

## Effects of Trichostatin A on the Histone Deacetylases (HDACs), Intrinsic Apoptotic Pathway, p21/Waf1/Cip1, and p53 in Human Neuroblastoma, Glioblastoma, Hepatocellular Carcinoma, and Colon Cancer Cell Lines

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### Abstract

**Background:** The aberrant and altered patterns of gene expression play an important role in the biology of cancer and tumorigenesis. DNA methylation and histone deacetylation are the most studied epigenetic mechanisms. Histone deacetylase inhibitors (HDACIs) such as valproic acid (VPA) and trichostatin A (TSA) are a group of anticancer compounds for the treatment of solid and hematological cancers. Previously, we reported the effect of two HDACIs, valproic acid (VPA) and TSA, on colon cancer and hepatocellular carcinoma (HCC), respectively. The aim of the current in vitro study is to investigate the effects of TSA on the intrinsic apoptotic pathway, p21/Waf1/Cip1 (p21), p53, and histone deacetylases (HDACs) 1, 2 and 3 in human neuroblastoma LAN-1, glioblastoma GBM-29, HCC SMMC7721, and colon cancer COLO 201 cell lines.

**Materials and methods:** In this lab-trial study, all three cell lines were seeded at the density of  $3 \times 10^5$  cells per well and incubated for 24 hours. Then, the cells were treated with TSA based on IC50 values for 24 hours except in the control groups; the control cells were treated with the equal amounts of the DMSO solvent. Subsequently, cell viability, cell apoptosis and gene expression were determined by three techniques including MTT assay, flow cytometry assay, and qRT-PCR.

**Results:** The result of qRT-PCR indicated that TSA could increase the expression levels of Bid, BimEL, Noxa, p21, and p53 genes and decrease those of Bcl-xL, RIP, Mcl-1, XIAP, HDACs 1, 2 and 3 significantly ( $P < 0.0001$ ) by which it inhibited cell growth and induced significant cell apoptosis in LAN-1, GBM-29, SMMC7721, and COLO 201 cell lines (p value < 0.001).

**Conclusion:** TSA can affect cell apoptotic via the intrinsic apoptotic pathway in LAN-1, GBM-29, SMMC7721, and COLO 201 cell lines.

**Keywords:** Apoptosis, Histone deacetylase, Mitochondrial, Trichostatin A

### Introduction

The aberrant and altered patterns of gene expression play a significant role in the biology of cancer and tumorigenesis. Gene alterations often occur at the epigenetic level that is not accompanied by changes in DNA structure. Epigenetic gene silencing is very important in cell differentiation, development, and imprinting. However, it can result in the development of numerous human cancers (1). Generally, epigenetic modifications result in the heritable silencing of genes without a change in the DNA sequence.

These modifications, i.e., DNA methylation and histone deacetylation, are the most studied epigenetic mechanisms. Histone acetylation and deacetylation are controlled by histone acetyltransferases (HATs) and HDACs, respectively. As gene expression regulators, HDACs remove an acetyl group from the N-terminal tails of histone proteins, resulting in chromatin compaction and gene silencing (2). To date, 18 different HDACs have been identified in mammalian cells (3). Even though DNA methylation and histone deacetylation are heritable, these

epi-genetic changes are reversible, which is an opportunity for cancer treatment. Histone deacetylase inhibitors (HDACIs) are a group of anticancer compounds for the treatment of cancers. They increase acetylation by inhibiting the HDAC activity. Structurally, these compounds are divided into the four groups of compressing hydroxamates (e.g., vorinostat), benzamides (e.g., MS-275), cyclic peptides (e.g., romidepsin), and aliphatic acids (e.g., valproic acid, or VPA). Alternatively, these compounds can be classified by their affinity for HDACs (4). The apoptotic effects of these agents have been reported in numerous human cancers. It has been shown that sodium butyrate (NaB), SAHA, and TSA are able to induce apoptosis in both S-type (SH-EP, SK-N-AS) and N-type (IMR32, LAN-1, and SH-SY5Y) neuroblastoma (NB) (5). In vitro studies have indicated that NaB and TSA induce strong apoptosis in the cancer cell lines of the breast, cervix, and brain (6). Several studies have also shown that TSA can induce apoptosis in the human glioblastoma cell lines U-343 MG, GBM-29, and U-343 MGa Cl. 2:6 (7). Some researchers have demonstrated that NaB can induce apoptosis in hepatocellular cancer (HCC) HepG2 and glioblastoma C6 cell lines (8). Previously, we reported the effects of TSA (9-13) and VPA (14, 15) on HCC and colon cancer, respectively. Recent studies have shown that HDACIs induce apoptosis via both the intrinsic and extrinsic pathways (16). Since epi-drugs such as TSA can inhibit cell growth and induce apoptosis in a tissue based on the concentration of drugs and the time of treatment, the effect of each dose of a compound, each time of treatment, or each experimental cell line could be a topic of novelty for research (17-20).

The aim of the current in vitro study is to investigate the effects of TSA on the intrinsic apoptotic pathway (Bid, BimEL, Noxa, Bcl-xL, RIP, Mcl-1, XIAP), p21/Waf1/Cip1 (p21), p53, and histone

deacetylases (HDACs) 1, 2 and 3 in human neuroblastoma LAN-1, human glioblastoma GBM-29, human HCC SMMC7721, and human colon cancer COLO 201 cell lines.

## Materials and Methods

### Materials

Neuroblastoma LAN-1, human glioblastoma GBM-29, human HCC SMMC7721, and human colon cancer COLO 201 cell lines were purchased from the National Cell Bank of Iran-Pasteur Institute. The TSA, kits and materials were obtained as provided for our previous works (21, 22). TSA was dissolved in dimethyl sulfoxide (DMSO) and prepared a work stock solution. The cells were maintained as explained in our previous works (21, 22).

