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Effect of Cassiopea andromeda Venom on P15INK4b, P21 WAF1/CIP1, P53, DNA methyltransferase 1, and Bcl-2 Genes Expression, Apoptosis Induction, and Cell Growth Inhibition in Acute Promyelocytic Leukemia NB4 Cell line

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Abstract

Background: One of the acute hematologic malignancies is acute promyelocytic leukemia (APL) that resulted in translocation of chromosomes 15 and 17, t (15; 17), and cessation in the maturation of myeloid cell line, and ultimate aggregation of neoplastic promyelocytes. Regarding that appetence of using herbal and marine medicine studies is increasing, and on the other hand, the features of Cassiopea andromeda Venom remained unclear; this study was conducted to determine its effects on NB4 cells as a model for APL.

Materials and Methods: In this experimental study, the cells were treated with C. andromeda Venom concentrations at different periods and times. Growth inhibition and toxic effects of C. andromeda Venom were evaluated through methyl thiazole tetrazolium salt reduction (MTT test). The flow cytometry analysis was carried out using 7AAD and Annexin V stains for evaluating this venom's effect on apoptotic pathways. Besides, Real-Time polymerase chain reaction was performed to evaluate the relative gene expression.

Results: C. andromeda Venom inhibited the growth of NB4 cells as correlated with concentration and time. Cell growth was inhibited by 49.1%, after 24 hours of treating NB4 cells with 1000µg/mL C. andromeda Venom. This venom increased the apoptotic process, which was then verified by 7AAD/AnnexinV staining. The fold change of p15INK4b, p21 WAF1/CIP1, P53, DNMT1, and Bcl-2 genes in the NB4 cell line were 144, 2.78, 1.75, 15.24, and 0.33, respectively, which meant that the expression level of p15INK4b, p21 WAF1/CIP1, P53, and DNMT1 were increased by 14400%, 278%, 175%, and 1524%, respectively and the expression of Bcl-2 was decreased by 67%.

Conclusion: Considering the inhibitory property of C. andromeda Venom, the authors recommended it as a part of combinational medication for treating APL in animal trials and for other leukemias' in vitro studies.

Keywords: Acute Leukemia, Apoptosis, Cassiopea venom, Epigenesis, Venom

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Introduction

Acute leukemias are characterized by a defect in maturation, leading to an imbalance between proliferation and maturation; since cells of the leukemic clone continue to proliferate without maturing to end cells and dying, there is a continued expansion of the leukemic clone, and immature cells predominate. Acute promyelocytic leukemia (APL) resulted from the translocation of chromosomes 15 and 17, t (15; 17). This disorder leads to the fusion of *promyelocytic leukemia* (PML) and *Retinoic Acid Receptor Alpha* (RARα) genes (1). This type of leukemia is known as clinical features such as the quick onset of symptoms and weak response to chemotherapy (2). In this leukemia, disseminated intravascular coagulation disorder is a major phenomenon that demonstrates decreased fibrinogen and thrombocytopenia. The another common prodrome is pallor, fatigue, and mild bleeding (3). Treatment of APL is an emergency medical action that needs to be times of admission, accurate, and

