Research Article:

The Effect of Honey Bee Venom on CD14 Protein Expression as Monocyte Marker in D-Alpha-Tocopheryl Succinate (Vitamin E)-Treated HL-60 Cells

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ABSTRACT

Background: The membrane form of a Cluster of Differentiation 14 (CD14) is anchored to the phospholipid bilayer via glycosylphosphatidylinositol anchor. This molecule is expressed on the intrinsic surface of monocytes and neutrophils. This protein is not expressed on the HL-60 cell surface. It is believed that the differentiation of HL-60 cells stops in the promyelocytic stage. The differentiation of HL-60 cells with compounds such as vitamin A, D, E results in membrane CD14 expression.

Objectives: The objective of this study was an evaluation of CD14 expression by Honey Bee Venom (HBV) in HL60 cells treated by D-alpha-Tocopheryl Succinate (D-α-TS). Methods: HL-60 cells were cultured in RPMI Media 1640 medium and treated with different concentrations of D-α-TS and HBV. Cellular differentiation was tested by Nitro Blue Tetrazolium (NBT) staining, immunocytochemistry, and flow cytometry. The studied data were analyzed using one-way analysis of variance and InStat 3 software.

Methods: HL-60 cells were cultured in RPMI medium and treated with different concentrations of D-α-TS and HBV. Cellular differentiation was tested by NBT staining, immunocytochemistry, and flow cytometry. The studied data were analyzed using one-way analysis of variance and InStat 3 software.

Results: MTT assay demonstrated that HBV and D-alpha-tocopheryl induce death in HL-60 cell lines in a time and dose-dependent manner. Also, Wright-Giemsa, NBT staining showed morphologically differentiation. Immunocytochemistry and flow cytometry analysis shows that cells treated with 6 µg/mL D-α-TS and 2.5 µg/mL HBV for 5 days significantly increase the expression of CD14 in HL-60 compared to cells treated with D-α-TS.

Conclusion: HBV can induce cell death and inhibit cell proliferation. Also, increase differentiation potency of D-α-TS via HBV can increase the differentiation by inhibiting NF-κB and COX-2 and increasing the expression of P21 that plays an essential role in increasing CD14 protein expression and subsequently induce differentiation in HL-60 cells to monocytes.

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Introduction

A Cluster of Differentiation 14 (CD14) exists in membrane surface and plasma soluble form proteins. The first form is linked to phospholipid bilayer membrane via glycosylphosphatidylinositol anchored protein [1]. This molecule is expressed on the intrinsic surface of monocytes and neutrophils [2, 3]. Today, the CD14 antigen is used as one of the singular markers to identify monocyte cells. This protein is not expressed on the HL-60 cell surface. It is believed that these cells are stopped in the promyelocytic stage of differentiation. But the differentiation of these cells with compounds such as vitamins A, D, and E further differentiate them into monocytes and neutrophils and the expression of membrane surface proteins (CD14) [4-6].

Normal hematopoiesis is a dynamic process in which the hematopoietic stem cells in bone marrow generate all blood cells, including red blood cells, white blood cells, platelets, and others. This process is dependent on the coordination between frequency regulating signal transduction pathways, where the regular function of these pathways leads to the production of healthy blood cells [7-9]. Meanwhile, abnormalities in any of these pathways lead to the creation of neoplastic cells, leading to impaired apoptosis, differentiation, and uncontrolled proliferation of blood cells and their precursors [7-11].