### Cell culture and cell viability

The LAN-1, GBM-29, SMMC7721, and COLO 201 cells were cultured in conditions similar to those mentioned previously (21, 22). The LAN-1 and GBM-29 cells were treated with TSA (0, 25, 50, 75, 100, and 200 nM), and the SMMC7721 and COLO 201 cells were treated with TSA (0, 1, 2.5, 5, 10, and 20  $\mu$ M) for 24 hours except in the control groups; the control cells were treated with the equal amounts of DMSO as a solvent. After 24 hours, the cell viability was determined by the MTT assay according to the standard protocols. Finally, the optical density was detected by a microplate reader at the wavelength of 570 nm.

### Cell apoptosis assay

To determine LAN-1, GBM-29, SMMC7721, and COLO 201 cell apoptosis, the cells were seeded at the density of  $3 \times 10^5$  cells per well and incubated for 24 hours. Then, the cells were treated with TSA based on IC50 values for 24 hours except in the control groups; the control cells were treated with the equal amounts of the DMSO solvent. Subsequently, as in our previous study

(13), the apoptotic cells were investigated by flow cytometry.

### Real-time Quantitative Reverse Transcription Polymerase Chain Reaction (qRT-PCR)

The qRT-PCR was done to determine the relative expression levels of the Bid, BimEL, Noxa, Bcl-xL, RIP, Mcl-1, XIAP, p21, p53, and HDACs 1, 2, and 3 genes. The LAN-1, GBM-29, SMMC7721, and COLO 201 cells were cultured at the density of  $3 \times 10^5$  cells per well and treated with TSA for 24 hours. This was done based on the concentrations and IC50 values demonstrated in Table I. The control groups were treated with the solvent. Then, qRT-PCR was done as in our previous work (13). The primer sequences (23-30) are indicated in Table II.

### Ethical Consideration

This study was supported and approved by the Ethics Committee of Jahrom University of Medical Science. The ethics certificate code number is IR.JUMS.REC.1399.078.

## Results

### Cell viability

The viability of the LAN-1, GBM-29, SMMC7721, and COLO 201 cells treated

with TSA was evaluated with the MTT assay as explained in Section 2. As indicated in Figure 1, TSA could induce significant cell growth inhibition in all the cell lines in a dose-dependent manner ( $P < 0.0001$ ).

### Cell apoptosis assay

To identify the apoptotic cells, LAN-1, GBM-29, SMMC7721, and COLO 201 cells were treated with TSA for 24 hours and then stained with annexin-V-(FITC) and PI. As indicated in Figures 2, 3 and 4 (part A), TSA induced cell apoptosis significantly ( $P < 0.0001$ ). In addition, the LAN-1 cell line was more sensitive to TSA than the other cell lines, and the percentage of the apoptotic cells in it was more than that in the other cell lines (Figure 4, part B). Based on the statistical analysis, a significant difference existed between the experimental (treated) and the control (untreated) groups (Figure 4, part C3).

### Gene expression

The result of qRT-PCR indicated that TSA could increase the expression levels of Bid, BimEL, Noxa, p21, and p53 genes and decrease those of Bcl-xL, RIP, Mcl-1, XIAP, HDACs 1, 2 and 3 significantly (Figure 5).

Table I: IC50 values of TSA was determined by MTT assay in different cell lines including LAN-1, GBM-29, and SMMC7721.

Cell line	Duration (in hours)	IC50 value	LogIC50	R squared
LAN-1	24	61.48 nM	1.789	0.9818
GBM-29	24	81.64 nM	1.911	0.9817
SMMC7721	24	4.726 $\mu$ M	0.6745	0.9224
COLO 201	24	4.906 $\mu$ M	0.6903	0.6567

Table II: The primer sequences of Bid, BimEL, Noxa, Bcl-xL, RIP-1, Mcl-1, XIAP, p21, p53, and HDACs 1, 2 and 3

Primer	Primer sequences (5' to 3')	Product lent/bp	Reference
<b>Bid</b>		169	23
<b>Forward</b>	TGGTGTGGCTTCCTCCAA		
<b>Reverse</b>	GAATCTGCCTCTATTCTTCCC		
<b>BimEL</b>		100	23
<b>Forward</b>	GAGCCACAAGACAGGAGC		
<b>Reverse</b>	CCATTGCACTGAGATAGTGG		
<b>Bcl-xL</b>		220	23
<b>Forward</b>	GCGACGACTTCTCCCGC		
<b>Reverse</b>	GCGATGTTGTCCACCAGG		
<b>Noxa</b>		233	24
<b>Forward</b>	ATGAATGCACCTTCACATTCTCT		
<b>Reverse</b>	TCCAGCAGAGCTGGAAGTCGAGTGT		
<b>RIP1</b>		106	25
<b>Forward</b>	AGAAAGTGTAGAAGAGGACGTG		
<b>Reverse</b>	AGGTACTGCCACACAATCAAG		
<b>Mcl-1</b>		79	26
<b>Forward</b>	GGGCAGGATTGTGACTCT CATT		
<b>Reverse</b>	GATGCAGCTTCTTGTTTATGG		
<b>XIAP</b>		368	27
<b>Forward</b>	GTGACTAGATGTCCACAAGG		
<b>Reverse</b>	GTTGAGGAGTGTCTGGTAAG		
<b>p21</b>		270	28
<b>Forward</b>	AAGACCATGTGGACCTGTCACTGT		
<b>Reverse</b>	AGGGCTTCCTCTTGAGAAAGATCA		
<b>P53</b>		121	28
<b>Forward</b>	TAACAGTTCCTGCATGGGCGGC		
<b>Reverse</b>	AGGACAGGCACAAACACGCACC		
<b>HDAC1</b>		374	29
<b>Forward</b>	AACCTGCCTATGCTGATGCT		
<b>Reverse</b>	CAGGCAATTCGTTTGTGAGA		
<b>HDAC2</b>		314	29
<b>Forward</b>	GGGAATACTTTCCTGGCACA		
<b>Reverse</b>	ACGGATTGTGTAGCCACCTC		
<b>HDAC3</b>		328	29
<b>Forward</b>	TGGCTTCTGCTATGTCAACG		
<b>Reverse</b>	GCACGTGGGTTGGTAGAAGT		
<b>GAPDH</b>		172	30
<b>Forward</b>	GAAGGTGAAGGTCGGAGTC		
<b>Reverse</b>	GAAGATGGTGTATGGGATTTC		