supportive therapy for decreased bleeding and death rate (3). According to the treatment protocols for cancer, administering chemotherapy and radiotherapy is considered the first medication (۲) . However, these medications have specific adverse effects. On the other hand, the natural and marine supply of organisms provides a chance for some researchers to recruit them as a possible cancer treatment (4). Progression in cell cycle phases (G1, S, G2, M) is controlled by a family of serine/threonine protein kinases. These heterodimer kinases consist of two catalytic subunits, the cyclin-dependent protein kinase (CDK) and the regulatory subunit, cyclin, which is responsible for CDK activation. So far, 21 CDK genes and 29 cyclin genes have been encoded in the human genome. The p15INK4b gene, also known as MTS2 and INK4b, is a representative of tumor suppressor genes with a structure and function similar to P16. Both p15INK4b and P16 proteins specifically inhibit CDK4 and CDK6 activity, which are key regulators of eukaryotic cell cycle progression in the G1 phase. The p21 $^{WAF1/CIP1}$ gene is another gene that inhibits the activity of CDKs, known as P21 $^{Waf/cip1}$ or P21/CDKN1A. p21 $^{WAF1/CIP1}$ is well known as a cell cycle inhibitor and can inhibit the cell cycle progression during the G1/S and G2/M transitions by inhibiting CDK4,6/Cyclin-D and CDK2/Cyclin-E activity, respectively. Another regulator of the cell cycle is the P53 gene. Tumor suppressor P53 exerts its biological activity by regulating the transcription of many genes involved in the cell cycle arrest, apoptosis, DNA repair, aging, and metabolism, such as transcription factors (5). Bcl-2 (B Cell Lymphoma 2) family proteins are the key to regulating apoptosis, including pro-apoptotic and anti-apoptotic proteins. The role of the Bcl-2 protein, the most well-known member of the Bcl-2 family of apoptosis-regulating proteins, was elucidated by inactivation of the apoptotic pathway and tumor spread (6).

DNA methylation is one of the major epigenetic changes in the mammalian genome that is constructed and maintained by DNA methyltransferase (DNM). DNA methylation involves the addition of the methyl group to the cytosine base in CpG dinucleotide-rich regions at the genome known as CpG islands. The family of DNMTs includes DNMT1, DNMT3A, DNMT3B, DNMT3L, and DNMT2, of which DNMT1 is mainly used as a protector of methyltransferase in every cell division (7). Researchers have found that the marine ecosystem has been identified as an unfinished bioactive source with extensive biological and pharmacological features; therefore, identifying and introducing new compounds with selective toxicity is advantageous. Because of chemical complexity and biological diversity, marine-derived compounds are major candidates for discovering new therapeutic agents (8). C. Andromeda that called “upside-down jellyfish,” is another member of the Cnidaria family (9), that was native to the eastern Mediterranean sea, but due to some anthropogenic effects, entered the coastal waters of the Persian Gulf in the Nayband lagoon from Bushehr-Iran in 2014 (10). Cnidarians are the primary phylum of typically toxic animals. Their main feature is the presence of nematocytes (Cnidocytes), which are specialized organs for the production and secretion of venom and are located in the tentacles of these organisms (11). Studies have shown that C. Andromeda venom is effective in suppressing the breast adenocarcinoma (12), and its raw venom at a dose of 0.21 mg/kg of protein caused death in mice, and 51 µg of protein after injection into the skin of mice, caused vascular permeability and skin necrosis. Also, its raw venom has been shown to have phospholipase A2 activity and induce lysis of mouse lymphocytes. Dosages of 1 µg of protein lysed 51% of human erythrocytes (13). C. Andromeda produces a very strong and rapid venom that is very harmful to humans and deadly to its victims (10). In addition, this venom

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can cause creation abnormalities in the liver, heart, kidney, and spleen tissue in the rat after injection (14). Since no study has evaluated the effect of C. Andromeda venom fraction on leukemia, the present study aimed to evaluate C. Andromeda venom fraction's effect on the viability, apoptosis, and necrosis of cell lineage of APL.

Materials and Methods

Materials

The acute promyelocytic leukemia cell line was purchased from the National Cell Bank of Iran-Pasteur Institute. RPMI-1640 with L-Glutamin was obtained from CBSA company (IRAN). The cells were maintained in RPMI supplemented with 10% fetal bovine serum (FBS) and antibiotics in a humidified atmosphere of 5% CO2 in air at 37°C. Separation of tentacles was performed according to Bloom et al. (1998) method (12). Briefly, the tentacles were excised manually from specimens, immediately after capturing by trawl, and directly placed into small glass containers filled with the third part of seawater and then transported in the ice bags to the Persian Gulf Marine Biotechnology Research Center laboratory from the Persian Gulf Biomedical Research Center, Bushehr University of Medical Sciences, Iran. Subsequently, after homogenization (IKA homogenizer, Germany), autolysis occurred after keeping containers at 4°C for two consequent days, then the toxins of tissues were released and then centrifuged (Eppendorf, Germany) at 12,000×g for 15 minutes in 4°C to remove the sediments. The resultant supernatant was freeze-dried (Christ, UK) and kept at −80°C until further analysis (15). This work was approved by the ethics committee of Bushehr University of Medical Science with a code number of IR.BPUMS.REC1397.042.