In many cancers, cells remain in the early stages of development and lose the controlling mechanisms of division, growth, and differentiation [7]. These cells do not express the surface molecules necessary for monocyte/macrophage function (CD14 molecules, etc.) [8]. They cannot convert to mature cells [7]. These cells are known as cancer stem cells that have been identified in many cancers [9]. The main characteristics of cancer stem cells include self-renewal and the potential to induce differentiation by various combinations in mature cells [9]. Acute promyelocytic leukemia is the most common and fatal cancer among adults. This cancer is cytogenetically characterized by a balanced reciprocal translocation between chromosomes 15 and 17 [t (15; 17) (q21; q21)], [10]. The displacement results in a protein complex known as Promyelocytic Leukemia protein–Retinoic Acid Receptor α (PML–RARα), which causes the loss of function of Promyelocytic Leukemia (PML) gene (proliferation tumor suppressor), eventually leading to myeloid cancer [11-15]. One of the functions of PML-RAR-α is to inhibit cell differentiation in the promyelocytic stage and prevent the uncontrolled proliferation of promyelocytes neoplasm cells in the bone marrow. The HL-60 cell line was established in 1977 by a patient with acute myeloid leukemia. This cell can respond to signal-inducing differentiation, and maturation is lost [12, 14, 15]. As a result, the cell surface antigen (such as CD14) is not expressed [15]. But differentiation of these cells by different compounds occurs along with the expression of surface markers [4, 15-17]. Chemotherapy is currently the most common treatment for cancers. However, due the unselective properties of chemotherapeutic agents, a new method known as differentiation therapy has been described in in vitro studies [12, 13]. Although there are differentiation therapy methods to erase the cancer cells, chemotherapy is considered the conventional therapy [11, 12, 16, 17-22]. Chemotherapeutic agents mainly affect rapidly dividing cells such as cancerous cells. They do not, however, differentiate between cancer cells and healthy cells, which physiologically have a high turnover, such as cells in the bone marrow, lung, and ovary. It is for this reason that differentiation therapy has become the prevalent method of treating cancer [9, 15, 23, 24]. Differentiation therapy is used successfully in patients suffering from acute promyelocytic leukemia [11, 12]. Several studies have shown that certain nutritional supplements such as vitamins A, C, and E can inhibit cell proliferation and differentiation in cancer cells and express the surface markers of specific adult cells such as CD14 and CD11 [1, 18]. It has also been found that the level of vitamin E in patients with leukemia is lower than that in healthy people [6, 8].

The HL-60 cell line belongs to acute promyelocytic cancer. These cells do not express the specific differentiation markers such as CD14 [2]. If differentiation in HL-60 cancer cells is induced, they express cell surface receptors associated with differentiated lineages, morphology, and enzyme activity. Cellular surface antigen differentiation cells are, therefore, obtained [1]. It has been demonstrated that the D-alpha-Tocopheryl Succinate (D-α-TS) can inhibit proliferation and induce differentiation in HL-60 cell class in monocyte cells [4, 6, 18]. However, the concentration of D-α-TS is required to induce differentiation, CD14 expression, and then programmed cell death has several harmful effects, including lack of sufficient absorption of vitamins A, D, and K and peptic ulcer formation. Therefore, achieving a compound by which we can increase the low levels of D-α-TS capabilities to inhibit the proliferation and induce differentiation while reducing the side effects is presented as a solution.

Honey Bee Venom (HBV) among these materials is noted. We stipulate that HBV is a factor, which inhibits cell proliferation. Its anti-proliferative and pro-apoptotic

effects have been proved on different cancer cell classes. Bee venom can induce apoptosis, necrosis, and cell lysis in cancer cells. It is also proposed that the melittin available in HBV can cause death in cancer cells by activating phospholipase [25-27]. It is also suggested that the melittin and phospholipase A2 available in the venom can inhibit kappa nuclear factor and cyclooxygenase 2 mRNA [28, 29]. Also, Moon et al. found that HBV can decrease the expression of anti-apoptosis proteins (Bcl2 and Bcl-X) and increase p21 protein expression in the U-937cell class [28, 30]. In 2014, Wang et al. investigated the role of p21 protein to induce differentiation in HL60 cells to monocytes by D-α-TS and have suggested that the increased expression of p21 protein plays an essential role in this process [17]. The role of p21 and p27 proteins in regulating differentiation and apoptosis and the p21 protein expression increase in the differentiation of leukemia cells have been already established. Because of the similarity in molecular pathways involved in the inhibition of cell proliferation and apoptosis by HBV and D-α-TS, it can be inferred that toxic and inhibitory doses of HBV can increase the proliferative inhibitory and differentiation effects of D-α-TS on HL-60 cancer cells by inhibiting the activity of kappa beta factor, decreased expression of cyclooxygenase 2 and Bcl2, and increased expression of p21 protein [6, 17, 21, 24, 28, 29].