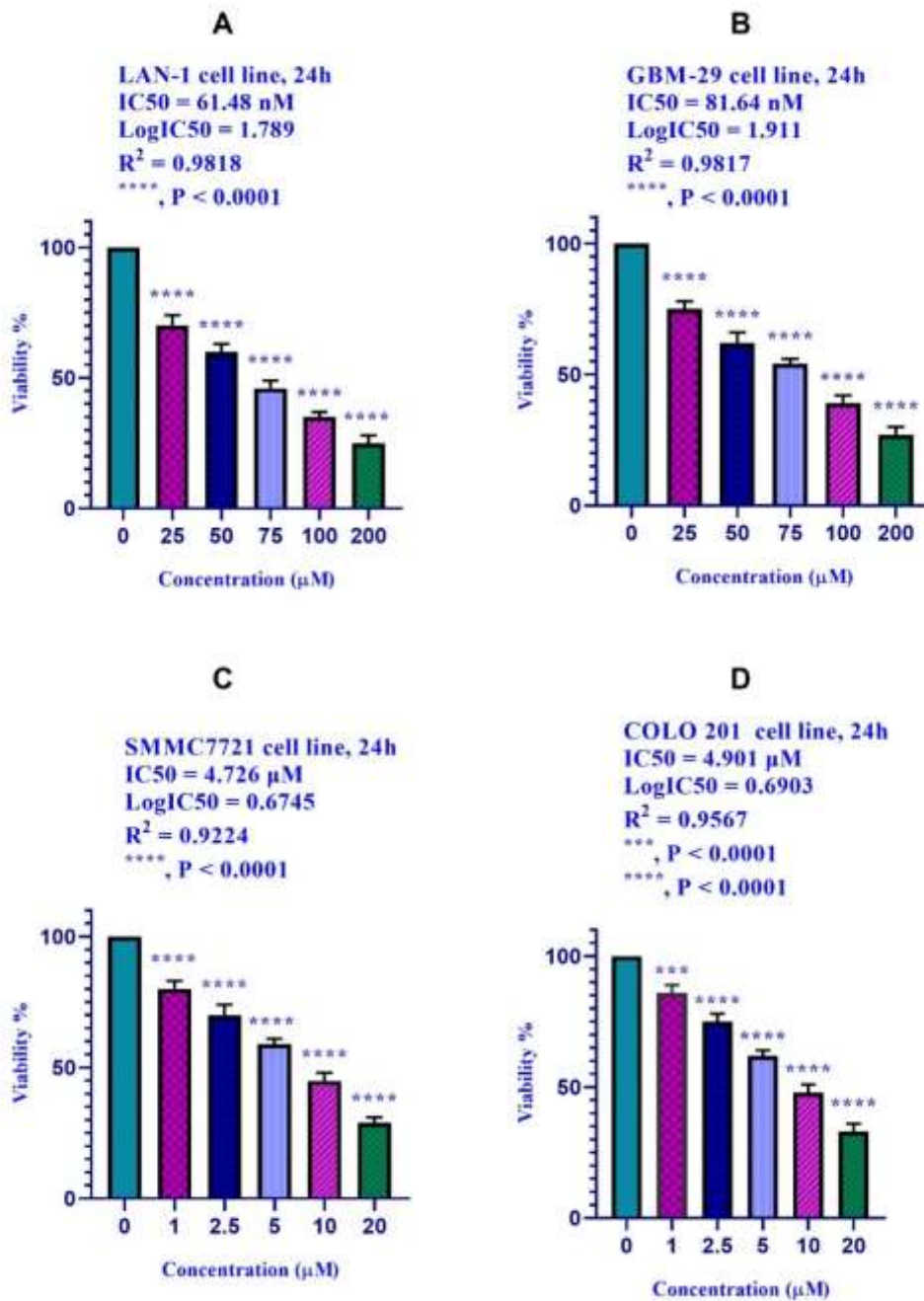
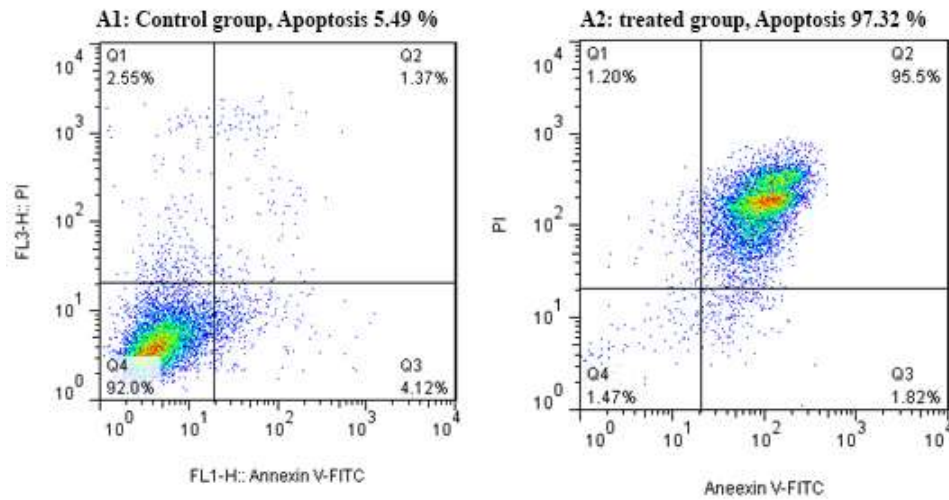