Cell culture and cell viability

Acute promyelocytic leukemia and PBMs cell lines were cultured in RPMI supplemented with 10% FBS and antibiotics at 37°C in 5% CO2. After survival reached more than 90%, cells were counted by Neubauer slide and then transferred to a 96-well plate (2× 10^4 cells per well). After 24 hours, the medium was replaced with an experimental medium containing various concentrations of C. Andromeda venom (31, 62, 125, 250,500, and 1000 µg/mL prepared in the culture medium). The control groups received RPMI only. After a period of 24, 48, and 72 hours, the cells were investigated by MTT assay according to Standard protocols to determine cell viability. A cell-free culture medium with the mentioned concentrations of venom was used as blank control. Therefore, MTT solution was added to each well for 4 hours at 37°C, and then the MTT solution was changed by DMSO and shaken for 10 minutes to dissolve all of the crystals. Finally, the optical density was detected by a microplate reader at a wavelength of 570 nM and 690 nM, for cells and blank control wells, respectively. Each experiment was repeated three times (triplicates). The percentage of cytotoxicity was calculated by the following equation:

$$\text{Cytotoxicity} = [(OD_{Test}-OD_{Blank}) - (OD_{Control}) \times 100]/ (OD_{Control})$$

Cell apoptosis assay

To determine NB4 apoptotic and necrotic cells, the cells were cultured (2×10^6 cells/well) and incubated overnight and then treated with C. Andromeda venom (1000 µg/mL) for 24 h. Subsequently, After incubation, cell suspension of each good transfer to the microtube and centrifuge at 600g for 5 minutes, and the supernatant was removed at last. Washed the cells by PBS (Invitrogen, Thermo Fisher Scientific), and finally, Annexin-V-(FITC) and 7AAD (Biolegend, USA) were used to determine the apoptotic cells by FACSCalibur flow cytometry (Becton Dickinson, Heidelberg, Germany).

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

To determine the relative expression level of p15INK4b, p21 $^{WAF1/CIP1}$, p53, DNMT1,

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and Bcl-2 genes, qRT-PCR was done. The NB4 was treated with C. Andromeda venom (1000 µg/mL) for 24 h, and then qRT-PCR was done. The primer sequences are indicated in Table I.

Statistical analysis

Descriptive data were reported in frequency, percent, mean, and standard deviation. Repeated measures ANOVA test was used to compare the mean of groups at three-time points, and one-way ANOVA was used to compare the means between the groups. Significance level was considered less than 0.05, and IBM SPSS v.19 and Graph Pad Prism 8.4.3 were used for data analysis and charting, respectively.

Results

Inhibiting proliferation and inducing cytotoxicity

The proliferative effect of C. andromeda Venom on the PBLs and NB4 cells line was examined. C. andromeda Venom-induced cell death in a concentration and time-dependent manner by MTT assay in the NB4 cell line. Studies between 24, 48, and 72 hours have shown that in NB4 cell line and normal lymphocytes, the difference between mean survival rates and dilution is an influential factor in the difference between time shifts ($P < 0.001$). After treatment of the cells in different periods and different dilutions, it was found that in normal lymphocytes, the difference in survival between different dilutions in the time interval of 48 and 72 was significant, and in the time interval of 24 hours, this difference was not significant. In the NB4 cell line, the difference in survival between different dilutions in the time interval of 24 and 48 hours was not significant and in 72 hours was significant (Figure 1). Normal lymphocyte cell count was able to regenerate 100% of their killed cells three times during the three encounters with venom than the survival of the first treatment (24 hours). However, the NB4 cell line was able to regenerate 28% of its killed cells during the first three encounters of venom compared to the survival of the first treatment (24 hours). According to studies, it was found that the amount of IC50 for NB4 cells is 1000µg/mLin 24 hours (Figure 2).

