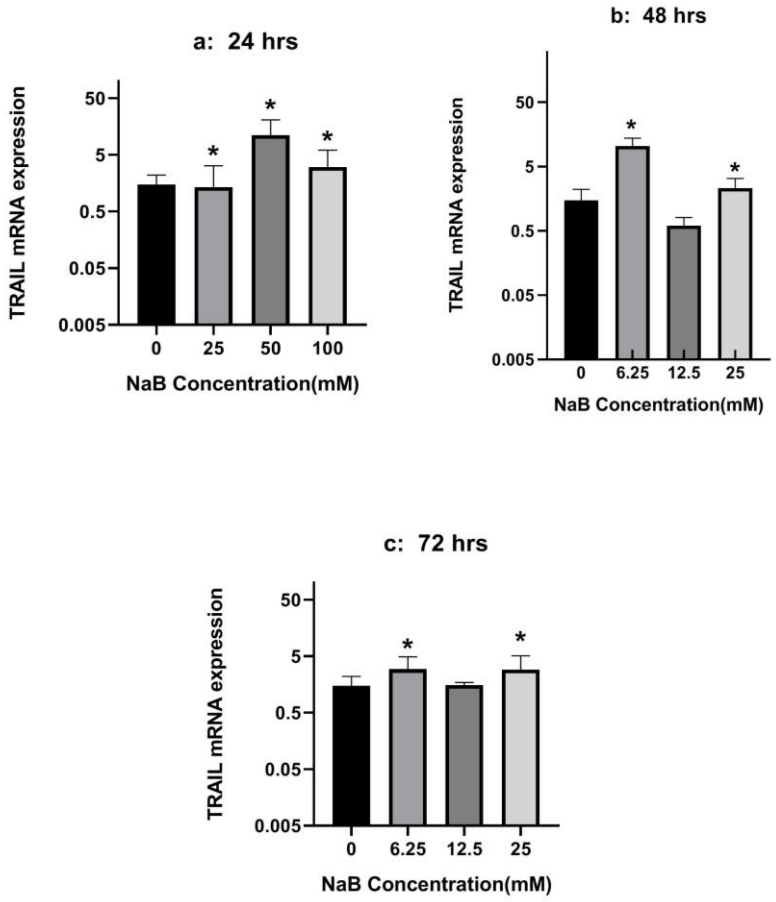

Figure 6. *Tumor necrosis factor-related apoptosis-inducing ligand* (*TRAIL*) gene expression; A) up regulation of *TRAIL* gene expression at concentrations of 25, 50, and 100 mM after 24 h of treatment with NaB; B) increase of *TRAIL* gene expression at concentrations of 6.25 and 25 mM after 48 h of treatment with NaB in NaB-treated HCT-116 cells, compared to that reported for the untreated group; C) significant increase of *TRAIL* gene expression at concentrations of 6.25 and 25 mM after 72 h of treatment with NaB, compared to that reported for the untreated control group; evaluation of *TRAIL* gene expression using quantitative real-time polymerase chain reaction; *P<0.05

can induce apoptosis in tumor cells with the regulation of apoptotic gene expression via extrinsic and intrinsic pathways (26).

The effect of NaB as an HDACi on gene expression in cancer cells has been indicated in several studies (27, 28). Owing to the important characteristic of sodium butyrate, it could be a reliable adoption for the assessment of its role in apoptotic gene expression. In the present study, the optimum concentration of sodium butyrate was evaluated on gene expression involved in extrinsic apoptosis pathways, including *TRAIL, DR4, DR5, CD95*, and *CD95L*. The obtained results of the MTT assay showed that IC_{50} values were 50, 6.25-12.5, and 6.25 mM for 24, 48, and 72 h of treatment, respectively.

Yamamura et al. have studied the effect of NaB at concentrations of 1 and 5 mM for 24 and 48 h of treatment, respectively. They indicated that NaB is capable of increasing apoptosis at certain concentrations in HCT and SW480 cell lines (29). In the current study, *FAS* gene expression significantly increased at determined IC_{50} in 24, 48, and 72 h of treatment resulting in a considerable increase in apoptosis induction. Furthermore, *FASL* gene expression reduced at 24, 48, and 72 h in the aforementioned concentrations. According to previous studies, *FASL* increased in tumor cells; therefore, its gene expression reduced when treated with certain concentrations of NaB in three incubation times.

The obtained results of the present study are in line with the findings of other studies carried out on other cancer cell lines. A study conducted by Bernard B et al. evaluated the effect of NaB on apoptosis induction with 4 mM of NaB in 24 h, and the results indicated that induction of *FAS*-mediated apoptosis would increase when treated with NaB at the specific concentration (30).