Figure 1. The effect of TSA on LAN-1, GBM-29, SMMC7721, and COLO 201 cell viability: Each experiment was conducted in triplicate. The mean values from the three experiments are reported with  $\pm$  standard errors of the mean. An asterisk (\*) indicates a significant difference between the experimental and the control groups. IC<sub>50</sub>s and P values are also shown in parts A, B, C and D.

**A: Neuroblastoma LAN-1 cell line**



**B: Human glioblastoma GBM-29**

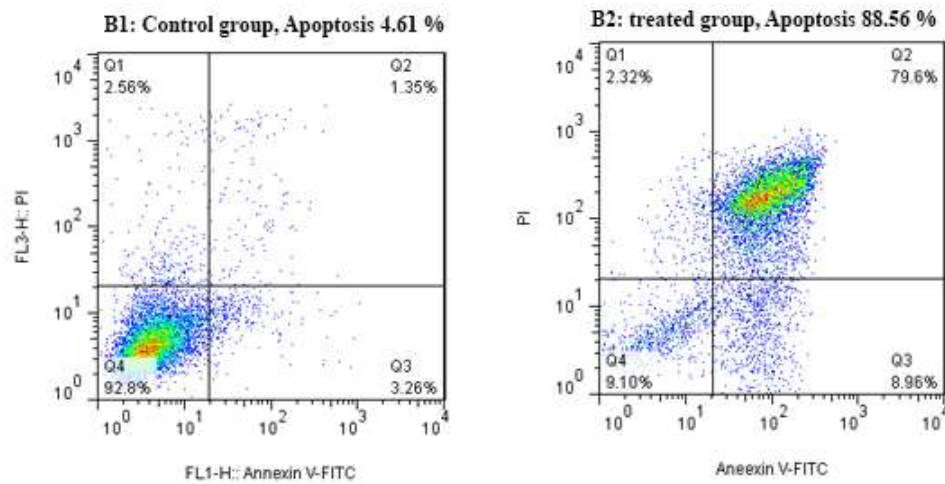
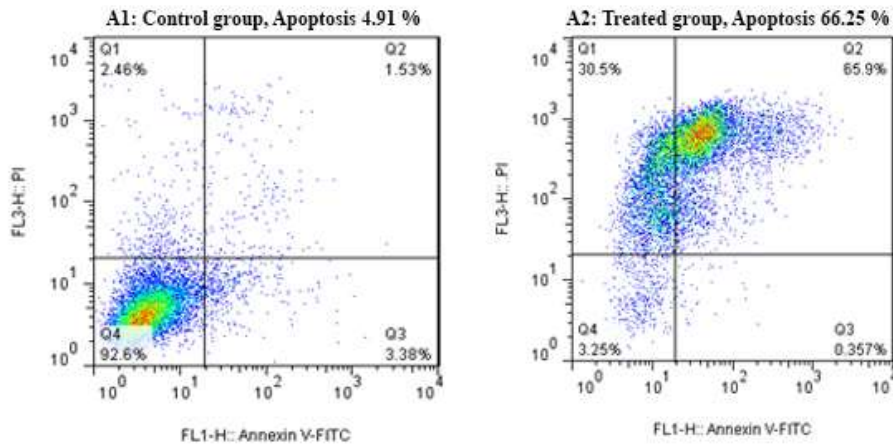


Figure 2. The apoptotic effect of TSA on LAN-1 and GBM-29 cell lines: TSA was demonstrated to induce significant cell apoptosis after 24 hours of treatment.

A: HCC cell line SMMC7721



B: colon cancer COLO 201

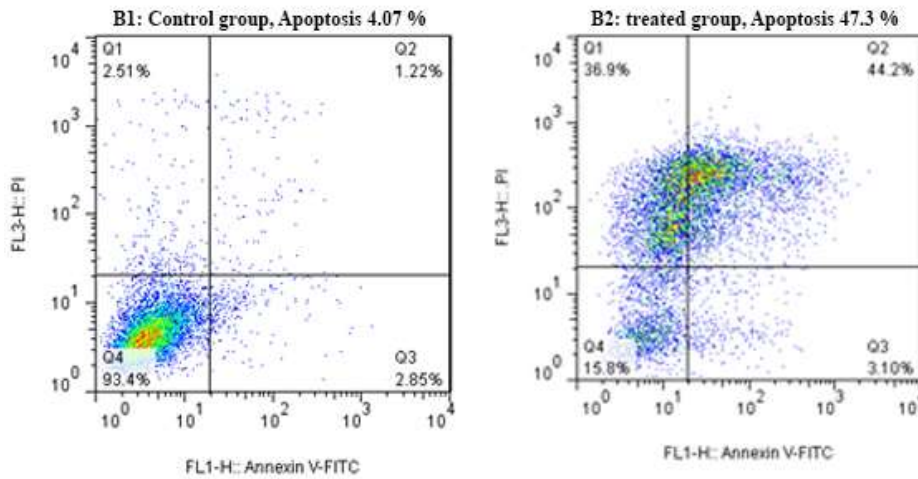


Figure 3. The apoptotic effect of TSA on SMMC7721 and COLO 201 cell lines: The results indicated that TSA could induce a significant cell apoptosis after 24 hours.