Measurement of cell apoptosis by flow cytometry

After treating NB4 cells with a concentration of 1000µg/mL of the C. andromeda Venom for 24 hours, the results were considered apoptosis or necrosis. By comparing the two groups of control and treated NB4 cells, it turned out that 90.2% of control cells were AnnexinV(neg)/7AAD(neg) and 45.4% of treated cells were AnnexinV(pos)/7AAD(pos), Which indicated the apoptotic effect of C. Andromeda Venom in NB4 cells (Figure 3).

Gene expression analysis by real-time PCR technique

The melting curve of real-time PCR revealed that nonspecific products were not produced during PCR. In this study, the CTs of each gene were normalized to HPRT genes. Quantitative real-Time PCR results were calculated using the gene expression formula ($2^{-\Delta\Delta CT}$), considering that the fold change of p15INK4b, p21 $^{WAF1/CIP1}$, P53, DNMT1, and Bcl-2 genes in the NB4 cell line were 144, 2.78, 1.75, 15.24, and 0.33 respectively, which meant that the expression level of p15INK4b, p21 $^{WAF1/CIP1}$, P53, and DNMT1 were increased 14400%, 278%, 175%, and 1524% respectively and the expression of Bcl-2 was decreased 67% (Figure 4).

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Table I: The primers sequences of p15INK4b, p21 $^{WAF1/CIP1}$, p53, DNA methyltransferase 1, and Bcl-2 Genes.

Primers	Primer sequences (5' to 3')	Product length
p53		**464**
Reverse	5'-AGGACAGGCACAAACACGCACC-3'	
Forward	5'-TAACAGTTCCTGCATGGGCGGC-3'	
p21 $^{WAF1/CIP1}$		**1425**
Reverse	5'-CAGCCGGCGTTTGGAGTGGTAGAA-3'	
Forward	5'-GAGGCCGGGATGAGTTGGGAGGAG-3'	
p15INK4b		**82**
Reverse	5'-GAGCAAAGGCCAGCATCCT-3'	
Forward	5'-GATGTGCAAGCGACGACAGA-3'	
BCL-2		**89**
Reverse	5'-CGGTTCAGGTACTCAGTCATCC-3'	
Forward	5'-GGTGGGGTCATGTGTGTGG-3'	
DNMT1		**1791**
Reverse	5'-CCTAGCCCCAGGATTACAAGG-3	
Forward	5'-ACTCATCCGATTTGGCTCTTTC-3'	
HGPRT		**88**
Reverse	5'-ATAGCCCCCCTTGAGCACAC-3	
Forward	**5'-GGACAGGACTGAACGTCTTG-3'**	

HGPRT, Hypoxanthine-guanine phosphoribosyltransferase; BCL-2, B-cell lymphoma2; DNMT, DNA Methyl Transferase

