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RESEARCH ARTICLE

Epigallocatechin-3-gallate enhances differentiation of acute promyelocytic leukemia cells via inhibition of PML-RARα and HDAC1

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The use of all-*trans* retinoic acid (ATRA) has dramatically improved the treatment and survival rate of patients with acute promyelocytic leukemia (APL). However, toxicity and resistance to this drug are major problems in the treatment of APL with ATRA. Earlier studies have suggested that the green tea polyphenol epigallocatechin gallate (EGCG) induces cell death in hematopoietic neoplasms without adversely affecting normal cells. In the present study, the potential therapeutic effect of EGCG in APL and the underlying molecular mechanisms were investigated. EGCG (100 μM) significantly inhibited proliferation and induced apoptosis in HL-60 and NB4 cells. This effect was associated with decreased expressions of multidrug resistance proteins ABCB1, and ABCC1, whereas the expressions of pro-apoptotic genes CASP3, CASP8, p21, and Bax/Bcl-2 ratio were significantly increased. EGCG, at 25 μM concentration, induced differentiation of leukemic cells towards granulocytic pattern in a similar manner to that observed for ATRA (1 μM). Furthermore, EGCG suppressed the expression of clinical marker PML/RARα in NB4 cells and reduced the expression of HDAC1 in leukemic cells. In conclusion, the results suggested that EGCG can be considered as a potential treatment for APL.

KEYWORDS
APL, apoptosis, differentiation, EGCG, MDR

1 | INTRODUCTION

Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia which is characterized by chromosomal translocation t(15;17)(q22; q21). This translocation leads to fusion between the retinoic acid receptor-α (RARα) and promyelocytic leukemia (PML) genes (Adams & Nassiri, 2015). This fusion also recruits histone deacetylases (HDACs) resulting in the repression of the differentiating

Abbreviations: ABC, ATP-binding cassette membrane transporters; APL, acute promyelocytic leukemia; ATRA, all-trans retinoic acid; EGCG, Epigallocatechin-3-gallate; GUSB, glucuronidase beta; HDACs, Histone deacetylases; MDR, multidrug resistance protein; NBT, nitro blue tetrazolium; PI, Propidium iodide; PMA, phorbol myristate acetate; PML/RARα, promyelocytic leukemia/retinoic acid receptor-α; TFRC, transferrin receptor.

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role of retinoic acid. Therefore, HDAC inhibitors are suggested as valuable therapeutic agents for this form of leukemia (Lo-Coco & Hasan, 2014; Minucci, Nervi, Coco, & Pelicci, 2001).

Introduction of all-*trans* retinoic acid (ATRA) for the treatment of APL patients has dramatically improved their survival. However, despite improvements in the prognosis of the majority of APL patients, relapse of disease and resistance to ATRA are still a critical problem (Tomita, Kiyoi, & Naoe, 2013). Overexpression of multidrug resistance (MDR)-associated proteins including ATP-binding cassette (ABC) membrane transporters (e.g., ABCB1 and ABCC1) in leukemic cells is an important contributor to chemotherapy resistance (Shaffer et al., 2012). Dietary phytochemicals and numerous natural products have emerged as potential antitumor, chemopreventive, and chemosensitizing agents (Chen, Qiu, Hu, Wang, & Wang, 2016; Hong, Ismail, Kang, Han, & Kwon, 2016; Safe and Kasiappan, 2016; Iranshahi et al., 2010; Iranshahi, Sahebkar, Takasaki, Konoshima, & Tokuda, 2009). Different natural compounds can improve the efficiency of chemotherapeutic agents, decrease resistance to chemotherapeutic drugs, and reduce adverse side effects of chemotherapy (Surh, 2003).

Green tea is a popular beverage in Asia. Epidemiological studies have suggested that drinking green tea is effective in the treatment of different diseases. Based on many in vivo and in vitro studies, biological activities of green tea is mediated by its major polyphenolic constituent, epigallocatechin gallate (EGCG), which is a potent antioxidant (Gupta, Hussain, & Mukhtar, 2003). The beneficial effects of EGCG are reported in the treatment of cardiovascular disease, diabetes, neurodegenerative diseases, and liver diseases. EGCG has also been shown to reduce the risk of cancer developing in the prostate, bladder, stomach, oesophagus, and lung (Li, Liu, Pang, Han, & Mao, 2012; Thielecke & Boschmann, 2009; Xiao et al., 2014; Yang, Landau, Huang, & Newmark, 2001; Zhou et al., 2014). More recently, EGCG has been reported to cause cell cycle arrest in various mouse, rat, and human cell lines. The mechanisms underlying the anticancer effect of flavonoids include antioxidant activity, inhibition of cell proliferation, induction of apoptosis, and stimulation of cell differentiation (Kanadaswami et al., 2005). In this study, we conducted a series of experiments to examine the effects of EGCG on proliferation, apoptosis, and differentiation of NB4 (which express PML-RARα) and HL60 (which are null for PML-RARα) cell lines. It was also aimed to determine whether antileukemic effects of EGCG are associated with any alteration in the expressions of HDAC1, PML-RARα, and ABC membrane transporters.

