ORIGINAL ARTICLE

Elevated Bax/Bcl-2 Ratio: A Cytotoxic Mode of Action of Kermanian Propolis Against an Acute Lymphoblastic Leukemia Cell Line, NALM-6

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Abstract Currently, alternative cancer remedies, especially herbal-derived medicines, have attracted great interest. Propolis, a honeybee-produced naturopathic formulation, is an available, affordable, and safe example of such remedies with different content according to its geographic location. Findings regarding the protective properties of this resinous substance across numerous pathological conditions are promising. Although the anti-tumor effects of propolis from different origins have been explored to some degree, yet there is no study on the effects of Kermanian propolis in the treatment of hematologic malignancies. Accordingly, the objective of the present experiment was to divulge the anti-tumor potential of this bioactive substance both as monotherapy and in combination with doxorubicin against an acute lymphoblastic leukemia cell line (NALM-6).The viability of cells treated with Kermanian propolis (5–500 μg/mL) and doxorubicin (5–100 μg/mL) was analyzed during 72 h. Based on the MTT results, the best incubation time, IC50 concentrations, and finally the cytotoxicity of the combination therapy were ascertained. Next, the apoptotic rate and expression of apoptosis-related genes (Bcl-2 and Bax) were assessed in mono and combination therapies using flow cytometry and real-time PCR assays, respectively. Kermanian propolis and doxorubicin have impressive tumor-suppressing activity in a dose-dependent manner (IC_{50} concentrations: 100 and 40 μg/mL respectively). The best incubation time was considered 48 h. For the combination approach, 50 and 10 μg/mL were determined as optimum concentrations of the compounds. The selected concentrations induced notable apoptosis in the studied cells through significant ($P < 0.01$) upregulation of Bax/Bcl-2 level. The present study clearly suggests that Kermanian propolis, as an adjunct treatment option, has a promising apoptosis-induced cell death potential in the NALM-6 cell line.

Keywords Kermanian propolis · Acute lymphoblastic leukemia (ALL) · NALM-6 · Doxorubicin · Bax/Bcl-2 ratio

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Introduction

ALL is the abbreviation for acute lymphocytic leukemia, a hematopoietic cancer that affects a noticeable percentage of people, especially in children [1–3]. The proliferation

and consequent accumulation of lymphoid progenitor cells in different tissues (especially bone marrow and blood) are the hallmarks of this malignancy, which includes approximately 80% of acute leukemia cases under the age of 15 [1–3]. Doxorubicin (DOX)-based chemotherapy is widely applied as a five-step treatment approach for this malignancy: induction, consolidation, interim maintenance, delayed intensification, and maintenance [3]. Two major mechanisms of action are proposed for this medication; as a drug of choice, this compound triggers cell death through topoisomerase II suppression-dependent DNA repair disruption and free radicals-induced oxidative stress [4]. In spite of therapeutic effectiveness, the remarkable dose-dependent DOX-induced toxicity for non-cancerous cells is the major obstacle for its extensive clinical application [5–7]. Therefore, it is vital to identify new approaches and/ or compounds that can improve the effectiveness and also reduce the cytotoxicity and chemo-resistance to this drug. In this regard, combined-modality therapy, the amalgamation of anticancer agents, is now accepted as a more efficient strategy [8]. Propolis (bee glue), a resinous honeybees-produced substance, has attracted the attentiveness of researchers due to its multiple biological/pharmacological potentials [9–12]. It has been evidenced that the color, chemical composition, and pharmacological activity of this aromatic waxy-like substance depend on several factors, such as location, plant sources, and extraction solvents [13]. Despite the variable compositions, propolis has gained popularity as an adjunct treatment option or a dietary supplement [13]. Nowadays, it is being extensively employed to ameliorate different illnesses such as cancers, bacterial infections, inflammation, and gastrointestinal tract disorders [9, 13, 14]. Although the antitumor efficacy of propolis from different origins has been explored to some degree [14–17], yet there is no study on the effects of Kermanian propolis in the treatment of hematologic malignancies. Due to this dearth and also the high frequency of ALL, the focus of the present research was to divulge the cytotoxic and apoptosis-inducing potentials of this bioactive substance (as monotherapy and/ or in combination with DOX) against an ALL cell line (NALM-6).

Materials and Methods

Substances and Drugs

In this experiment, Kermanian propolis was acquired from a local market and its nature was confirmed by the Department of Pharmacognosy, School of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. After washing (thrice) and drying, it was crushed, treated

with methanol (500 mL; Merck), and incubated at 37 °C for 3 days. The extract was collected on different days, pooled, and then concentrated using a rotary evaporator (Heidolph). To prepare the stock solution (1.6×10^3 μg/ mL), 0.016 g of Kermanian propolis was dissolved in 10 μL dimethyl sulfoxide (DMSO, Sigma Aldrich), mixed well, and then the final volume of 10 mL was obtained using the cell culture medium. The stock solution was filtered using a sterile filter (0.22 μm; Orange Scientific Company) and the final concentrations were prepared using the cell culture medium. Regarding DOX (EBEWE Pharma), intended concentrations were attained by the addition of a relevant amount of stock solution (2 mg/mL) to the cell culture medium.