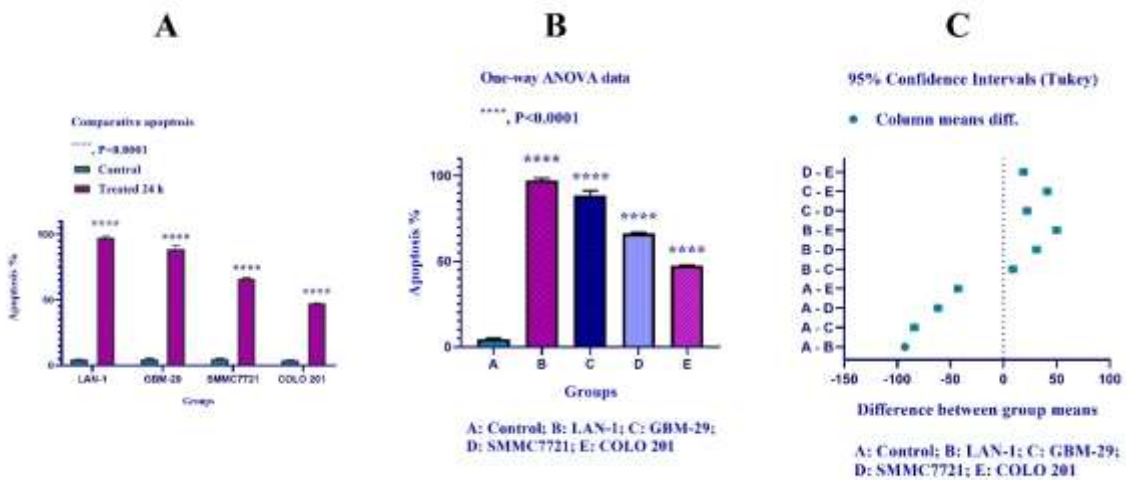


Figure 4. The apoptotic effect of TSA on LAN-1, GBM-29, SMMC7721, and COLO 201 cell lines: TSA induced significant cell apoptosis in all the cell lines after 24 hours of treatment (A). Also, the LAN-1 cell line was more sensitive to TSA than the other cell lines (B). Based on the statistical analysis, there was a significant difference between the experimental and the control groups (C).