The differentiation of blood cancer cells, including cells trapped in the G0/G1 phase of the cell cycle, is related to an increase in p21 and p27 proteins (an essential factor in the differentiation of monocytes and consequently the expression of CD14). According to signaling pathways (message transfer) involved in the D-α-TS effect on the proliferation and differentiation of HL-60 cells and the known mechanisms in the HBV function, it seems that the HBV effect overlaps with D-α-TS function in several mechanisms [31, 32]. Sach suggested the differentiation therapy theory in 1987 for the first time. He could differentiate mouse myeloid cells in experimental conditions by differentiation factor in the monocytes and granulocytes [14]. Recently, the effect of vitamin E on inhibiting proliferation and inducing apoptosis in cancer cells has been proved [18, 19]. Tocopherol is the most abundant and prevalent form of vitamin E in nature. D-α-TS is a synthetic derivative of vitamin E [20]. The necessary high concentrations of this compound to inhibit proliferation and induce differentiation and subsequent expression of differentiation markers (such as CD14, CD11b) have shown harmful effects [21, 22]. In this study, we examined the synergistic effects of HBV and low concentration of D-α-TS on the amount of CD14 protein expression.

Materials and Methods

Cell culture

The human HL-60 blood cancer cell line was provided by NCBI (National Cell Bank of Iran). The cells were cultured in RPMI Media 1640 (GIBCO-Invitrogen) with 15% (v/v) Fetal Bovine Serum (FBS, GIBCO-Invitrogen) and antibiotics (100 U/mL penicillin and 100 mg/mL streptomycin). The cells were maintained at 37°C in a humidified incubator containing 5% CO_2 (v/v). The medium was changed every 48 h.

MTT cytotoxicity assay

Cell viability was measured by colorimetric MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium-bromide) assay [33]. Briefly, HL-60 cells were plated in 24-well tissue culture plates at a density of 5 × 104 cells/mL. After 24 h of culture, the cells were treated with medium alone, ethanol, and different concentrations of D-α-TS, HBV (from Semnan Province in Iran) for an additional 24 hours, 48 hours, and 72 hours. At each point time, cell survival was determined by MTT assay: 100 µL MTT (5 mg/mL) was added to each well, and the cultures were incubated for an additional 4 hours at 37°C in the dark. The formazan crystals in cells were then dissolved in isopropanol 1 mL/well. Absorption was measured at 570 nm using a spectrophotometer (Milton Roy Spectronic 21D).

Morphological analysis and nitroblue tetrazolium reduction assay

To study the combined effect of HBV and D-α-TS on the induction of cell differentiation, the HL-60 cells were treated with 2.5 µg/mL of HBV and 6 µg/mL of D-α-TS. Nitro Blue Tetrazolium (NBT) reduction test and Wright-Giemsa staining showed morphological differentiation in the presence of HBV. Morphological studies of single-cell suspensions were prepared, and 2 × 105 cells were loaded into a cytofunnel and centrifuged at 27 g in a cytospin centrifuge. The slides were fixed with methanol and dried. The slides were stained with WrightGiemsa (Sigma) stain solution for 20 min and rinsed in deionized water, air dried, and observed under a microscope with a camera [34].

Monocytic differentiation of HL-60 cells was analyzed by NBT reduction. HL-60 cells (untreated and treated with HBV and of D-α-TS) were analyzed for their ability to produce superoxide radicals in response to phorbol esters (a functional parameter of mature granulocytes and macrophages). The production of superoxide radi-

cals was measured by reducing NBT to formazan. The NBT test was performed as previously described [16]. HL-60 cells were briefly pelleted by centrifugation and re-suspended to 1 x106 in cell culture media. A solution containing 0.2% (w/v) NBT and Phorbol-Myristate-Acetate (PMA) (200 ng/mL) was prepared in the same medium. Then, 100 µL of the cell suspension and 100 µL of the NBT-PMA solution were plated into 96-well µL plate wells and incubated at 37°C in CO_2 for 45 minutes. Wet cell preparations were examined microscopically and counted using a hemocytometer. A minimum of 200 cells per treatment was scored. Cells scored as positive contained black formazan deposits within their cytoplasm.

Immunocytochemistry

HL-60 cells were coated with poly l-lysine 24-well tissue culture. The cells were then treated with 6 µg/mL D-α-TS and 2.5 µg/mL HBV. Cells were washed with Phosphate-Buffered Saline (PBS) and fixed in 4% formalin at 4°C for 15 minutes. They were then blocked with 1% Bovine Serum Albumin (BSA) at room temperature for 45 minutes followed by incubation with monoclonal mouse anti-CD14 antibody (Abcam182032, diluted 1:100 in 0.2% PBST- BSA) overnight at 4°C, washed in 0.1% PBST and incubation with a Fluorescein Isothiocyanate (FITC) -conjugated goat anti-mouse Ig (Sigma 12506) diluted 1:16 in 0.2% BSA–PBST for 30 minutes at 37°C. The cells were also incubated with 1 µg/mL 4, 6-Diamidino-2-Phenylindole (DAPI) for 1 min for nuclear staining and were washed 3 times with PBS. They were then observed in the fluorescence microscope (Fargene, Iran) control cells labeled with FITC without primary antibody, were used as the negative control.