2 | MATERIAL AND METHODS

2.1 | Chemicals and reagents

High-glucose Roswell Park Memorial Institute (RPMI 1640) medium and fetal bovine serum were purchased from Gibco (USA). TRIzol was purchased from Invitrogen (USA). Penicillin–streptomycin solution, ATRA, propidium iodide (PI), nitro blue tetrazolium (NBT), phorbol myristate acetate, 7-hydroxy-3H-phenoxazin-3-one-10-oxide (resazurin), and EGCG (>95%) were obtained from Sigma-Aldrich (USA). Giemsa-staining solution was purchased from Merck (Germany). Human promyelocytic leukemia cell lines, HL60, and NB4, were obtained from cell bank of Pasteur Institute (Iran). Real-time PCR

TABLE 1 Sequences of primer and probe selected for real-time PCR quantification using TaqMan probes

GENE	Forward primer	Reverse primer	PROBE	Product size (BP)
ABCB1	TAGCGAAACATTGAAAATAC	AGTCGGAGTATCTTCTTC	CAGTTGGTTTCTTTTCCTTCTTATCTT	189
ABCC1	GGAAGAGCATCAGTAACTAA	CTCCCAAGTATTACCAGTG	AAGTTCTCCACAATGCTGCC	150
HDAC1	CTGCTGCTTATTAAGTTTC	GCGATGACTACATTAAATTC	CACAGAACCACCAGTAGACAA	179
PML-RAR	CCG ATG GCT TCG ACG AGT T	GCT TGT AGA TGC GGG GTA GAG	AGT GCC CAG CCC TCC CTC GC	147
GUSB	GGCTTCTGATACTTCTTATACCA	TCGCTCACACCAAATCCTT	ACTACTCTTGGTATCACGACTACGGG	205
TFRC	ACAGTCTCCTTCCATATTCCCAAA	CCTTCCTTCAATCACACTCAGTT	ACCATCTCGGTCATCAGGATTGCC	120

Note. ABCB1 and ABCC1 = ATP-binding cassette membrane transporters; HDAC1 = Histone deacetylases; PML-RAR = promyelocytic leukemia/retinoic acid receptor; GUSB = glucuronidase beta, TFRC = transferrin receptor; PCR = polymerase chain reaction.

TABLE 2 Sequences of primer selected for real-time PCR quantification using SYBR Green

GENE	Forward primer	Reverse primer	Product size (BP)
P53	GGAACTCAAGGATGCCCAG	CAAGAAGTGGAGAATGTCAGTC	155
P21	AACGGCGGCAGACCAGCAT	GAGACTAAGGCAGAAGATGTAGAGCG	150
PTEN	AGTAGAGGAGCCGTCAAATC	ATCAGAGTCAGTGGTGTCAG	109
CASP3	AGAACTGGACTGTGGCATT	GCTTGTCGGCATACTGTTT	191
CASP9	CTTTGTGTCCTACTCTACTTTCC	AACAGCATTAGCGACCCTA	151
CASP8	TGTTGGAGGAAAGCAATCTG	CCTGGTGTCTGAAGTTCCCT	124
PI3K	TGCGGAAACTGACGGACGATGA	CGGAGCGGAGGTGCCAGAA	162
AKT	GCACCTTCATTGGCTACA	CCGCTCCGTCTTCATCAG	104
BCL2	CCAAGAAAGCAGGAAACC	GGATAGCAGCACAGGATT	170
BAX	GCCTCCTCTCCTACTTTG	CTCAGCCCATCTTCTTCC	102
GAPDH	GAAGTCAGGTGGAGCGAGG	TGGGTGGAATCATATTGGAACAT	200
BACTIN	GCCTTTGCCGATCCGC	GCCGTAGCCGTTGTCG	90