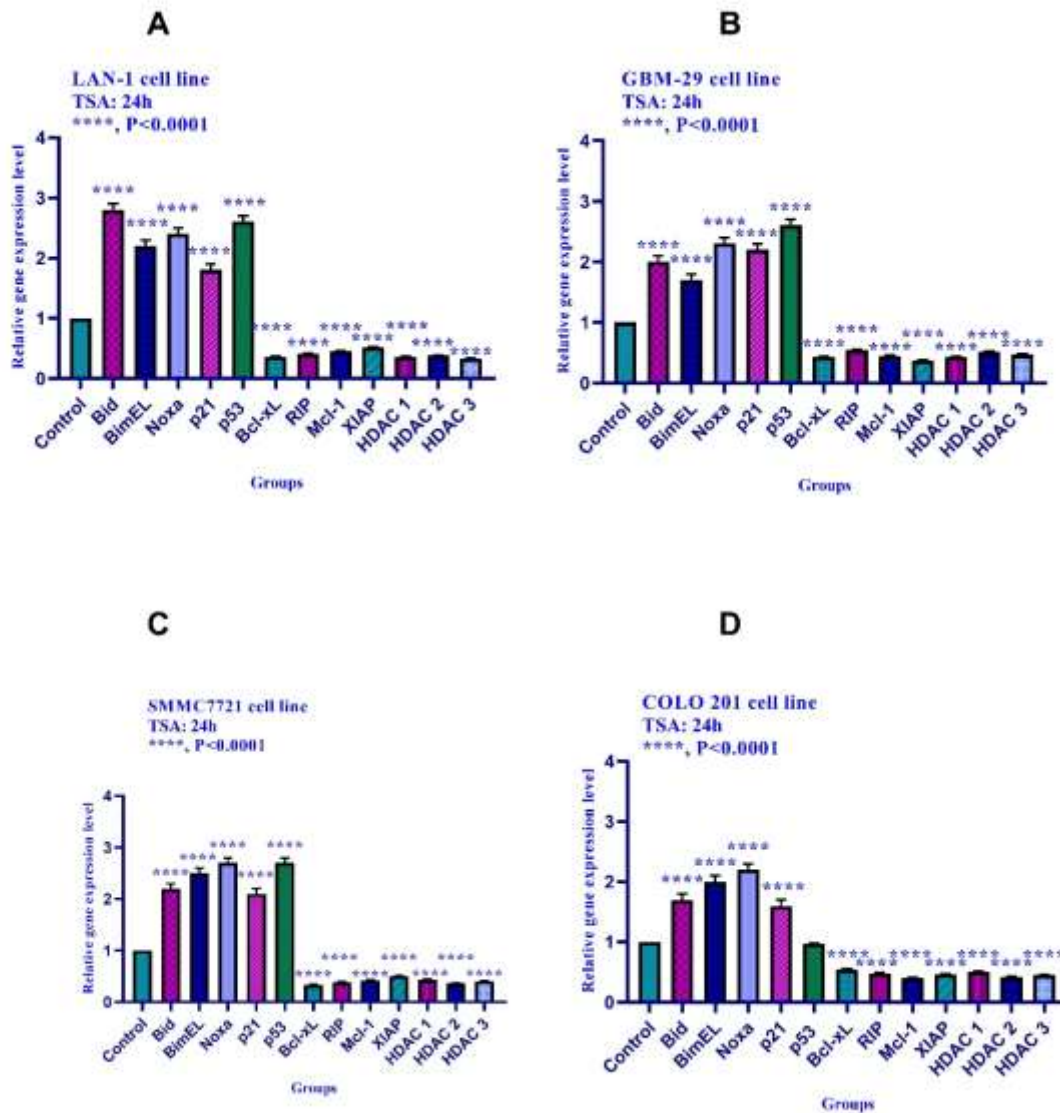


Figure 5. The relative expression levels of Bid, BimEL, Noxa, Bcl-xL, RIP, Mcl-1, XIAP, p21, p53, and HDACs 1, 2 and 3 genes in the LAN-1, GBM-29, SMMC7721, and COLO 201 cells treated with TSA versus control groups: As indicated, there were significant differences between the experimental and the control groups. An asterisk (\*) indicates a significant difference.  $P < 0.0001$ .

## Discussion

Several molecular apoptotic pathways have been reported for HDACIs, one of which is the intrinsic/mitochondrial pathway. Recent experimental works have shown that these compounds enhance mitochondrial apoptosis in a p53-dependent manner in the medulloblastoma cell line (31). In this regard, it has been shown that HDACIs activate the intrinsic apoptotic pathway via the upregulation of

several Bcl-2 family genes (32). As observed in this study, TSA induced significant apoptosis and cell growth inhibition in all the treated cell lines. This compound up-regulated the expression levels of Bid, BimEL, Noxa, p21, and p53 genes but down-regulated the expression levels of Bcl-xL, RIP, Mcl-1, XIAP, HDACs 1, 2 and 3 significantly. According to the findings of the present study, TSA can induce cell apoptosis via a



mitochondrial (intrinsic) apoptotic pathway in LAN-1, GBM-29, SMMC7721, and COLO 201 cell lines. Furthermore, TSA had no significant effect on the p53 gene expression in colon cancer COLO 201 line. Previously, we reported that valproic acid and TSA could induce apoptosis through the inhibition of histone deacetylase 1 and the re-activation of CIP/KIP family genes (p21, p27, and p57) in the SW480 colon cancer cell line [15]. In the present study, TSA was found to induce apoptosis by regulating the intrinsic apoptotic pathway, p21/Waf1/Cip1, and p53 in human neuroblastoma, glioblastoma, hepatocellular carcinoma, and colon cancer cell lines. Inconsistent with this finding, several researchers have reported that TSA plays its role by up-regulating the expression of pro-apoptotic genes (such as BIM) and down-regulating anti-apoptotic genes in the pancreatic cancer cell lines HPAF, CFPAC1, Panc1 PC, PSN1, Miapaca2, RT45P1, Paca44, and T3M4 (33). Similarly, it has been shown that TSA mediates its apoptotic effect via the mitochondrial pathway in the human prostatic cancer DU145 cell line (34). The same effect has been reported for sodium butyrate (NaBt), as another histone deacetylases inhibitor in pancreatic cancer cell lines (ASPC-1 and PANC-1) (35). Regarding the lung cancer H157 cell line, a decreased expression of anti-apoptotic proteins has been observed in TSA-treated cells (36). In the lung cancer H157 cell line, TSA triggers apoptosis by activating the intrinsic pathway along with the Fas/FasL system and the extrinsic pathway along with mitochondrial dysfunction (37). In MM1S myeloma cells, TSA treatment down-regulates the expression of the anti-apoptotic Bcl-2 proteins (38). Similar to our results, it has been reported that HDACIs activate the mitochondrial pathway in neuroblastomas (39). They induce apoptosis in human glioblastoma cells with a decrease in the anti-apoptotic Bcl-x1 protein and an increase in the pro-

apoptotic factor (40). Besides, Apoptosis is induced via the mitochondrial pathway by a shift in the bax/bcl-2 ratio in HCC (41). As reported in this article, TSA up-regulated the p53 gene expression in LAN-1, GBM-29, and SMMC7721 cell lines, whereas it had no significant effect on this gene in the COLO 201 cell line. It means that TSA can play its role through two apoptotic pathways, including p53-dependent, and independent pathways. Additionally, a minimal apoptotic effect was seen in the COLO 201 cell line. It may be concluded that TSA can induce a strong apoptotic effect through a p53-dependent pathway.

Contrary to our results, several experimental studies have reported that HDACIs can induce apoptosis through the activation of the extrinsic apoptotic pathway (42). Meanwhile, numerous in vivo studies have shown that HDACIs mediate their apoptotic effects via both the intrinsic and extrinsic apoptotic pathways in the colon, breast, hematopoietic, central nervous system, melanoma, lung, ovarian, renal, prostatic, and stomach cancer cell lines (43).

Taken together, HDACIs can affect cell apoptosis through extrinsic and intrinsic pathways. Therefore, the evaluation of the extrinsic apoptotic pathway on human neuroblastoma LAN-1, human glioblastoma GBM-29, human HCC SMMC7721, and human colon cancer COLO 201 cell lines is recommended.

## Conclusion

In summary, our findings demonstrated that TSA can play its apoptotic role via an intrinsic (mitochondrial) apoptotic pathway in human neuroblastoma LAN-1, human glioblastoma GBM-29, human HCC SMMC7721, and human colon cancer COLO 201 cell lines. Furthermore, it induces apoptosis through both p53-dependent and independent pathways.

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## Conflict of interest

The authors declare no conflict of interest.