Flow cytometry

Flow cytometry detection of cell differentiation of HL-60 cells into monocytes was determined following 5 days of treatment with D-α-TS and HBV. The cells were then centrifuged (1500 rpm for 5 min) and fixed with ethanol, washed twice with PBS and 1% BSA at 4°C. CD14 monoclonal antibodies (Abcam 182032, diluted 1:100 in 0.2% PBST-BSA) were added to 100 µL cell suspension (106 of living cells/mL) in PBS containing 1% BSA and 0.1% NaN3. The cell suspension was incubated for 45 min at 4°C. Finally, 500 µL of 0.01% (v/v) formaldehyde was added to each tube, and CD14 expression was measured immediately using a Becton Dickinson FACScan analyzer.

Statistical analysis

The results are expressed as the Mean±SEM. The statistical analysis was performed using either the Independent t test or one-way ANOVA followed by post hoc comparisons. The Student's t test was employed to compare the difference between two groups and one-way ANOVA to compare more than two groups. The differences between groups were accepted to be statistically significant at $P<0.05$, $P<0.01$, and $P<0.001$. The SPSS software 22.0 was used for statistical analyses. All experiments were performed a minimum of 3 times.

Results

MTT assay of cell viability

My previous experiment demonstrated that HBV and D-α-TS induce death in HL-60 cell lines in a time and dose-dependent fashion [19]. To examine the cytotoxic effects of HBV and D-α-TS alone and synergetic effects (HBV/VES) on human HL-60 cancer cell line, cells were cultured with different concentrations of HBV and D-α-TS for 24, 48, and 72 h. Cell viability was determined by MTT assay. As shown in Figure 1, they both inhibit the proliferation of HL-60 cells in a time and dose-dependent manner. The highest inhibition of cell proliferation was observed at a concentration of 2.5 µg/mL of HBV and 6 µg/mL D-α-TS for 72 hours, so that this increase in the rate of inhibition of proliferation in the presence of bee venom, D-α-TS at 72 hours after treatment were significant compared to the control sample at the level of $P<0.001$.

Viability was determined via MTT assay. The cells were exposed to medium alone as a control, and 2.5 µg/mL of honey bee venom and 6 µg/mL D-α-TS for 24, 48, and 72 hours. The results are expressed as means±SEM of data obtained in three independent experiments. The data are statistically significant in comparison to the vehicle control by one-way ANOVA.

Morphological analysis and nitro blue tetrazolium reduction assay

To study the combined effect of HBV and D-α-TS on the induction of cell differentiation, the HL-60 cells were treated with 2.5 µg/mL of HBV and 6 µg/mL of D-α-TS. Wright-Giemsa staining showed morphological differentiation in the presence of HBV. The results of the morphological analysis showed that the number of differentiated cells in the presence of bee venom and D-α-TS 5 days after treatment increased significantly compared to the control sample, which was significant (Figure 2).