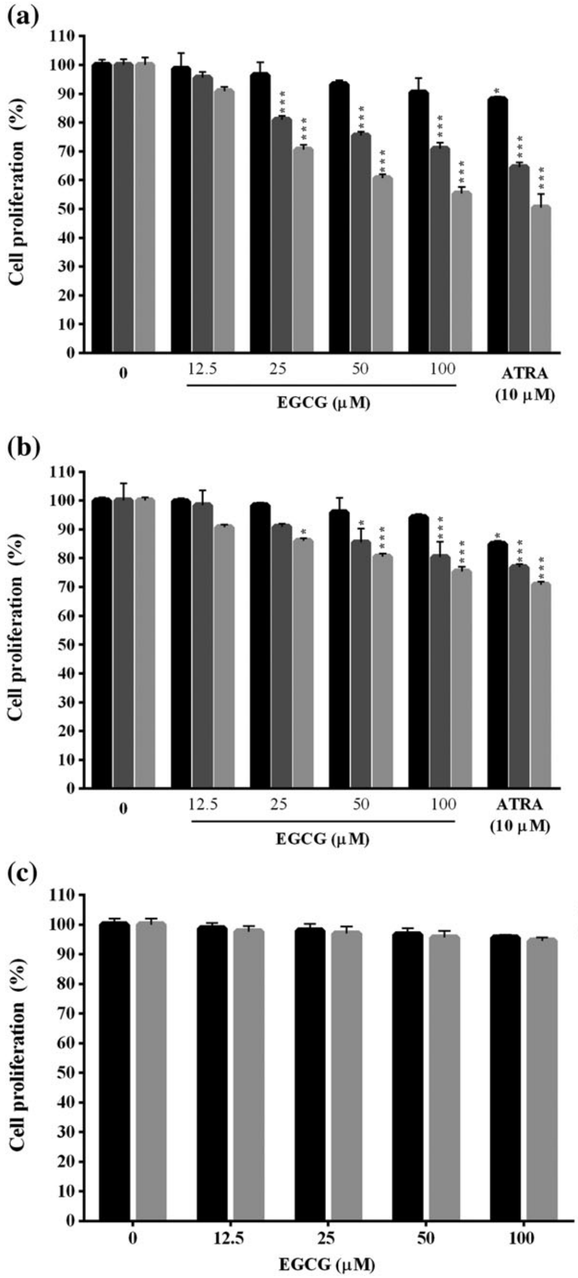

FIGURE 1 Effects of epigallocatechin-3-gallate (EGCG) on proliferation of leukemic cells. (a) HL60 cells, (b) NB4 cells, and (c) normal polymorph nuclear cells were treated up to 72 hr with different concentrations of EGCG (12.5–100 μM), or all-trans retinoic acid (ATRA). Cell proliferation was determined using resazurin assay. Data are expressed as the mean ± SEM of three independent experiments performed in triplicate. *$p < .05$, **$p < .01$, and ***$p < .001$ versus corresponding untreated control cells (concentration of 0)

Master Mix and cDNA synthesis Kit were obtained from Roche Diagnostic (Switzerland) and Fermentas (Lithuania), respectively.

2.2 | Human normal cells isolation and cell culture

Polymorphonuclear cells (PMNs) from healthy volunteers were isolated under sterile conditions by two consecutive Ficoll-Hypaque (Pharmacia) density gradient centrifugations (Cassatella et al., 1988). PMN was purified to more than 97%, as determined by morphological examination of Wright-stained smears. After washing with sterile phosphate-buffered saline, the cells were resuspended in RPMI medium (RPMI with 10% (*v*/v) fetal bovine serum, 100 units/ml penicillin, and 100 μg/ml streptomycin). HL60, NB4, and PMN cells were maintained at 37 °C in a humidified atmosphere (90%) containing 5% CO_2. The cells were incubated with various concentrations of EGCG (12.5–100 μM) and ATRA (1, 10 μM) for 24, 48, and 72 hr. For each concentration and time course of study, there was a control sample that remained untreated and received the equal volume of medium. All experiments were carried out in triplicate.

2.3 | Cell proliferation assay

Cell proliferation was determined using resazurin reduction by live cells to resorufin, a highly fluorescent compound. After treatment of NB4, HL60, and PMN cells with EGCG (12.5–100 μM) or ATRA (10 μM), 20 μl of resazurin reagent [300 μM resazurin, 78 μM methylene blue, 1 mM potassium Hexacyanoferrate III, and 1 mM potassium Hexacyanoferrate II] was added to each well. After 4 hr, fluorescence intensity was measured by a fluorescence Victor X5 2030 Multilabel Plate Reader (PerkinElmer, Shelton, Connecticut) at an excitation wavelength of 530 nm and an emission wavelength of 590 nm (Mashkani, Tanipour, Saadatmandzadeh, Ashman, & Griffith, 2016; Kashafi, Moradzadeh, Mohamadkhani, & Erfanian, 2017).

2.4 | Apoptosis assay

Effects of EGCG on apoptosis of HL60 and NB4 cells were evaluated using PI staining method. The cells were treated with EGCG (12.5–100 μM) or ATRA (10 μM), and then incubated with PI reagent. The quantification of apoptosis and necrosis was carried out using FACS Calibur (BD Biosciences) flow cytometer followed by analysis with Flowjo software (TreeStar Inc., USA; Rangarajan et al., 2015).

2.5 | Differentiation assay

Cell differentiation was evaluated using morphological observation with Giemsa staining and NBT reduction assay. To perform NBT assay, HL60 and NB4 cells treated with EGCG (6.5–50 μM) or ATRA (1 μM) were washed with phosphate-buffered saline and suspended in NBT solution (2 mg/ml) containing 200 ng/ml phorbol myristate acetate. After 25 min incubation at 37 °C in the dark, cytospin slides were prepared and stained with Giemsa. Differentiated cells were recognized by their intracellular reduced dark blue formazan granules (300 cells were scored for the presence of the granules, Moradzadeh, Hosseini, Erfanian, & Rezaei, 2017; Moradzadeh et al., 2017).