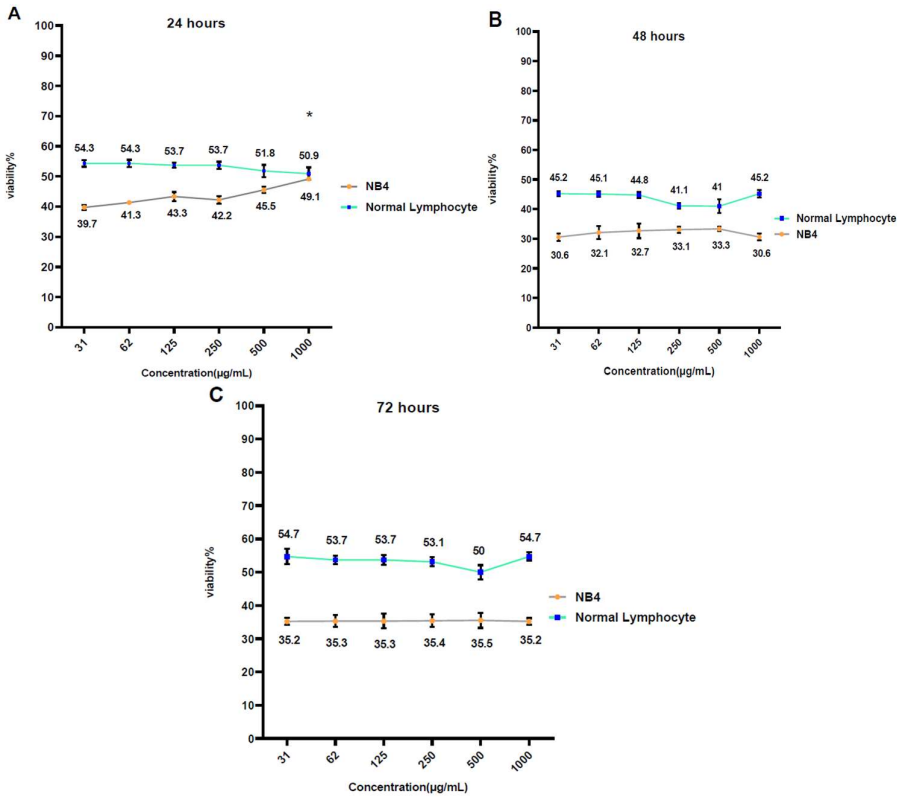

Figure 1. A) Comparison of NB4 cell survival and normal lymphocytes at different dilutions in 24 hours. The difference in survival rate between the different dilutions of cellular NB4 (P <0.001) was significant and normal lymphocytes (P = 0.071) were not significant. B) Comparison of NB4 cell survival and normal lymphocytes at different dilutions in 48h. The difference in survival rate between different dilutions of cell NB4 (P = 0. 204) was not significant and was significant in normal lymphocytes (P = 0.004). C) Comparison of NB4 cell survival and normal lymphocytes at different dilutions in 72 hours. The difference in survival rate between the different dilutions of cell NB4 (P = 0.999) was not significant and normal lymphocytes (P = 0.049) were significant.

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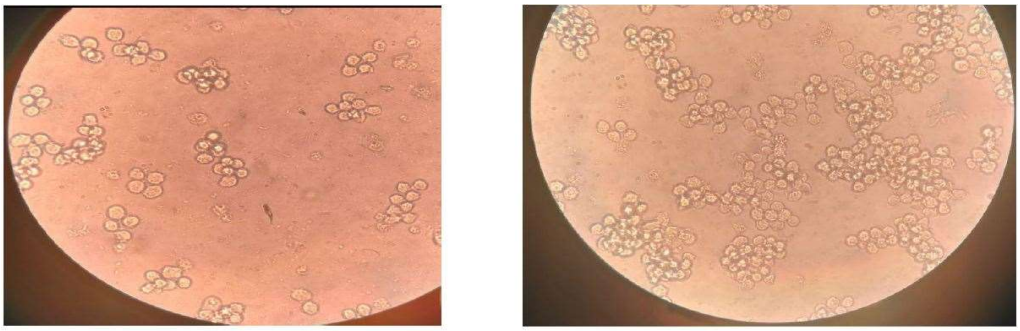
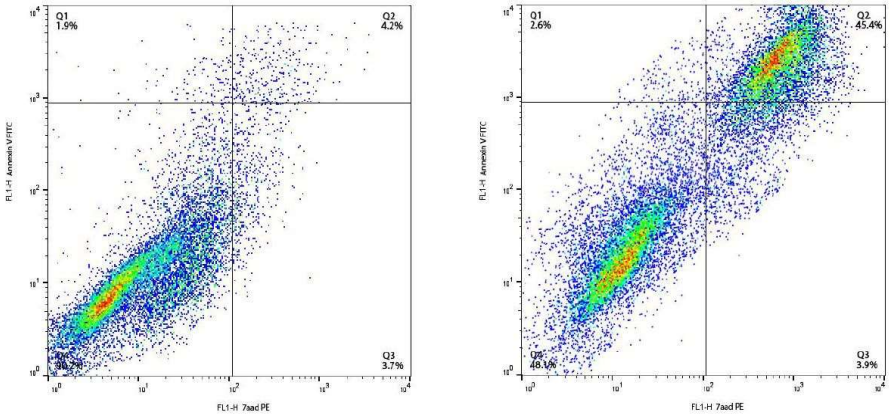


B A

Figure 2. Comparison of the effect of IC50 dilution with Cassiopea andromeda Venom in NB4 cells. (×40). A: NB4 control cell, B: NB4 cell after 24 hours of treatment with 1000 µg/mL dilution.