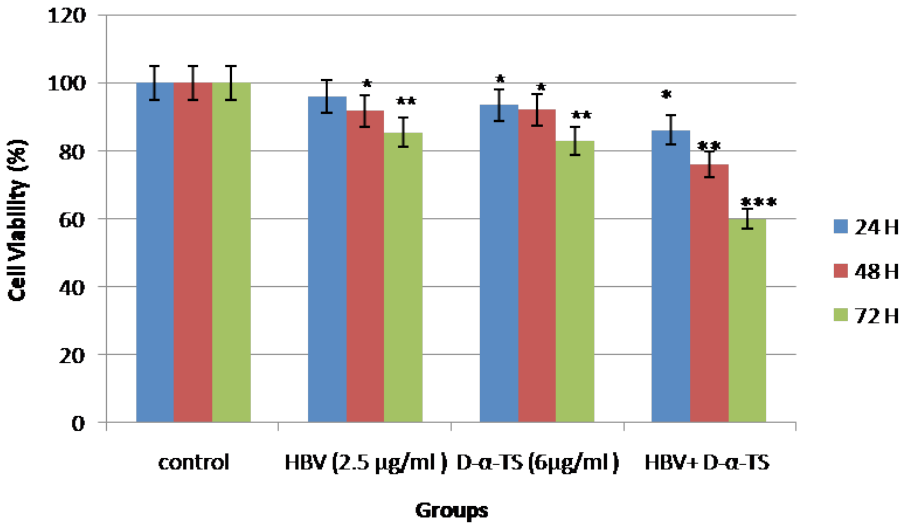

Figure 1. Time and dose-dependent effect of honey bee venom and D-alpha-tocopherol succinate HL-60 cells
*P<0.05, **P<0.01, and ***P<0.001

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Results of the synergistic effect of HBV and D-α-TS on the differentiation of HL-60 cells based on nitro blue tetrazolium assay

Differentiation of cells into neutrophils and monocytes is associated with increased production of hydrogen peroxide. Under these conditions, the cell can reduce NBT and form formazan crystals. NBT is a yellow salt that is deoxidized by phagocytic cells such as neutrophils and monocytes due to the production of the enzyme peroxidase and becomes a dark-colored substance. This dark substance is visible inside the differentiated cells. Therefore, in this study, the NBT reduction test was used to indicate cell differentiation into monocytes. The results of the NBT assay showed that the number of NBT + cells in the presence of bee venom and D-α-TS at 5 days after treatment increased significantly compared to the control sample, which was significant at P<0.001 (Figure 3).

Immunocytochemistry

İn this study, immunocytochemistry showed that HBV increases the expression of CD14 in HL-60 cells treated with D-α-TS. The negative control group of cells was incubated with a secondary antibody. Due to the absence of primary antibody, the link between the primary and secondary antibodies cannot be seen. Immunocytochemical data in this study showed that a concentration of 2.5 µg/mL HBV increased the expression of CD14 protein in cells treated with 6 µg/mL D-α-TS compared to cells treated with 6 µg D-α-TS alone (Figure 4).

Flow cytometry

In this study, in addition to a qualitative evaluation of immunocytochemistry, changes in the expression of CD14 protein levels in differentiated cells were quantitatively estimated by flow cytometry. In this study, HL-60 cells were incubated for 5 days with a combined concentration of 2.5 µg/mL HBV and 6 µg/mL D-α-TS, and then changes in CD14 expression was compared to the control sample by the specific antibody of this protein via flow cytometry. The results showed that CD14 expression in the presence of HBV, D-α-TS at 5 days after treatment increased significantly compared to the control sample, which was significant at P<0.001 (Figure 5).

The results revealed that the number of CD14-expression cells increased significantly compared to other groups, which was significant at P<0.001. In the chart, the population of cells located on the bottom left did not express CD14, and the population of cells located on the bottom right expressed CD14 protein.

Discussion

Although there are different therapies to erase the pernicious cancer cells, chemotherapy is considered as the conventional therapy [11, 12, 16, 23]. Chemotherapeutic agents mainly affect rapidly dividing cells such as cancerous cells. They do not, however, differentiate between cancer cells and healthy cells, which physiologically have a high turnover, such as cells in the bone marrow, lung, and ovary. So differentiation therapy has become the prevalent method of treating cancer [9, 15, 17, 24].