Another study was performed on the *FAS* gene expression in the MCF-7 cell line undergoing the treatment of 2.5-mM NaB in 48 h of treatment. It was observed that *FAS* gene expression was elevated by the treatment with NaB (31). In addition, the obtained results of the present study from *TRAIL* gene expression showed a significant increase in the

three times of incubation, especially at 25 mM that was a common concentration among the treatment times.

Moreover, *DR4* gene expression significantly increased at concentrations of 6.25 and 25 mM for 48 h and only at a concentration of 6.25 mM for 72 h of treatment with NaB. The *DR5* gene expression also significantly increased after 24 h of treatment at concentrations of 25, 50, and 100 mM and at concentrations of 6.25 and 25 mM for 48 h of treatment. Hernandez et al. have observed a significant increase in *TRAIL* gene expression when the KM12C, KML4A, and KM20 cancer cell lines (i.e., colon cancer cells of different metastatic potential) were treated with NaB after 24 and 48 h of incubation (32). Furthermore, in another study conducted by Kim et al., NaB could not show a significant effect on *DR4* gene expression. However, there was a significant increase in *DR4* gene expression based on the obtained results of the present study (33).

Kim et al. in another study have evaluated the effect of NaB on *TRAIL* and their specific receptors that are *DR4* and *DR5* in the HT-29 cancer cell line. Their results showed that *TRAIL*-mediated apoptosis increased at a concentration of 4 mM for 24 h of treatment, and the elevation of *DR5* gene expression was observed using the western blot and real-time PCR despite the *DR4* gene expression not showing a clear difference. Therefore, Kim et al. observed that NaB could alter the gene expression with an effect on transcription factor SP1 (29). In the present study, *TRAIL* gene expression remarkably increased at three studied concentrations after 24 h and at concentrations of 6.25 and 25 mM after 48 h of incubation. Moreover, concentrations of 6.25 and 25 mM demonstrated a significant increase after 72 h of incubation.

The results of the present study indicated that the expression of *FAS, TRAIL, DR4*, and DR5 were upregulated, and the expression of *CD95L* was downregulated. These findings are supported by the experiments showing the enhancement of *FAS, TRAIL, DR4*, and *DR5* and reduction of *CD95L* in CRC cells. Moreover, abnormal proliferation and excessive growth of cancer cells would occur by a defect in apoptosis accompanied by the deregulation of the cell cycle. As a result, NaB as an HDACi could promote apoptosis induction. On the other hand, counteracting the innate immune cells against tumors has been indicated.

Plasmacytoid dendritic cells express *TRAIL* and induce cytotoxicity T cells (CTLs) to kill the tumor cells. In addition, NK cells could exert cytotoxicity effects on cancer cells through the interaction of *FAS/FASL* and *TRAIL* signaling pathways. The *FASL* and *TRAIL* were expressed in adaptive immune cells, such as T cells and CTLs, which have cytotoxicity activities against tumor cells. Furthermore, *FAS/FASL* expression by cancer cells represents that these cells are resistant to *FAS*-mediated apoptosis. The most common mechanism utilized to evade apoptosis is the regulation of receptor surface expression (31, 32).

The *FAS* expression was downregulated in CRC that could lead to apoptosis resistance; therefore, tumor growth of colorectal carcinoma increases (30). It seems that NaB may have an effective role in the induction of the apoptosis process in CRC via the reduction of *FASL* gene expression and increase of *TRAIL, DR4, DR5*, and *FAS* gene expression, along with reinforcement of innate and adaptive immune responses, resulting in the facilitation of the apoptosis process.

6. Conclusion

Sodium butyrate as an HDACi could affect the gene expression of the extrinsic apoptotic pathway and stimulate apoptosis induction in a treated HTC-116 cell line. Overall, the findings of the current study indicated that sodium butyrate has the maximum effect on the optimum concentrations and certain treatment times in CRC. Consequently, the cytotoxicity effect of sodium butyrate is a treatment time-dependent and concentration-dependent manner.

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Footnotes

Authors' Contribution: F.F. participated in study design. F.F., A.M., and M.M. participated in data collection, evaluation, and drafting. F.F. and A.M. participated in reverse transcriptase quantitative polymerase chain reaction and statistical analyses. F.F. and A.M. performed sample collection, prepared laboratory working, and conducted molecular experiments. F.F. and A.M. extensively contributed to interpreting the data and drawing conclusions. All the authors edited the final version of this paper for submission, participated in the finalization of the manuscript, and approved the final draft.
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