A B

Figure 3. The apoptotic effect of Cassiopea andromeda Venom (1000 µg/mL) on NB4 cell versus control group after 24 hours that AnnexinV (neg)/7AAD(neg), AnnexinV(pos)/7AAD(neg), nnexinV(neg)/7AAD(pos), and AnnexinV(pos)/7AAD(pos), were considered as viable, early apoptotic, necrotic and late apoptotic, respectively. A) NB4 control. B) NB4 treated.

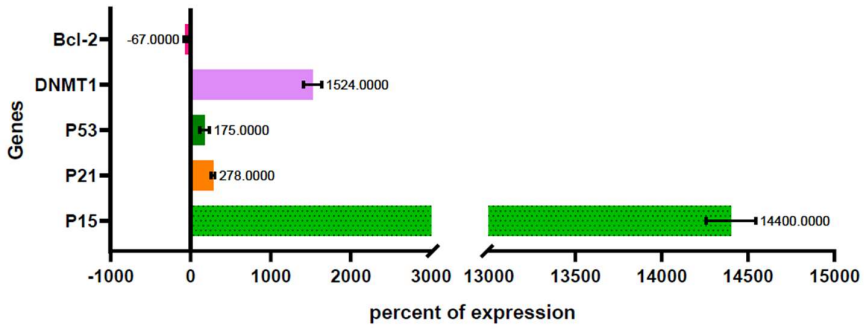


Figure. 4. Gene expression fold change in NB4 cells

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Discussion

Aberrant DNA methylation of the promoter region and mutations in apoptosis and the cell cycle control points are the most agents in numerous cancers (16). Agents and drugs that increase the expression of p15INK4b, p21 $^{WAF1/CIP1}$, p53 genes and, decrease the expression of Bcl-2 and DNMT1 genes can prevent the progression of cancers (7, 17-21). Although cytotoxicity of jellyfish venom has been so far reported in different kinds of tumoral cells(12), the cytotoxic mechanism of jellyfish venom is unknown but mitochondrial malfunction and oxidative stress have been suggested as a major mechanism for the cell damage caused by jellyfish venom in few published works(22, 23). In the present study, it was shown that C. Andromeda venom inhibited cell growth and induced apoptosis in the NB4 cell line. Genes expression assessment was performed to evaluate the mechanisms of apoptosis and cell growth after C. Andromeda venom treatment. It was found that C. Andromeda venom increases p15INK4b, p21 $^{WAF1/CIP1}$, p53 and, DNMT1 gene expression and decreases Bcl-2 gene expression in the NB4 cell line. In line with the present report, it has been demonstrated that C. Andromeda venom-induced cytochrome c release in mitochondria obtained from breast adenocarcinoma patients(12). It has already been well established that as the p53 increased, the cell underwent apoptosis, for this reason, many treatments seek to increase p53 expression (24). If p53 and p21 $^{WAF1/CIP1}$ increase their expression at the same time, the cell cycle arrests (19). But if p53 or p21 $^{WAF1/CIP1}$ expression predominates, apoptosis or cell cycle arrest occurs, respectively (19, 25). In this study, C.Andromeda venom increased the simultaneous expression of these two genes, indicating inhibition of the NB4 cell proliferation. Previous studies have shown a direct relationship between p15INK4b hypermethylation or reduced expression with malignancies (5). Besides, if there is an increase in p15INK4b expression, a better prognosis and greater effect of chemotherapy are indicated (26). It was found that C. Andromeda venom caused a sharp increase in p15INK4b expression and it was possible that by inhibiting the CDK4/6 (cyclin-dependent kinase 4/6), the NB4 cells have arrested in the G1-phase of the cell cycle. Furthermore, other researchers have reported that Bcl-2 has increased expression in most cancers and by reducing Bcl-2 expression, the prognosis was improved (27). Bcl-2 is an anti-apoptotic protein. In addition to all the above, C. Andromeda venom can lead to cellular apoptosis by reducing Bcl-2 gene expression. On the other hand, in line with the increased expression of DNMT1, it is possible that the Bcl-2 gene expression is reduced by hypermethylation by C. Andromeda venom, or this venom independently increases DNMT1 expression and decreases Bcl-2 expression. In this case, and considering the increased expression of DNMT1 gene in other cancers (21), further studies are needed on the effect of C. Andromeda venom on the DNMTs family.