Cell Culture

The ALL cell line, NALM-6 (CRL-3273[TM]), was purchased from the Iranian cell bank of Pasteur Institute and then cultured in RPMI-1640 medium (Gibco Company) enriched with 2 mM L-glutamine, 10% fetal bovine serum (Gibco Company), and 1% penicillin/streptomycin (Sigma Aldrich) in a moistened atmosphere (5% CO_2 at 37 °C). The medium was replaced each 48 h and the cells were given subculture after reaching 80–90% confluency. The morphological assessment was done daily using an inverted microscope (ECLIPSE E100; Nikon).

Cell Viability Evaluation

Cells (10×10^3/well) were seeded into wells of 96-well plate containing 0.1 mL of culture medium enriched with pre-defined concentrations of the selected compounds. Desired concentrations for Kermanian propolis were 5, 10, 25, 50, 75, 100, 250, and 500 μg/mL and for DMSO-solved DOX were 5, 10, 20, 40, 80, and 100 μg/mL. Following the stipulated time [24, 48, and 72 h], the plate was centrifuged (500 × g/5 min), the medium was eliminated, and the cell survival rate was analyzed using the MTT assay kit (Sigma Aldrich) according to the instruction. Concisely, after the addition of 100 μL of the 5 mg/mL MTT solution to the wells, incubation (4 h at 37 °C), and centrifugation (700 × g/10 min), the supernatant was discarded and DMSO (100 μL) was added to each well. The plate was shaken and the optical densities (OD) of the wells were read at 570 nm using the ELISA reader ELX808 device (BioTek Company). After the determination of cell viability percentages [18, 19], the IC$_{50}$ concentrations were specified. It is worth mentioning that the control group contained 0.1% DMSO-treated cells. To evaluate the combined toxic effects and determination of optimal concentrations, NALM-6 cells were treated (48 h) with DOX (5, 10, 20, and 40 μg/mL) and Kermanian propolis (10, 25,

50, 75, and 100 µg/mL) simultaneously. In order to explore possible toxic effects of Kermanian propolis on normal cells, human peripheral blood mononuclear cells (PBMCs) were cultured in the same condition applied for NALM-6 cells and then treated with IC_{50} concentration of the corresponding extract. All experiments were performed in triplicate (independently), each being repeated 3 times.

Determining the Combination Index (CI) Value

The CompuSyn software version 1.0 (Ting Chao Chou and Nick Martin, Paramus, NJ, 2005) was used to investigate the synergistic effects of Kermanian propolis and DOX based on the results of MTT assays. This software applies the multiple drug effect equation by Chou and Talalay to calculate the CI values: $[CI = (D)_1/(Dx)_1 + (D)_2/(Dx)_2]$, where $(Dx)_1$ and $(Dx)_2$ indicate the doses of Kermanian propolis and DOX in a combination required to achieve the same efficacy as that of Kermanian propolis (D_1) and DOX (D_2) when used singularly. CI values < 1, $= 1$ and > 1 indicate synergistic, additive, and antagonistic effects, respectively.

Apoptosis Assay

In order to better evaluate the NALM-6-suppressing potential of Kermanian propolis in vitro, ApoFlowEx® FITC kit (ExBio Company) was used. Cells were seeded into cell culture flasks (10×10^4 cells/flask), and 48 h after treatment with Kermanian propolis (100 µg/mL), DOX (40 µg/mL), and Kermanian propolis + DOX (50 and 10 µg/mL), the cells were extracted, washed, suspended in binding buffer, and labeled using propidium iodide (PI) and Annexin V based on the manufacturer's recipes [18]. After elapse of the incubation time (15 min), the percentage of apoptotic cells (Annexin V^+/PI^- and Annexin V^+/PI^+ cells) was quantified using CyFlow® Space flow cytometer (Sysmex Partec). As a general rule, unlabeled cells were used to remove autofluorescence. Trials were repeated 3 times.

RNA Segregation and cDNA synthesis

As per the instructions given by the manufacturers, the harvest of total RNA and then cDNA synthesis from the treated and control groups (following 48 h treatment) were done using TRIzol (Invitrogen) and RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, K1621), respectively. Quality and purity of extracted RNA were evaluated by agarose gel electrophoresis [20] and optical density measurement (A_{260}/A_{280} ratio) using NanoDrop 1000 Spectrophotometer (Thermo Fisher Scientific), respectively.

Expression Profiling of Apoptosis-Related Genes

The relative gene expression analysis was conducted by real-time PCR and using RealQ Plus 2 × Master Mix Green (Ampliqon Company, 5 µL), 1 µL of the cDNA product, 0.5 µL of each primer, and 3 µL of nuclease-free water (total volume of 10 µL). The PCR amplification program using Rotor Gene 6000 Real-time PCR System (Corbett Research) was as follows: denaturation (95 °C/15 min), 40 cycles (95 °C/30 s), and 60 °C for 1 min. A melting curve assessment was applied to affirm the specificity of the products [20]. The housekeeping gene, β-actin, was used as the internal control and the relative Bax and Bcl-2 genes expression was calculated using the comparative Ct method. All trials were repeated 3 times and the list of used primers sequence is available in Table 1.