2.6 | Real-time polymerase chain reaction (PCR) quantification with TaqMan probe and SYBR Green

HL60 and NB4 cells treated with EGCG (25, 100 μM) or ATRA (1, 10 μM) were subjected to RNA extraction using TRIzol according to manufacturer's instructions. RNA concentration and purity were determined using spectrophotometry. cDNA was synthesized from total RNA (100 ng) of each sample using cDNA synthesis kit with random hexamer primer. Primers and probes were designed using Beacon software (Applied Biosystems, USA; Tables 1 and 2). Gene

TABLE 3 IC_{50} values of epigallocatechin-3-gallate in HL60, NB4, and PMN cells at 72 hr

Cell lines	HL60 cells			NB4 cells			PMN cells	
Treatment	24 hr	48 hr	72 hr	24 hr	48 hr	72 hr	24 hr	48 hr
Epigallocatechin-3-gallate (μM)	856.7 ± 0.14	188.4 ± 0.04	91.0 ± 0.04	1509.0 ± 0.14	359.0 ± 0.08	242.2 ± 0.04	1876.0 ± 0.13	1442.0 ± 0.10

expression changes were measured for genes involved in differentiation (PML-RARα and HDAC1), and ABC membrane transporters using TaqMan-based real-time PCR, and for genes of apoptosis pathways (PI3K, AKT, BCL2, BAX, p53, p21, PTEN, CASP3, CASP8, and CASP9) using SYBR Green-based real-time PCR technology with an Applied Biosystems Step One plus detection system (ABI, USA). The reaction mixture consisted of 2 μl of cDNA (250–400 ng), 1 μl of primers (100 pmol), 10 μl of 2 × master mixes [0.4 μl of the probe (250 nM) for Taq-Man method], and dH_2O to bring the volume to 20 μl. The optimized parameters used for the