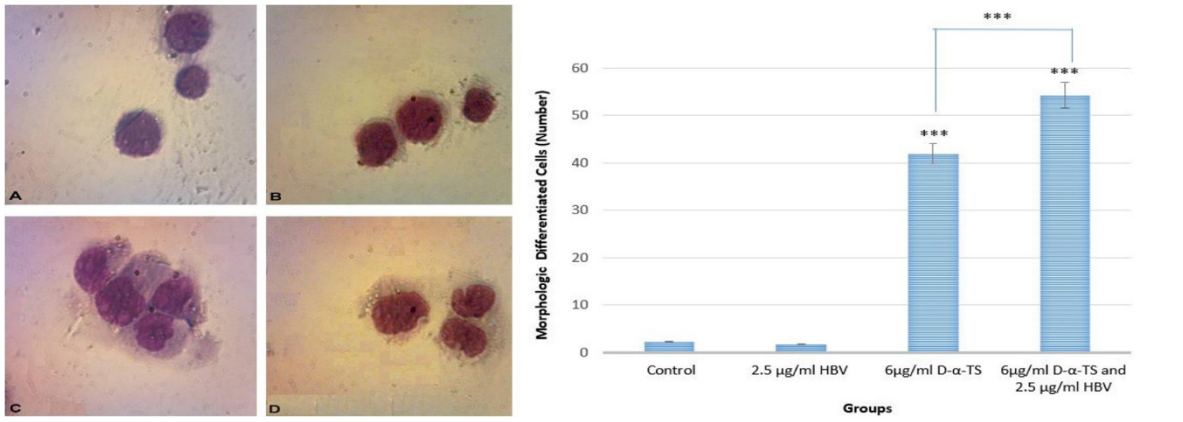

Figure 2. A: HL-60 cell (control), B: HL-60 cells treated with 2.5 µg/mL bee venom, C: cells treated with 6 µg/mL D-alpha-tocopherol succinate, D: cells treated with 6 µg/mL D-alpha-tocopherol succinate and 2.5 µg/mL honey bee venom for 5 days (Wright-Giemsa staining) x100.

The diagram shows the number of differentiation cells in the presence of 2.5 g/mL honey bee venom, 6 µg/ml D-alpha-tocopherol succinate and both simultaneously after 5 days, Cell count with light microscope, (n=100),

***P <0.001, **P <0.01, *P<0.05

Differentiation therapy is used successfully in patients suffering from acute promyelocytic leukemia [11, 12, 25]. The objective of this study was the evaluation of CD14 expression by Honey Bee Venom (HBV) in HL-60 cells treated by D-α-TS. Several studies have shown that certain nutritional supplements such as vitamins A, C, and E can inhibit cell proliferation and differentiation in cancer cells and therefore express the surface markers of specific adult cells such as CD14 and CD11 [1, 18, 20, 26]. It has also been found that the level of vitamin E in patients with leukemia is lower than that in healthy people [6, 8]. The HL-60 cell line belongs to acute promyelocytic cancer. These cells do not express the specific differentiation markers such as CD14 [2].

If differentiation in HL-60 cancer cells is induced, they express cell surface receptors associated with differentiated lineages, morphology, and enzyme activity; therefore, cellular surface antigen differentiation cells are obtained [1]. It has been demonstrated that D-α-TS can inhibit proliferation and induce differentiation in HL-60 cell class in monocyte cells [4, 6, 18].

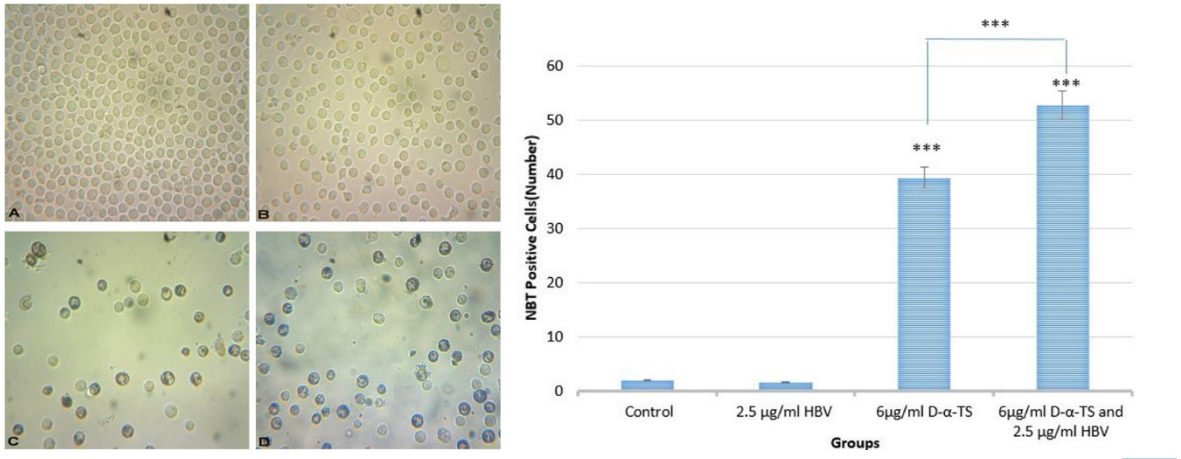

Figure 3. Effect of D-alpha-Tocopherol Succinate (D-α-TS) and Honey Bee Venom (HBV) on Nitro Blue Tetrazolium (NBT) reduction