## References

1. Abbas A, Gupta S. The role of histone deacetylases in prostate cancer. *Epigenetics* 2008; 3(6): 300-309.
2. Machado Vieira R, Ibrahim L, Zarate J, Carlos A. Histone deacetylases and mood disorders: epigenetic programming in gene environment interactions. *CNS Neurosci Ther* 2011; 17(6): 699-704.
3. Delcuve GP, Khan DH, Davie JR. Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors. *Clin Epigenetics* 2012; 4(1): 1-13.
4. Damaskos C, Valsami S, Kontos M, Spartalis E, Kalampokas T, Kalampokas E. Histone deacetylase inhibitors: an attractive therapeutic strategy against breast cancer. *Anticancer Res* 2017; 37(1): 35-46.
5. Deubzer HE, Ehemann V, Kulozik AE, Westermann F, Savelyeva L, Kopp-Schneider A. Anti-neuroblastoma activity of *Helminthosporium carbonum* (HC)-toxin is superior to that of other differentiating compounds in vitro. *Cancer Lett* 2008; 264(1): 21-28.
6. Egler V, Korur S, Faily M, Boulay JL, Imber R, Lino MM. Histone deacetylase inhibition and blockade of the glycolytic pathway synergistically induce glioblastoma cell death. *Clin Cancer Res* 2008; 14(10): 3132-3140.
7. Svechnikova I, Almqvist PM, Ekström TJ. HDAC inhibitors effectively induce cell type-specific differentiation in human glioblastoma cell lines of different origin. *Int J Oncol* 2008; 32(4): 821-827.
8. Joachimiak R, Kaznica A, Drewa T. Influence of sodium butyrate on hepatocellular carcinoma (hepG2) and glioblastoma (C6) cell lines in vitro. *Acta Pol Pharm* 2007; 64(6): 561-563.
9. Sanaei M, Kavooosi F, Salehi H. Genistein and trichostatin a induction of estrogen receptor alpha gene expression, apoptosis and cell growth inhibition in hepatocellular carcinoma HepG 2 cells. *APJCP* 2017; 18(12): 3445-3450.
10. Sanaei M, Kavooosi F, Roustazadeh A, Golestan F. Effect of genistein in comparison with trichostatin a on reactivation of DNMTs genes in hepatocellular carcinoma. *JCTH* 2018; 6(2): 141-146.
11. Kavooosi F. Effect of curcumin and trichostatin a on the expression of DNA methyltransferase 1 in hepatocellular carcinoma cell line hepa 1-6. *IJPHO* 2018; 8(4): 193-201.
12. Sanaei M, Kavooosi F, Arabloo M. Effect of Curcumin in Comparison With Trichostatin A on the Reactivation of Estrogen Receptor Alpha Gene Expression, Cell Growth Inhibition and Apoptosis Induction in Hepatocellular Carcinoma Hepa 1-6 Cell ILine. *Asian Pac J Cancer Prev* 2020; 21(4): 1045-1050.
13. Kavooosi F, Sanaei M. Comparative analysis of the effects of valproic acid and tamoxifen on proliferation, and apoptosis of human hepatocellular carcinoma WCH 17 cellin. *IJPHO* 2018; 8(1): 12-20.
14. Sanaei M, Kavooosi F, Mansoori O. Effect of valproic acid in comparison with vorinostat on cell growth inhibition and apoptosis induction in the human colon cancer SW48 cells in vitro. *Exp Oncol* 2018; 40 (2): 95-100.
15. Sanaei M, Kavooosi F. Effect of 5-aza-2'-deoxycytidine in comparison to valproic acid and trichostatin A on histone deacetylase 1, DNA methyltransferase 1, and CIP/KIP family (p21, p27, and p57) genes expression, cell growth inhibition, and apoptosis induction in colon cancer SW480 cell line. *Adv. Biomed. Res* 2019; 8: 52-59.
16. Bolden JE, Peart MJ, Johnstone RW. Anticancer activities of histone