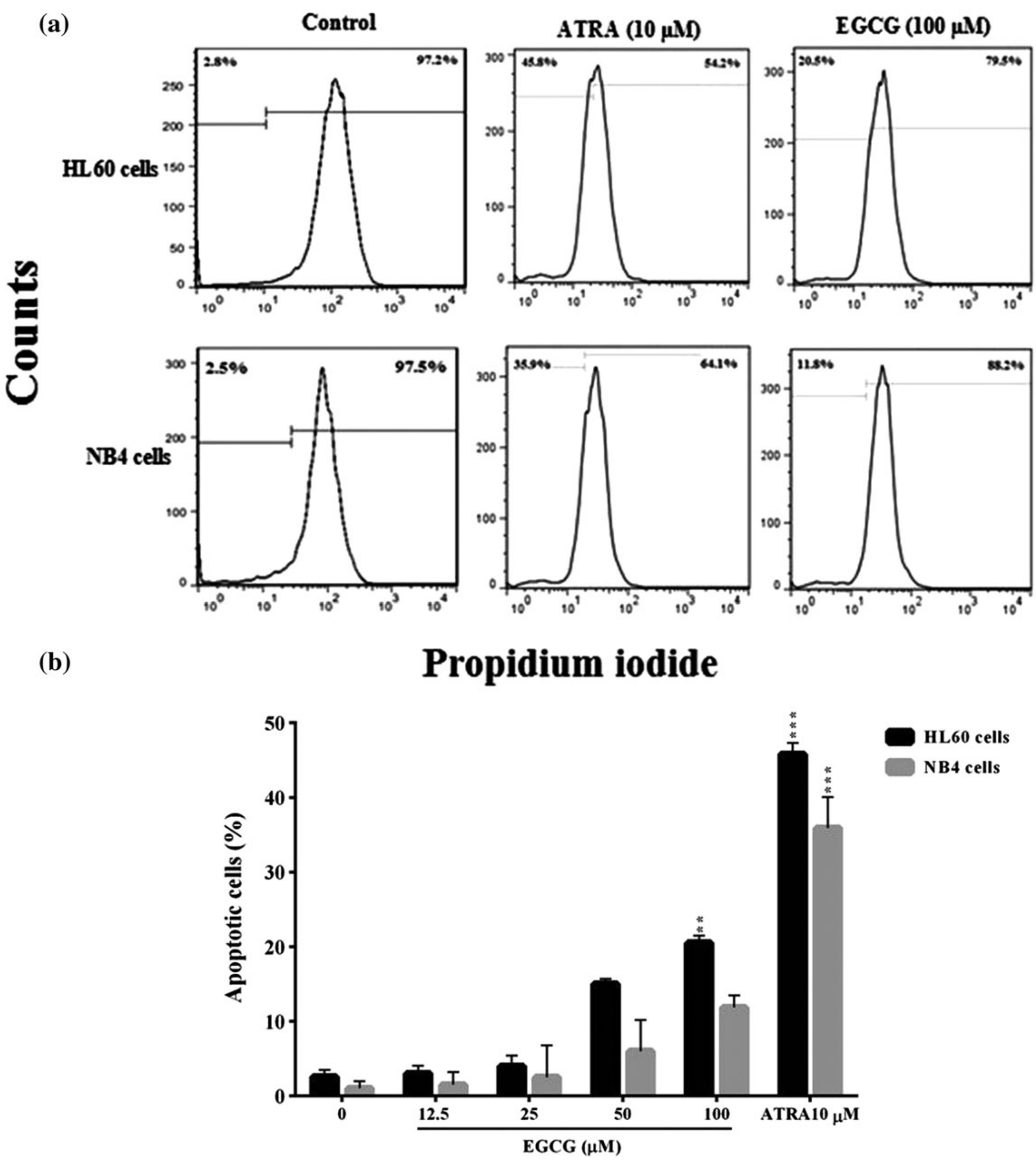

FIGURE 2 Effects of epigallocatechin-3-gallate (EGCG) on cell cycle of leukemic cells as evaluated by quantifying the sub-G1 population obtained from propidium iodide staining. The HL60 and NB4 cells were treated for 72 hr with EGCG (12.5–100 μM), or all-trans retinoic acid (ATRA). (a) Representative histogram of the fluorescence intensity of PI-stained leukemic cells. The sub-G1 region is made by cells with reduced DNA content (apoptotic cells). (b) Quantitative analysis of apoptosis as shown in (a). Data are expressed as the mean ± SEM of three independent experiments performed in triplicate. $^{**}p < .01$ and $^{***}p < .001$ versus untreated control cells (concentration of 0)

thermocycler were short hot-start at 95 °C for 15 min followed by 40 cycles, each consisting of denaturation at 95 °C for 15 s, annealing at 60 °C for 1 min, and extension at 72 °C for 20 s. As the final step of SYBR Green real-time PCR, melting curves were incorporated from 60 to 90 °C rising by 0.3 °C. Samples were run in triplicate, and the fold difference of expression in treated and untreated samples was calculated using the $2^{-\Delta\Delta Ct}$ method (Pfaffl, 2006). The expressions of genes that measured using TaqMan probes method were normalized to glucuronidase beta and transferrin receptor genes. The expressions of genes that measured using SYBR Green method were normalized to GAPDH and β-actin as housekeeping genes.

2.7 | Statistical analysis

Data are presented as the mean ± SEM and analyzed by one-way analysis of variance and Tukey's multiple comparisons posttest. The *p* values less than .05 were considered as statistically significant. Statistical analysis was performed using GraphPad PRISM software (Version 6, Graph Pad Software, CA).

3 | RESULTS

3.1 | EGCG inhibited proliferation of leukemia cells

As shown in Figure 1a, EGCG at concentrations of 25–100 μM significantly reduced proliferation of HL60 cells at 48 and 72 hr ($p < .001$). Such a decrease in proliferation was seen in NB4 cells after 72 hr (Figure 1b, $p < .05$). Similarly, a significant decrease in proliferation was seen in NB4 and HL60 cells incubated for 48 and 72 hr with 10 μM of ATRA ($p < .01$). EGCG had no effect on the proliferation of the PMN cells at concentrations of 12.5–100 μM (Figure 1c). Table 3 shows the IC_{50} values of EGCG in HL60, NB4, and PMN cells at 72 hr incubation.

3.2 | EGCG enhanced apoptosis of leukemia cells

Figure 2 shows the effects EGCG on cell cycle progression of HL60 and NB4 cells as evaluated by quantifying the sub-G1 population obtained from PI staining. In the presence of 100 μM of EGCG, the percent of HL60 cells in sub-G1 phase was increased at 72 hr ($p < .01$). ATRA also increased the percent of HL60 and NB4 cells in sub-G1 phase at concentration of 10 μM after 72 hr ($p < .001$).

3.3 | EGCG modulated genes involved in survival and apoptosis in leukemia cells

Figure 3a shows the effects of 72 hr incubation with EGCG on the expression of genes involved in survival (PI3K, AKT, and Bcl2) and apoptosis (p53, p21, PTEN, Bax, CASP3, CASP8, and CASP9). In HL60 cells, EGCG significantly increased the expressions of p21, PTEN, Bax, CASP3, and CASP8 ($p < .05$, Figure 3a). In HL60 cells, ATRA significantly increased the expressions of p21, CASP3, CASP9, PTEN, and Bax and decreased Bcl2, PI3K, and AKT ($p < .05$, Figure 3a). In NB4 cells, ATRA significantly increased the expressions of CASP3, p21, p53, PTEN, and Bax and decreased Bcl2 and AKT ($p < .05$, Figure 3a). As illustrated in Figure 3b, it was demonstrated that the Bax/Bcl2 ratio was significantly increased in leukemic cells after treatment with EGCG and ATRA ($p < .01$).