A: Control cells (NBT negative); B: Cells treated with HBV (NBT negative); C: Cells treated with 6 µg/mL D-α-TS (NBT positive), D: Cells treated with 6 µg/mL D-α-TS and 2.5 µg/mL HBV (NBT positive) for 5 days. The diagram shows the number of differentiated cells NBT positive in the presence of 2.5 g/mL HBV, 6 µg/mL D-α-TS separately and both simultaneously after 5 days, Cell count with light microscope, (n=100), ***P<0.001, **P<0.01, *P<0.05

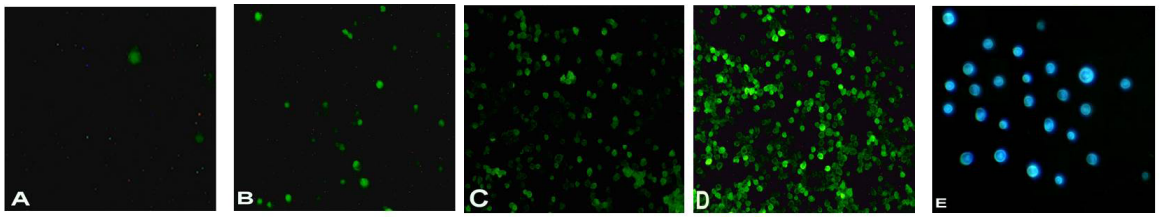

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Figure 4. Immunocytochemistry to qualitatively evaluate

A: The negative control group of cells that have been incubated with secondary antibody; B: The positive control group of cells that are untreated, but have received primary and secondary antibodies; C: Cells treated with 6 µg/mL D-alpha-tocopheryl succinate; D: Cells treated with 6 µg/mL D-alpha-tocopheryl succinate and 2.5 µg/mL bee venom for 5 days; E: DAPI staining shows the position of the nucleus in control groups.

However, the concentration of D-α-TS capabilities to inhibit the proliferation and induce differentiation, while reducing the side effects, is presented as a solution. HBV among these materials is noted.

We stipulate that HBV is a factor that inhibits cell proliferation. Its anti-proliferative and pro-apoptotic effects have been proved on different cancer cell classes. HBV can induce apoptosis, necrosis, and cell lysis in cancer cells. It is also proposed that the melittin available in HBV can cause death in cancer cells by activating phospholipase [26, 27]. It is also suggested that the melittin and phospholipase A2 available in the HBV can inhibit kappa nuclear factor and cyclooxygenase 2 mRNA [28, 29]. Also, Moon et al. Found that HBV can decrease the expression of anti-apoptosis proteins (Bcl2 and Bcl-X) and increase p21 protein expression in the U-937cell class [28, 29, 30].

In 2014, Wang et al. investigated the role of p21 protein to induce differentiation in HL-60 cells to monocytes by D-α-TS. They suggested that the increased expression of p21 protein plays a vital role in this process [17]. The role of p21 and p27 proteins in the regulation of differentiation and apoptosis and the p21 protein expression increase in differentiation of leukemia cells has already been established. Also, because of the similarity in molecular pathways involved in the inhibition of cell proliferation and apoptosis by HBV and D-α-TS, it can be stated that toxic and inhibitory doses of HBV can increase the proliferative inhibitory effect and differentiation effect of D-α-TS on HL-60 cancer cells by inhibiting the activity of kappa beta factor, decreased expression of cyclooxygenase 2 and Bcl2, and increased expression of p21 protein [6, 17, 21, 24].

The differentiation of blood cancer cells, including cells trapped in the G0/G1 phase of the cell cycle, is related to an increase in p21 and p27 proteins (an essential factor in the differentiation of monocytes and consequently the expression of CD14). According to signaling pathways (message transfer) involved in the D-α-TS effect on proliferation and differentiation of HL-60 cells and the known mechanisms in the HBV, it seems that