- deacetylase inhibitors. *Nat. Rev. Drug Discov* 2006; 5(9): 769-684.
17. Cao Q, Qian S, Wang N, Zhang L, Wang W, Shen H. TRPM2 mediates histone deacetylase inhibition-induced apoptosis in bladder cancer cells. *Cancer Biother Radiopharm* 2015; 30(2): 87-93.
  18. Wang D, Jing Y, Ouyang S, Liu B, Zhu T, Niu H. Inhibitory effect of valproic acid on bladder cancer in combination with chemotherapeutic agents in vitro and in vivo. *Oncol. Lett* 2013; 6(5): 1492-1498.
  19. Son MS, Park CH, Kim JW. Effect of valproic acid on nitric oxide and nitric oxide synthase in trabecular meshwork cell. *J. Korean Ophthalmol. Soc* 2018; 59(6): 543-548.
  20. Chateauvieux S, Morceau F, Dicato M, Diederich M. Molecular and therapeutic potential and toxicity of valproic acid. *J. biotechnol. biomed* 2010; 5: 1-18.
  21. Sanaei M, Kavooosi F, Roustazadeh A, Shahsavani H. In vitro effect of the histone deacetylase inhibitor valproic acid on viability and apoptosis of the PLC/PRF5 human hepatocellular carcinoma cell line. *APJCP* 2018; 19(9): 2507–2510.
  22. Sanaei M, Kavooosi F. Effect of curcumin and trichostatin a on the expression of DNA methyltransferase 1 in hepatocellular carcinoma cell line hepa 1-6. *IJPHO* 2018; 8(4): 193-201.
  23. Turrel-Davin F, Guignant C, Lepape A, Mouglin B, Monneret G, Venet F. Upregulation of the pro-apoptotic genes BID and FAS in septic shock patients. *Crit Care* 2010; 14(4):1-10.
  24. Zhu H, Yang W, He L, Ding W, Zheng L, Huang P. Upregulating Noxa by ER stress, celastrol exerts synergistic anti-cancer activity in combination with ABT-737 in human hepatocellular carcinoma cells. *PLoS One* 2012; 7(12): 52333-52339.
  25. Safari-Arababadi M, Modarressi MH, Arababadi MK. Up-regulation of RIP1 and IPS-1 in chronic HBV infected patients. *Genet. Mol. Biol* 2019; 42(2): 337-343.
  26. Rosato RR, Almenara JA, Kolla SS, Maggio SC, Coe S, Giménez MS. Mechanism and functional role of XIAP and Mcl-1 down-regulation in flavopiridol/vorinostat antileukemic interactions. *Mol Cancer Ther* 2007; 6(2): 692-702.
  27. Yin Z, Zhou Y, Ma T, Chen S, Shi N, Zou Y. Down-regulated lncRNA SBF2-AS1 in M2 macrophage-derived exosomes elevates miR-122-5p to restrict XIAP, thereby limiting pancreatic cancer development. *J Cell Mol Med* 2020; 24(9): 5028-5038.
  28. Chew YC, Adhikary G, Wilson GM, Xu W, Eckert RL. Sulforaphane induction of p21Cip1 cyclin-dependent kinase inhibitor expression requires p53 and Sp1 transcription factors and is p53-dependent. *J Biol Chem* 2012; 287(20): 16168-16178.
  29. Jin KL, Pak JH, Park J-Y, Choi WH, Lee J-Y, Kim J-H, et al. Expression profile of histone deacetylases 1, 2 and 3 in ovarian cancer tissues. *J Gynecol Oncol* 2008;19(3):185-190.
  30. Tan W, Lim SG, Tan TM. Up-regulation of microRNA-210 inhibits proliferation of hepatocellular carcinoma cells by targeting YES1. *World J Gastroenterol* 2015; 21(46): 13030-13036.
  31. Häcker S, Karl S, Mader I, Cristofanon S, Schweitzer T, Krauss J. Histone deacetylase inhibitors prime medulloblastoma cells for chemotherapy-induced apoptosis by enhancing p53-dependent Bax activation. *Oncogene* 2011; 30(19): 2275-2281.
  32. Matthews GM, Newbold A, Johnstone RW. Intrinsic and extrinsic apoptotic pathway signaling as determinants of histone deacetylase inhibitor antitumor activity. *Adv. Cancer Res* 2012; 116: 165-197.
  33. Moore PS, Barbi S, Donadelli M, Costanzo C, Bassi C, Palmieri M. Gene expression profiling after treatment with the histone deacetylase inhibitor trichostatin A reveals altered expression of both pro-and anti-apoptotic genes in pancreatic adenocarcinoma cells. *Biochim*

*Biophys Acta Mol Cell Res* 2004; 1693(3): 167-176.

34. Taghiyev AF, Guseva NV, Sturm MT, Rokhlin O, Cohen MB. Trichostatin A (TSA) sensitizes the human prostatic cancer cell line DU145 to death receptor ligands treatment. *Cancer Biol Ther* 2005; 4(4): 388-396.

35. Natoni F, Diolordi L, Santoni C, Montani MSG. Sodium butyrate sensitises human pancreatic cancer cells to both the intrinsic and the extrinsic apoptotic pathways. *Biochim. Biophys. Acta - Mol. Cell Res* 2005; 1745(3): 318-329.

36. Kim HR, Kim EJ, Yang SH, Jeong ET, Park C, Lee JH. Trichostatin A induces apoptosis in lung cancer cells via simultaneous activation of the death receptor-mediated and mitochondrial pathway. *Exp. Mol. Med* 2006; 38(6): 616-624.

37. Ruefli AA, Ausserlechner MJ, Bernhard D, Sutton VR, Tainton KM, Kofler R. The histone deacetylase inhibitor and chemotherapeutic agent suberoylanilide hydroxamic acid (SAHA) induces a cell-death pathway characterized by cleavage of Bid and production of reactive oxygen species. *Proc Natl Acad Sci USA* 2001; 98: 10833-10838.

38. Fandy TE, Srivastava RK. Trichostatin A sensitizes TRAIL-resistant myeloma cells by downregulation of the antiapoptotic Bcl-2 proteins. *Cancer Chemother Pharmacol* 2006; 58(4): 471-477.

39. Mühlethaler-Mottet A, Flahaut M, Bourlourd KB, Auderset K, Meier R, Joseph JM. Histone deacetylase inhibitors strongly sensitise neuroblastoma cells to TRAIL-induced apoptosis by a caspases-dependent increase of the pro-to anti-apoptotic proteins ratio. *BMC cancer* 2006; 6(1): 1-13.

40. Dokmanovic M, Marks PA. Prospects: histone deacetylase inhibitors. *J. Cell. Biochem* 2005; 96(2): 293-304.

41. Gahr S, Peter G, Wissniowski TT, Hahn EG, Herold C, Ocker M. The histone-deacetylase inhibitor MS-275 and

the CDK-inhibitor CYC-202 promote anti-tumor effects in hepatoma cell lines. *Oncol. Rep* 2008; 20(5):1249-1256.

42. Rosato RR, Almenara JA, Dai Y, Grant S. Simultaneous activation of the intrinsic and extrinsic pathways by histone deacetylase (HDAC) inhibitors and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) synergistically induces mitochondrial damage and apoptosis in human leukemia cells. *Mol. Cancer Ther* 2003; 2(12): 1273-1284.

43. Hwang JJ, Kim YS, Kim MJ, Jang S, Lee JH, Choi J. A novel histone deacetylase inhibitor, CG0006, induces cell death through both extrinsic and intrinsic apoptotic pathways. *Anti-cancer drugs* 2009; 20(9): 815-821.