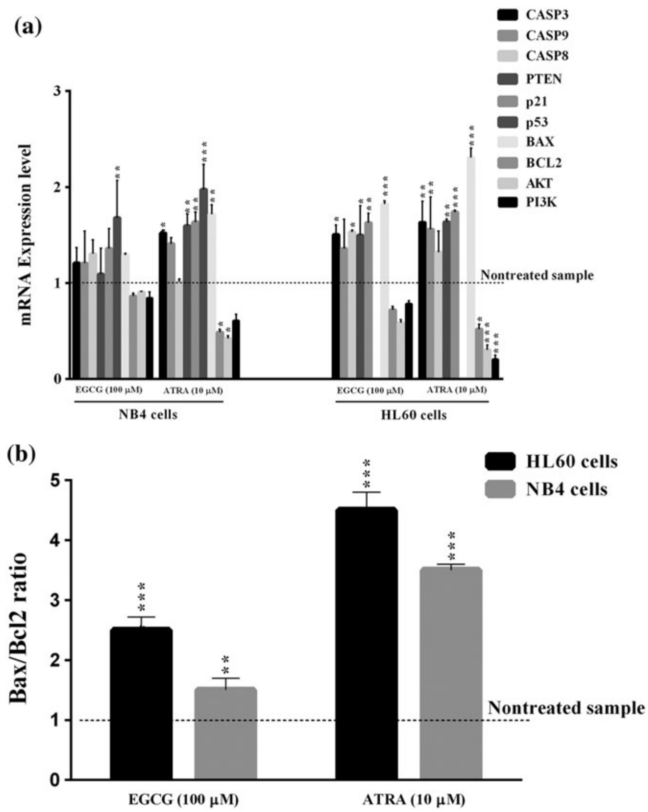

FIGURE 3 Effects of epigallocatechin-3-gallate (EGCG) on the expression of apoptotic and antiapoptotic genes in leukemic cells. (a) HL60 and NB4 cells were treated for 72 hr with EGCG, or all-trans retinoic acid (ATRA), and the expression of apoptotic (p53, p21, PTEN, CASP3, CASP9, CASP8, and Bax) and anti-apoptotic (PI3K, AKT, and Bcl2) genes were determined by real time polymerase chain reaction. Data are expressed as the mean ± SEM of three independent experiments performed in triplicate. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ versus nontreated control cells

3.4 | EGCG induced differentiation of leukemia cells

Morphological analysis of the leukemic cells using Giemsa staining is shown in Figure 4a. Untreated APL cells had promyelocytic characteristics containing a large nucleus and granules in the cytoplasm. After treatment with EGCG (less than 25 μM), the cells began to undergo morphological changes towards having hollow nuclei and larger zones of clear cytoplasm. After 72 hr of treatment, the granulocytic maturation pattern was seen in EGCG- and ATRA-treated cells; therefore, the ratio of nuclei to cytoplasm was decreased, and nuclei demonstrated a range of remodeling from simple indentations to polylobular nuclei.

In leukemic cells, and compared to untreated control cells, EGCG-treated cells displayed increased NBT reduction ability (the hallmark of granulocytic maturation) similar to the positive control (Figure 4b). The effect of EGCG on NBT reduction capacity of HL60 and NB4 cells was