Statistical analysis

The mean ± standard deviation (mean ± SD) was calculated and the statistical differences between the treatment and the control groups (flow cytometry and real-time PCR) were assessed by *t-test* using SPSS 20 software. Two-way ANOVA was applied for MTT results analysis and P values of ≤ 0.05 were regarded as statistically significant. Log-Logistic Nonlinear Regression Models were used for the dose–response analysis determining the optimal concentration. To enhance detection, models with 2, 3, 4, and 5 parameters were evaluated and among them, a Log-Logistic Nonlinear Regression with 4 parameters presented the best fit and the least error. Additionally, this model had the least AIC and BIC among all studied models.

Results

Kermanian Propolis Decreases the Metabolic Activity of NALM-6 Cells

The effect of different concentrations of Kermanian propolis, DOX, and Kermanian propolis + DOX on the

Table 1 The sequence of used primers for real-time PCR

Primers	Sequences (5'-3')
Bcl-2.S	ATCGCCCTGTGGATGACTGAG
Bcl-2.A	CAGCCAGGAGAAATCAAACAGAG
Bax.S	AGGATCGAGCAGGGCGAATG
Bax.A	TCAGCTTCTTGGTGGACGCA
β-actin.S	CCAACCGCGAGAAGATGA
β-actin.A	TCCATCACGATGCCAGTG

S Sense sequence; *A* Antisense sequence

metabolic activity of cells (an indicator of cell viability) was evaluated by MTT assay. As illustrated in Fig. 1, the viability of the cultured cells after monotherapy was reduced in a dose-dependent mode. As evident from Fig. 2, suggested half-maximal inhibitory concentrations (IC$_{50}$s) for Kermanian propolis and DOX were 72.99 and 20.39 respectively. Surprisingly, the viability of cells after treatment with these concentrations was more than 50%. Therefore, 100 and 40 µg/mL were selected for subsequent tests instead. In addition, 48 h was selected as the optimum exposure time for the next experiments. During this exposure time, the cytotoxic effect of the combination therapy was concentration-dependent, with approximately 30.9 ± 3.4, 28.2 ± 5.5, 56.6 ± 1.9, 68.1 ± 3.5, 45.6 ± 1.22, 49.4 ± 2.22, 62.1 ± 2.8, 74.5 ± 4.2, 50.9 ± 2.9, 55.6 ± 1.9, 67.9 ± 1.2, 78 ± 0.8, 63.8 ± 1.26, 68.5 ± 2.57, 82.8 ± 1, 90.5 ± 3.04, 84.37 ± 1.9, 89 ± 0.77, 92.38 ± 1.52, and 96.9 ± 1.08% reduction in metabolic activity after treatment with 10 + 5, 10 + 10, 10 + 20, 10 + 40, 25 + 5, 25 + 10, 25 + 20, 25 + 40, 50 + 5, 50 + 10, 50 + 20, 50 + 40, 75 + 5, 75 + 10, 75 + 20, 75 + 40, 100 + 5, 100 + 10, 100 + 20, and 100 + 40 µg/mL of Kermanian propolis along with DOX. Moreover, treating PBMCs with IC$_{50}$ concentration of Kermanian propolis induced no significant apoptotic effect

($P > 0.05$, Fig. 1d). This finding suggests the tumor-selective anti-proliferative effect of Kermanian propolis.

Combination of Kermanian propolis and DOX synergistically decreases cell viability of Nalm-6 cells

Based on MTT results and in order to achieve 50% cell death with the lowest possible concentrations of DOX, 4 pairs of concentrations were selected for detecting synergy indices: P(25) + D(5), P(25) + D(10), P(50) + D(5), and P(50) + D(10). CI analysis and normalized isobolograms indicated synergistic effect ($CI < 1$) in concentrations 50 µg/mL of Kermanian propolis + 10 µg/mL of DOX in NALM-6 cells (Fig. 3).

The Combination of Kermanian Propolis and DOX Induces Higher Apoptosis in NALM-6 Cell Line