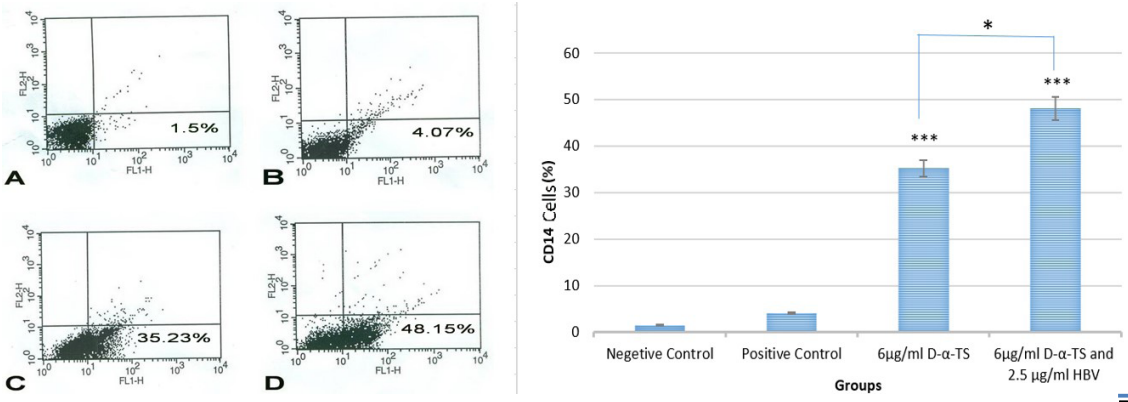

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Figure 5. Flow cytometry analysis

A: The negative control group of cells that have been incubated with secondary antibody; B: The positive control group of cells that are untreated, but have received primary and secondary antibodies; C: Cells treated with 6 µg/mL D-α-TS; D: Cells treated with 6 µg/mL D-α-TS and 2.5 µg/mL bee venom for 5 days.

the HBV effect overlaps with D-α-TS function in several mechanisms [31, 32].

HBV can increase the differentiation potency of D-α-TS by inhibiting the activity of NF-κB and COX-2 and increasing the expression of p21 [32, 35-38]. It can also increase the differentiation potency of D-α-TS on HL-60 cells to monocytes and increase CD14 protein expression. The results of this study confirmed the inhibitory effect of HBV and D-α-TS on the proliferation of HL-60 cell lines and increased D-α-TS differentiation potency, increasing CD14 expression. Comparing the synergistic impact of HBV and D-α-TS with the individual effect of these compounds showed that HBV could increase the expression of CD14 monocyte markers. Increased proliferation, inhibition, and differentiation induction, and generally the molecular mechanisms of monocyte differentiation, were seen.

The effect of increasing CD14 expression of D-α-TS by HBV on HL-60 cancer cells has not been examined yet, and it was evaluated in this study for the first time. It should be noted that the results were obtained via in vitro examinations, and the need for further research is emphasized. A CLuster of Differentiation 14 (CD14) exists in membrane surface and plasma soluble form proteins. The first form is linked to phospholipid bilayer membrane via glycosylphosphatidylinositol anchored protein [1]. This molecule is expressed on the intrinsic surface of monocytes and neutrophils [2, 3]. Today, the CD14 antigen is used as one of the singular markers to identify monocyte cells. This protein is not expressed on the HL-60 cell surface. It is believed that these cells have been stopped in the promyelocytic stage of differentiation. But the compounds such as vitamins A, D, and E will further differentiate these cells into monocytes and neutrophils and the expression of membrane surface proteins (CD14) [4-6]. Recently, the effect of vitamin E on inhibiting proliferation and inducing apoptosis in cancer cells has been proved [18, 19]. D-α-TS is the most abundant and prevalent form of vitamin E in nature. D-α-TS is a synthetic derivative of vitamin E [20]. The necessary high concentrations of this compound to inhibit proliferation and induce differentiation and subsequent expression of differentiation markers (such as CD14, CD11b) have shown harmful effects [21, 22].

Conclusion

In this study, we examined the synergistic effects of HBV and low concentration of D-α-TS on the amount of CD14 protein expression. The effect of increasing CD14 expression of D-α-TS by HBV on HL-60 cancer cells has not been examined yet, and it was evaluated in this study for the first time. HBV can increase the differentiation potency of D-α-TS by inhibiting NF-κB and COX-2 and increasing the expression of p21 [32-37, 39]. It can also increase the differentiation potency of D-α-TS on HL-60 cells to monocytes and increase CD14 protein expression. It should be noted that the results were obtained via in vitro examinations, and the need for further research is emphasized.

Ethical Considerations

Compliance with ethical guidelines

There were no ethical considerations to be considered in this research.

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Authors' contributions

All authors equally contributed to preparing this article.

Conflict of interest

The authors declared no conflict of interest in this study.

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