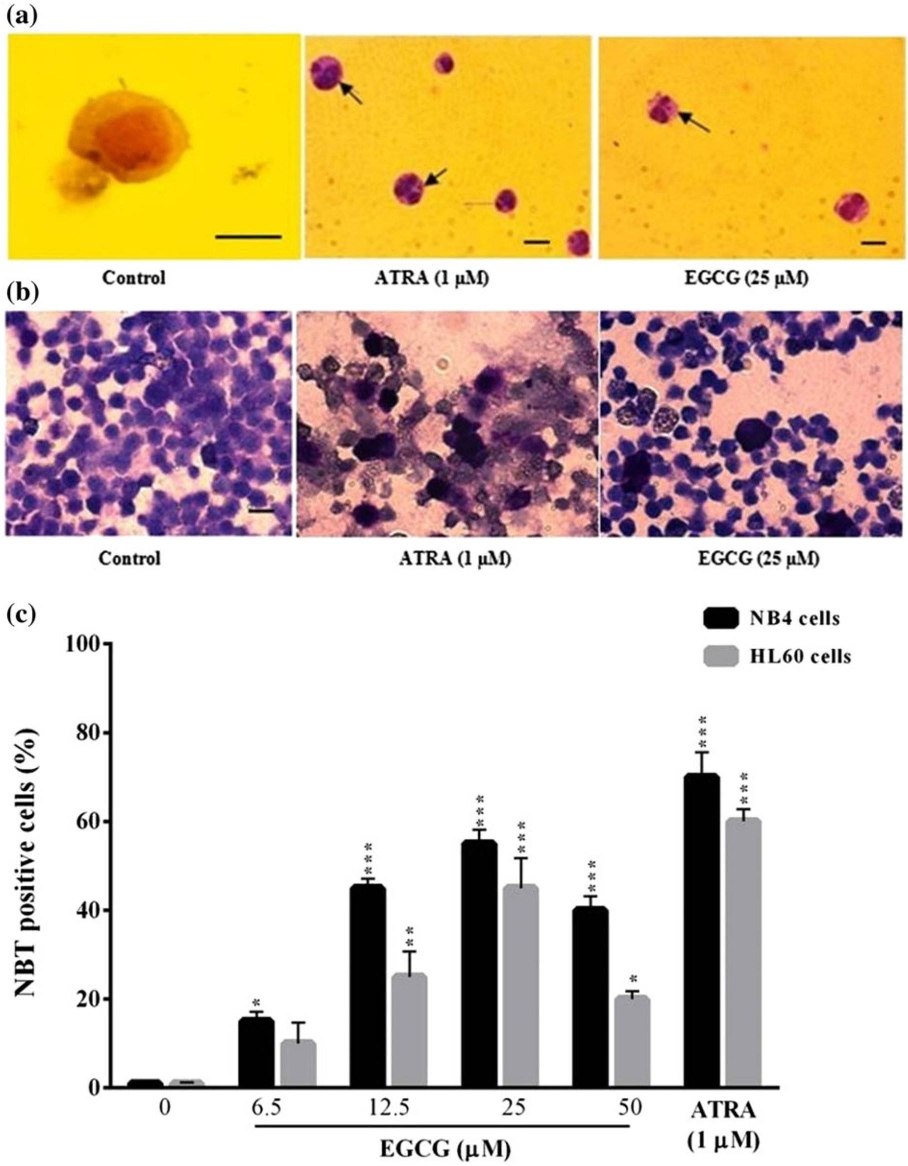

FIGURE 4 Effects of epigallocatechin-3-gallate (EGCG) on granulocytic morphology and differentiation of leukemic cells as determined by Giemsa staining and nitro blue tetrazolium (NBT) assays, respectively. (a) The HL60 and NB4 cells were treated for 72 hr with epigallocatechin gallate (EGCG, 6.5–50 μM), or all-trans retinoic acid (ATRA). Control promyelocytic leukemia cells show promyelocytes characteristic with cytoplasmic granules. Arrows in the treated cells demonstrate polymorphonuclear morphology of granulocyte (bar represents 0.01 mm). (b) Representative photomicrographs of the NBT stained leukemic cells treated 72 hr with EGCG, or ATRA. Black spots showed dark blue formazan deposits in the differentiated cells (bar represents 0.01 mm). (c) Quantitative analysis of differentiated cells as shown in (b). A minimum of 300 cells were scored. Results are means ± SEM of three independent experiments performed in triplicate. *$p < .05$, **$p < .01$, and ***$p < .001$ versus nontreated control cells [Colour figure can be viewed at wileyonlinelibrary.com]

comparable to that of ATRA. The EC_{50} values of EGCG were 20.46 ± 0.05 and 35.58 ± 0.07 in NB4 and HL60 cells at 72 hr incubation, respectively ($p < .001$, Figure 4c).

3.5 | EGCG decreased the expressions of PML-RARα and HDAC1 genes in leukemia cells

Figure 5a shows that 72 hr treatment with EGCG (25 μM) and ATRA (1 μM) significantly decreased the expression of PML-RARα gene in NB4 cells ($p < .001$, Figure 5). It is noteworthy to mention that HL60 cells were checked for PML-RARα expression and found to be null. Also, EGCG and ATRA significantly reduced the expression of HDAC1 gene in NB4 and HL60 cells when compared to untreated control cells ($p < .001$, Figure 5a).

3.6 | EGCG decreased the expression of MDR genes in leukemia cells

Real-time PCR assay showed that 72 hr incubation with EGCG (100 μM) decreased the expressions of ABCB1 and ABCC1 genes in HL60 and NB4 cells ($p < .001$, Figure 5b). On the contrary, no significant decrease in ABCB1 and ABCC1 expressions were observed in the cells treated with 10 μM ATRA.