To further assess the cytotoxic effects of Kermanian propolis and DOX, the impact of these substances on cell apoptosis was examined both individually and in combination. To this end, the studied cells were analyzed for Annexin V binding and PI uptake by the corresponding kit and using a flow cytometer. As illustrated in Fig. 4, 48 h incubation of cells with the selected concentrations (100, 40, and 50 + 10 µg/mL), significantly ($P < 0.01$)
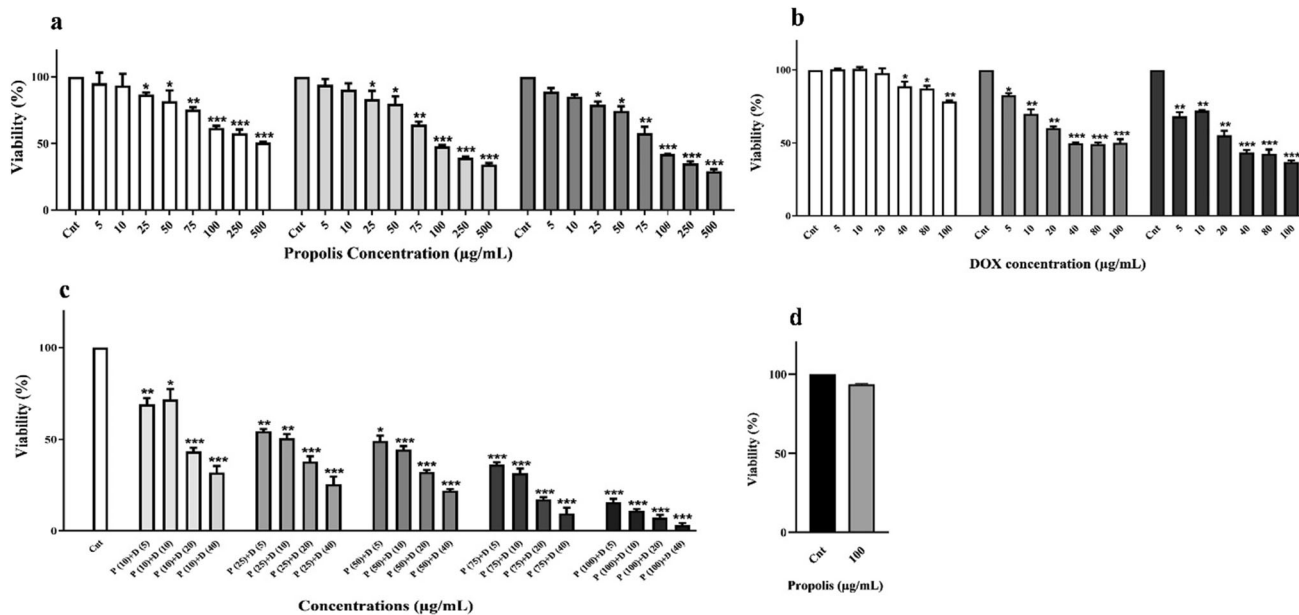

Fig. 1 Kermanian propolis decreases viability (%) of the NALM-6 cells. The cells were treated (24, 48, and 72 h) with different concentrations of Kermanian propolis (a) and DOX (b) and the MTT assay was applied. After exposure, the metabolic activity (%) of the treated cells was determined relative to the control cells (Cnt) which were set as 100%. As represented, the viability was reduced significantly (compared to the control value) in a dose-dependent mode (IC$_{50}$ concentrations: 100 and 40 µg/mL). As presented in (c),