It should be noted that this study is the first report of the evaluation of the effect of C. Andromeda venom on p15INK4b, p21 $^{WAF1/CIP1}$, p53, Bcl-2 and DNA methyltransferase 1 gene expression, apoptosis, and cell growth inhibition induction in APL NB4 cell line. Therefore, it is the novelty of the current work.

Conclusion

In conclusion and based on the reasons given above, in the NB4 cell line, after treatment of cells with C. Andromeda venom, increased apoptosis (flow cytometry results) and concomitant expression of p15INK4b, p21 $^{WAF1/CIP1}$, P53, and DNMT1 proteins and decreased Bcl-2 expression were observed. So it can be concluded that C. Andromeda venom in NB4 cell line or APL by stopping the cell cycle and controlling its uncontrolled proliferation reduced the number of cells

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and can be considered as a new treatment against this disease.

In this regard, It is recommended that in further studies, the synergistic properties of chemotherapeutic drugs with C. Andromeda venom be investigated on cell lines of different leukemias.

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Conflict of interest

The authors declared no conflict of interest.

References

1. Sabbaghi M, Kazemi A, Hassani S, Alimoghaddam K, Ghavamzadeh A, Ghaffari SH. Evaluation Of BIBR1532 And ATRA Combination In NB4 Cell Line. Payavard Salamat 2014;8(4):279-297.
2. Assadollahi V, Parivar K, Roudbari NH, Khalatbary AR, Motamedi M, Ezatpour B, et al. The effect of aqueous cinnamon extract on the apoptotic process in acute myeloid leukemia HL-60 cells. Adv Biomed Res 2013;2:1-26.
3. Walker DK, Held-Warmkessel J. Acute promyelocytic leukemia: an overview with implications for oncology nurses. Clin J Oncol Nurs 2010;14(6):747-759.
4. Wang E, Sorolla MA, Krishnan PDG, Sorolla A. From Seabed to Bedside: A Review on Promising Marine Anticancer Compounds. Biomolecules 2020;10(2):1-43.
5. Lim S, Kaldis P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. Development 2013;140(15):3079-3093.
6. Chipuk JE, Moldoveanu T, Llambi F, Parsons MJ, Green DR. The BCL-2 family reunion. Mol Cell 2010;37(3):299-310.
7. Singh V, Sharma P, Capalash N. DNA methyltransferase-1 inhibitors as epigenetic therapy for cancer. Curr Cancer Drug Targets 2013;13(4):379-399.
8. Leone A, Lecci RM, Durante M, Piraino S. Extract from the zooxanthellate jellyfish Cotylorhiza tuberculata modulates gap junction intercellular communication in human cell cultures. Mar Drugs 2013;11(5):1728-1762.
9. Ramires M, Rotundo MM, Begossi A. The use of fish in Ilhabela (São Paulo/Brazil): preferences, food taboos and medicinal indications. Biota Neotropica 2012;12(1):21-29.
10. Nabipour I, Moradi M, Mohebbi G. A first record on population of the alien venomous jellyfish, Cassiopea andromeda (Forsskål, 1775)(Cnidaria: scyphozoa: Rhizostomea) in the Nayband Lagoon from Bushehr-Iran (Persian Gulf). J Chem Pharm Res 2015;7(3):1710-1713.
11. Taheri N, Mohebbi G, Vazirizadeh A, Nabipour I. Clinical manifestations and managements in jellyfish envenomation A systematic review. ISMJ 2013;16(5):338-358.
12. Mirshamsi MR, Omranipour R, Vazirizadeh A, Fakhri A, Zangeneh F, Mohebbi GH, et al. Persian Gulf Jellyfish (Cassiopea andromeda) venom fractions induce selective injury and cytochrome c release in mitochondria obtained from breast adenocarcinoma patients. Asian Pac J Cancer Prev 2017; 18(1): 277–286.
13. Mariottini GL, Pane L. Mediterranean jellyfish venoms: a review on scyphomedusae. Mar Drugs 2010;8(4):1122-1152.
14. Mohebbi G, Farzadinia P, Vatanpour H, Hashemi A, Vazirizadeh A, Akbarzadeh S, et al. Sub-acute toxicity of the alien Cassiopea andromeda (forsskal, 1775) jellyfish venom, in rats. Ent Appl Sci Lett 2016;3(2):65-71.
15. Doonan LB, Lynham S, Quinlan C, Ibiji SC, Winter CE, Padilla G, et al. Venom composition does not vary greatly