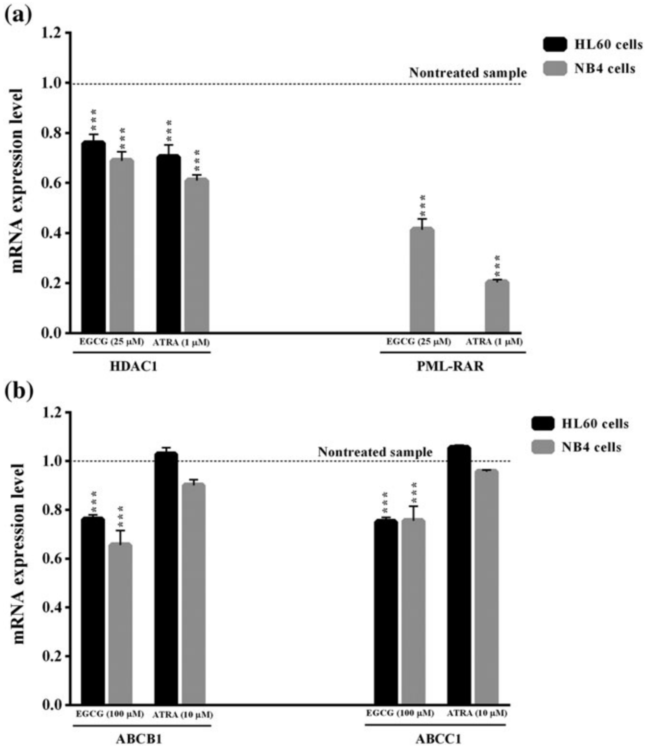

FIGURE 5 Effects of epigallocatechin-3-gallate (EGCG) on the expression of promyelocytic leukemia/retinoic acid receptor-α (PML-RARα), HDAC1, and ATP-binding cassette (ABC) membrane transporters genes in leukemic cells. The HL60 and NB4 cells were treated for 72 hr with EGCG, or all-trans retinoic acid (ATRA), and the expressions of PML-RARα, HDAC1 (a); ABCB1 and ABCC1 (b) genes were determined by real time polymerase chain reaction. Results are mean ± SEM of three different experiments. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ versus untreated control cells

4 | DISCUSSION

Patients with APL are successfully treated with ATRA. However, resistance to this drug and its toxicity are major problems in the treatment of APL (Tomita et al., 2013). Plant-derived bioactive compounds have been shown in numerous studies to exert a variety of antitumor actions, making them potential candidates for adjuvant therapy of different types of cancer (Karthick & Tandon, 2016; Mirzaei et al., 2016; Momtazi et al., 2016; Moradzadeh, Sadeghnia, Tabarraei, & Sahebkar, 2017). In the previous study, we showed that EGCG increased apoptosis and inhibited telomerase activity in breast cancer cells (Moradzadeh, Sadeghnia, Tabarraei, & Sahebkar, 2017). In this study, we showed that EGCG decreased proliferation, induced apoptosis, and promoted differentiation in the promyelocytic leukemia cells via inhibition of MDR and PML-RARα/HDAC1. EGCG was able to decrease the proliferation rate of APL cells, and this finding is consistent with previous reports on the growth inhibitory effect of EGCG in various cancer cells (Benyahia et al., 2004; Khan, Adhami, & Mukhtar, 2010; Ly et al., 2013; Wang et al., 2015). Although the obtained IC50 value for the antiproliferative effect of EGCG on leukemic cells was higher than that of ATRA, the antiproliferative effect was observed at concentrations with no obvious toxic effect on normal polymorphonuclear cells, suggesting the specificity of EGCG action in leukemic cells.

Failure to differentiate is one of the main characteristics of the promyelocytes in APL patients (Petrie, Zelent, & Waxman, 2009). Results of Giemsa staining and NBT assay showed that EGCG, such as ATRA, induces morphological changes in leukemic cells toward granulocytic pattern and increases their NBT reduction ability which is a hallmark of granulocytic maturation. In comparison with its pro-apoptotic effect, pro-differentiating action of EGCG was observed at <25 μM concentration. This observation is in agreement with previous findings that showed EGCG induced differentiation at various cancer cells (Annabi, Currie, Moghrabi, & Béliveau, 2007; Britschgi, Simon, Tobler, Fey, & Tschan, 2010; Chokor, Lamy, & Annabi, 2014; Huang et al., 2013; Lea et al., 1993). In contrast, Vézina, Chokor, and Annabi (2012) showed that EGCG may not pharmacologically affect differentiated macrophages in neuroinflammatory diseases. The inhibitory effect of EGCG on the level of PML-RARα expression may explain the pro-differentiating effect on NB4 cells. It is well known that fusions between PML and RAR recruit HDACs resulting in the suppression of differentiation-related genes (Kretsovali, Hadjimichael, & Charmpilas, 2012). Our results showed that treatment with EGCG and ATRA reduced the expression of HDAC1 in the leukemic cells. Interestingly, EGCG was able to induce differentiation in HL60 cells which is PML-RARα null, suggesting that the HDAC1 inhibition could be the major mechanism responsible for myeloid differentiation in such cells. The previous study also showed that EGCG induced differentiation via downregulation DNMT1, HDAC1, and HDAC2 in APL cells (Lung et al., 2002). Here, we have shown that EGCG at 100 μM concentration is able to decrease the expression of ABCB1 and ABCC1 in HL60 and NB4 cells, whereas ATRA failed to show such an effect. This effect is in agreement with previous findings that showed EGCG reduced MDR at various cancer cells (Lyn-Cook et al., 1999; Mei, Wei, & Liu, 2005; Nowakowska & Tarasiuk, 2016; Qian, Wei, Zhang, & Yang, 2005; Wen et al., 2017) and supports the notion that EGCG may suppress drug resistance via downregulation of ABC transporters.

In conclusion, the present results demonstrated that EGCG has antiproliferative, pro-apoptotic and pro-differentiating effects on human leukemia cells by inhibiting the expressions of PML-RARα, HDAC1, and MDR-associated proteins. These findings provide evidence supporting the potential application of EGCG in the treatment of APL patients.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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