48 h exposure of cells with the combination of Kermanian propolis (P) and DOX (D) had a cytotoxic effect on NALM-6 cells, in a concentration-dependent manner. Interestingly, no dramatic apoptotic effect was seen after treatment of PBMCs with IC$_{50}$ concentration of Kermanian propolis (d). Data are given as the mean ± standard deviation of three independent examinations. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$
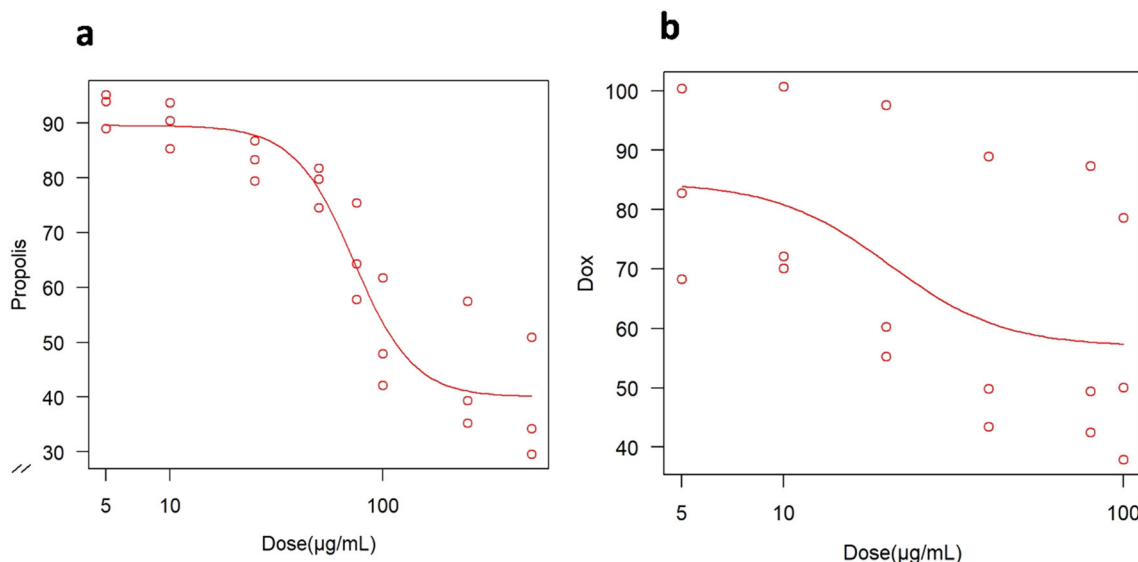
Fig. 2 Dose–response curves. The effect of different concentrations of Kermanian propolis (**a**) and DOX (**b**) on the viability of Nalm-6 cells. The x-axis represents concentrations and the Y-axis is corresponding to percentages of viability. The IC_{50} concentrations of Kermanian propolis and DOX were 72.99 and 20.39 µg/mL, respectively
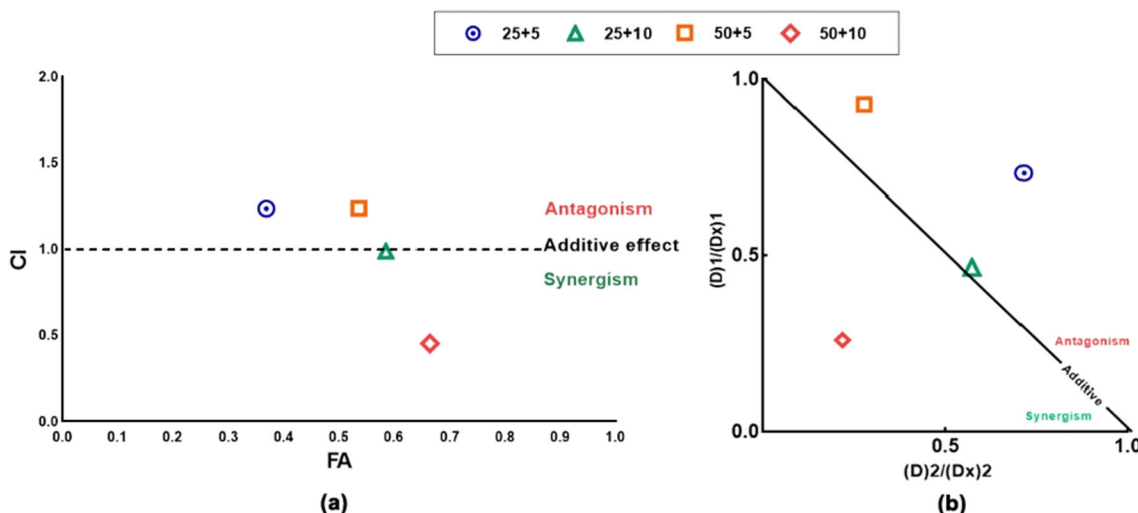

Fig. 3 Synergistic effect of Kermanian propolis (25 and 50 µg/mL) and DOX (5 and 10 µg/mL) determined by the Compusyn software. The CI/FA curve (**a**) and concentration-normalized isobologram analysis (**b**) of Kermanian propolis and DOX combination showed that one of the selected concentration sets was below the additive line and showed CI < 1, confirming a synergistic effect (Kermanian propolis: 50 µg/mL and DOX: 10 µg/mL). $(Dx)_1$ and $(Dx)_2$ refer to the concentration of Kermanian propolis and DOX in a combination needed to achieve the same efficiency as that of compounds used alone (D_1 and D_2)

increased Annexin V^+/PI^- and Annexin V^+/PI^+ cells in the treated groups compared with the control ones, implying the apoptotic effect of Kermanian propolis, DOX, and Kermanian propolis + DOX on NALM-6 cells. Although DOX, with or without Kermanian propolis, had the ability to induce apoptosis in the cells, the level of the impact was greater in the combination treatment. Indeed, the 50% cell death was obtained at a lower concentration of DOX in the combined state. It should be noted that

Kermanian propolis had neither cytotoxic nor apoptotic effects against PBMCs.