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between different nematocyst types isolated from the primary tentacles of Olindias sambaquiensis (Cnidaria: Hydrozoa). Biol Bull 2019;237(1):26-35.
16. Miao Z, Wu L, Lu M, Meng X, Gao B, Qiao X, et al. Analysis of the transcriptional regulation of cancer-related genes by aberrant DNA methylation of the cis-regulation sites in the promoter region during hepatocyte carcinogenesis caused by arsenic. Oncotarget 2015; 6(25): 21493–21506.
17. Lane DP, Cheok CF, Lain S. p53-based cancer therapy. Cold Spring Harb Perspect Biol 2010;2(9):1-24.
18. De Braekeleer M, Douet-Guilbert N, De Braekeleer E. Prognostic impact of p15 gene aberrations in acute leukemia. Leuk Lymphoma 2017;58(2):257-265.
19. El-Deiry WS. p21 (WAF1) mediates cell-cycle inhibition, relevant to cancer suppression and therapy. Cancer Res 2016;76(18):5189-5191.
20. Adams JM, Cory S. The Bcl-2 apoptotic switch in cancer development and therapy. Oncogene 2007;26(9):1324-1337.
21. Zhang W, Xu J. DNA methyltransferases and their roles in tumorigenesis. Biomarkers 2017;5(1):1-8.
22. Ayed Y, Boussabbeh M, Zakhama W, Bouaziz C, Abid S, Bacha H. Induction of cytotoxicity of Pelagia noctiluca venom causes reactive oxygen species generation, lipid peroxydation induction and DNA damage in human colon cancer cells. Lipids Health Dis 2011;10(1):1-10.
23. Morabito R, Condello S, Currò M, Marino A, Ientile R, La Spada G. Oxidative stress induced by crude venom from the jellyfish Pelagia noctiluca in neuronal-like differentiated SH-SY5Y cells. Toxicol In Vitro 2012;26(5):694-699.
24. Marchenko ND, Wolff S, Erster S, Becker K, Moll UM. Monoubiquitylation promotes mitochondrial p53 translocation. EMBO J 2007;26(4):923-934.
25. Aubrey BJ, Kelly GL, Janic A, Herold MJ, Strasser A. How does p53 induce apoptosis and how does this relate to p53-mediated tumour suppression? Cell Death Differ 2018;25(1):104-113.
26. Matsuno N, Hoshino K, Nanri T, Kawakita T, Suzushima H, Kawano F, et al. p15 mRNA expression detected by real-time quantitative reverse transcriptase-polymerase chain reaction correlates with the methylation density of the gene in adult acute leukemia. Leuk Res 2005;29(5):557-564.
27. Callagy GM, Pharoah PD, Pinder SE, Hsu FD, Nielsen TO, Ragaz J, et al. Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. Clin Cancer Res 2006;12(8):2468-2475.

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