Kermanian Propolis Regulates the Expression of Apoptosis-Related Genes

To better understand the mechanisms underlying the cytotoxic effect of Kermanian propolis, DOX, and their combination on NALM-6 cells, the expression levels of Bcl-2 and Bax at mRNA level were examined in treated
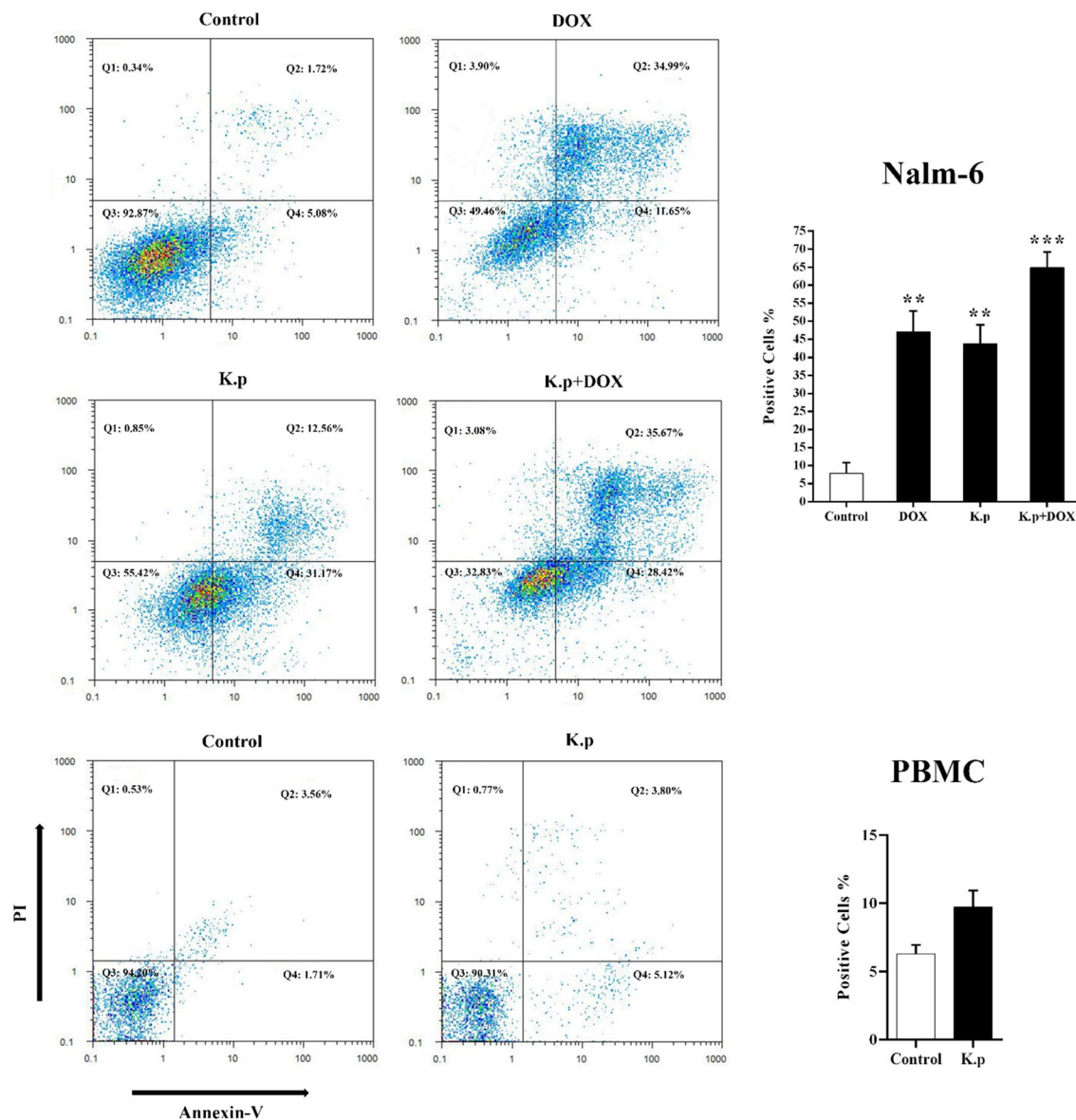
Fig. 4 Apoptotic cell death induction by single and combined therapy approaches in NALM-6 cells. NALM-6 cells were exposed to the selected concentrations of Kermanian propolis (K.p; 100 μg/mL), DOX (40 μg/mL), and K.p + DOX (50 + 10 μg/mL) for 48 h and then the levels of Annexin V binding and PI uptake were assessed using flow cytometer. As shown, combination therapy

(Kermanian propolis 100 μg/mL, DOX 40 μg/mL, and Kermanian propolis + DOX 50 + 10 μg/mL) and control (DMSO-treated) cells. Based on the results, the expression levels of the studied genes were significantly altered in treated cells compared with the control ones (Fig. 5); the expression levels of Bcl-2 and Bax genes were down- and up-regulated, respectively.

Taken together, these findings suggest that Kermanian propolis triggers an apoptotic pathway in NALM-6 cells, thereby reduces tumor cells viability and progression. As another cornerstone finding, the amalgamation of

$(64.9 \pm 4.35\%)$ increased the Annexin V$^+$/PI$^-$ and Annexin V$^+$/PI$^+$ cells compared to monotherapy with DOX $(47.03 \pm 5.8\%)$ and K.p $(43.72 \pm 5.29\%)$, respectively. Interestingly, no significant apoptotic effect was seen after treating PBMCs with IC$_{50}$ concentration of Kermanian propolis. One representative analysis among three independent ones (n = 3) is shown. $^{**}P \leq 0.01$ and $^{***}P \leq 0.001$

Kermanian propolis and DOX further enhances the anti-NALM-6 activity of these substances compared with the monotherapy strategy, probably due to a/an synergistic or additive interaction.

Discussion

Currently, recognition of non-toxic and more effective substances has taken the spotlight in cancer drug development. In this context, honeybee products have attracted a
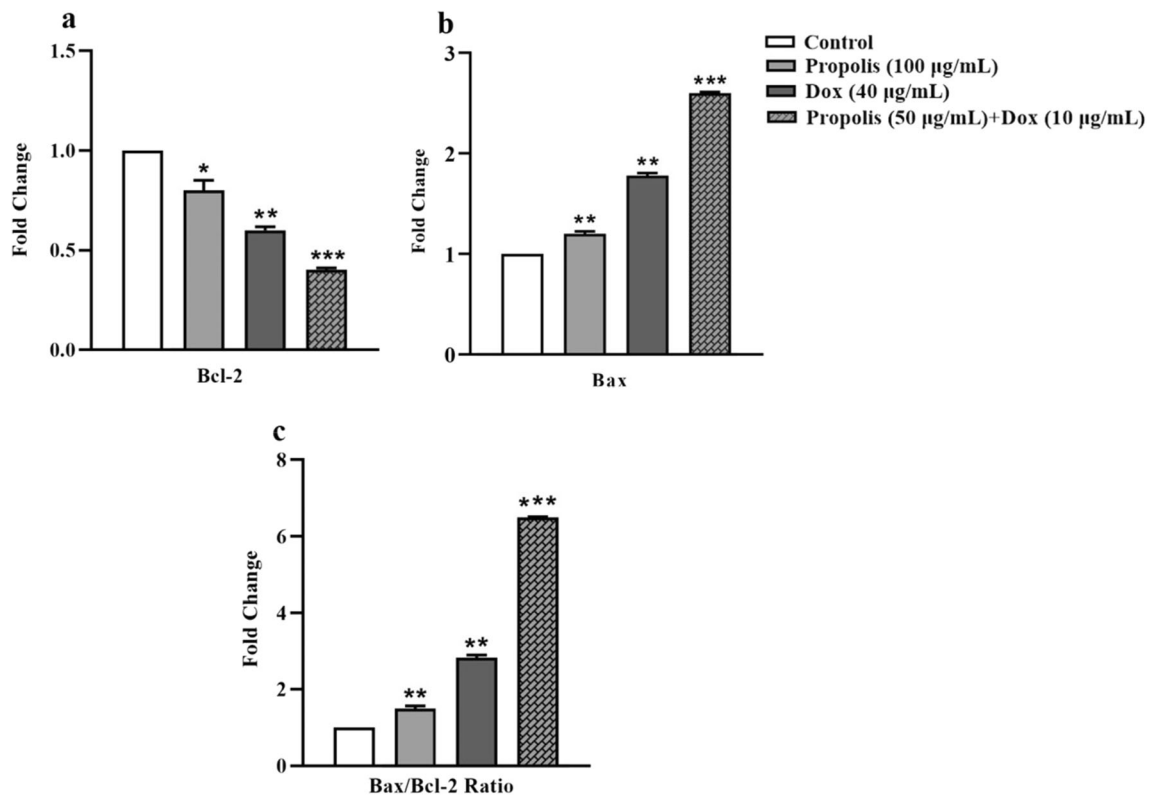
Fig. 5 The expression of the studied mRNAs in the control and treated groups. The fold change was measured relative to the control and calculated after adjusting for the ß-actin housekeeping gene using the comparative Ct ($2^{-\Delta\Delta CT}$) method. After 48 h exposure, the expression of Bcl-2 (**a**) and Bax (**b**) genes was significantly reduced and increased, respectively, compared with the corresponding control group. Interestingly, the alteration of Bax/Bcl-2 ratio was more prominent in combination therapy, followed by DOX at selected concentrations (**c**). The expression of the studied genes in NALM-6 cells underwent modification in DOX and Kermanian propolis treatment alone, but when cells were treated with DOX and Kermanian propolis in combination, the extent of alteration was clearly higher. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$. For each gene, we had 3 independent replicates (n = 3)

lot of attention [11, 12, 21, 22]. Accordingly, several studies are targeted toward evaluating the health benefits of propolis and have recommended this material for different applications related to gynecological, oral, oncological, and dermatological care [11, 13]. Based on these studies, propolis has been introduced as an immunomodulator and alleviator of oxidative stress, inflammation, sepsis, ulcer, and bacterial infections [9, 13, 14]. This honey-derived substance is mainly composed [11, 23] of vitamins, minerals, resin, wax, enzymes, essential oils, pollen, and various organic elements (esters, flavonoids, terpenes, ß-steroids, aromatic aldehydes, phenolic acids, and alcohols). Although it has been suggested that the bioactive potential of this substance mainly depends on the presence of polyphenols [9, 10, 15], especially flavonoids (quercetin, pinocembrin, acacetin, chrysin, rutin, luteolin, kaempferol, apigenin, myricetin, catechin, naringenin, and galangin), yet the exact mechanisms of actions have not been fully elucidated and further experiments are warranted to explain the exact effective factors. About the tumor-suppressing potential of honey, propolis, and other derivatives,

modulation of angiogenesis, insulin signaling, oxidative stress, and inflammation, as well as induction of apoptosis, cell cycle arrest, and mitochondrial outer membrane permeabilization were introduced as the main molecular mechanisms [11, 13]. Regarding propolis, Desamero and colleagues evidenced that crude ethanolic extract of Philippine stingless bee propolis had selective in vitro/*vivo* anti-cancer effects against differentiated-type human gastric cancer cell lines [17]. The researchers attributed the cytotoxicity to significant modulation of cell cycle-related genes and the consequent cell cycle arrest at the G_0/G_1 phase. Likewise, another study delineated apoptosis-dependent anti-breast tumor activity of propolis. Another fascinating finding of this experiment was the selective toxicity of this material against tumor cells and not normal cells [24]. Similarly, the ability of Turkish propolis for selective cytotoxicity against human lung cancer cells through the induction of endoplasmic reticulum stress, apoptosis, and caspase activity was approved [16].

Despite current knowledge on the biological activities of propolis and its ingredients, no study has been performed

regarding the potential anti-hematopoietic cancer properties of Kermanian propolis and the involved mechanisms. Therefore, the exploration of its apoptosis-dependent cytotoxic effects on NALM-6 cells was aimed in the present experiment. The MTT, flow cytometry, and real-time PCR assays were employed to assess the metabolic activity, apoptotic rate, and expression levels of target genes, respectively, in the treatment and control groups. As illustrated in Figs. 1 and 3, MTT outcomes implied that the Kermanian propolis, DOX, and Kermanian propolis + DOX reduced the viability of the cells in a dose-dependent manner. The IC_{50} concentrations were 100, 40, and 50 + 10 μg/mL, respectively. To reinforce the obtained results and to better explicate the cytotoxicity mechanisms of the studied compounds, especially Kermanian propolis, the apoptotic rate was determined. The considerable Annexin-PI labeling in treated cells unequivocally supported the MTT findings. Exposure to defined concentrations caused significant apoptosis ($P < 0.01$) in cells treated with Kermanian propolis, DOX, and Kermanian propolis + DOX (Fig. 4). As apoptosis induction is a desirable characteristic for an anti-cancer drug candidate [18], the observed increase in both early and late apoptotic cells indicated that apoptosis induction was, at least partially, responsible for the cytotoxic effect of the studied compounds. Considering this finding and also to ascertain the mechanism of apoptosis, we decided to analyze the expression of apoptosis-related genes following treatments. Based on the findings (Fig. 5), the underlying mechanism seemed to be the elevated ratio of Bax/Bcl-2 ($P < 0.01$). Bcl-2 family members, as the cell death regulator proteins, can exert anti-apoptotic or pro-apoptotic functions [25]. Bcl-2 protein is localized to the outer membrane of mitochondria and plays a pivotal role in supporting cell survival and suppressing the functions of pro-apoptotic proteins [25]. In contrast, Bax (a pro-apoptotic protein of the Bcl-2 family) promotes permeabilization of the mitochondrial membrane and also the release of apoptotic signals [25]. A large and growing body of data supports the application of Bax/Bcl-2 ratio as a predictive marker that determines cancer cells' susceptibility to apoptosis [26, 27]. Interestingly, here we hinted at significant down-regulation of Bcl-2 as well as notable up-regulation of Bax, and thereby the increment of Bax/Bcl-2 ratio in the Kermanian propolis-, DOX-, and noticeably in Kermanian propolis+DOX-treated cells.

The cytotoxic effects of green and red propolis (G12 and G13) on different human leukemic cells (K562, HL60, NB4, Ramos human Burkitt lymphoma, Raji human Burkitt lymphoma, NALM-16, NALM-6, RS4, B15, and REH) has been compared by Franchi and colleagues [28]; higher cytotoxicity of red propolis, induction of 50% apoptosis after 24 and before 48 h as well as different IC_{50}

concentrations (< 40 and > 30 μg/mL for G12; < 20 and > 15 μg/mL for G13) were the most important findings of their study about NALM-6. Unfortunately, these researchers did not investigate the apoptosis-related mechanisms of these compounds. Another interesting study on the propolis-induced apoptosis mechanism was reported by Gunduz et al. [29]. They suggested that inhibition of telomerase function played a notable role in Turkish propolis (ethanolic extract)-induced apoptosis in a T-cell lymphoblastic leukemia (CCFR-CEM) cell line. More fascinating experiments were carried out by Cogulu and Eom. The former evidenced the significant decrease in the expression of human telomerase reverse transcriptase (hTERT) in bone marrow-derived leukemic cells, following treatment with 60 ng/mL Turkish propolis [30]. Eom et al. appraised the cytotoxic and apoptotic effects of ethanolic extract of the Korean propolis on HL-60 cells; they introduced dose-dependent inhibition of cell proliferation, apoptosis induction, caspase-3 activation, and increased levels of cytosolic cytochrome c as the main antineoplastic mechanisms of Korean propolis [31].

In summary, our findings unveil that Kermanian propolis, individually or in combination with DOX, is an effective NALM-6-suppressor compound that activates the apoptosis pathway. However, further molecular and cellular investigations are demanded to recognize other possible anti-tumor mechanisms of this natural product.

Declarations

Conflict of interest The authors have no conflicts of interest to declare that are relevant to the content of this article.

Ethics Approval The study gained the approval of the ethical committee of Kerman University of Medical Sciences with the Ethics approval code of IR.KMU.REC.1398.397.

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