World Cancer Research Journal WCRJ 2019; 6: e1293

VANADIUM COMPLEX INDUCED APOPTOSIS IN HEPG2 CELLS BY THE UP-REGULATION OF P53, P21, AND CASPASE-8

H. BAKHSHI ALIABAD[1], S. KHANAMANI FALAHATI-POUR[2], H. AHMADIRAD[1], M. MOHAMADI[2], M. REZA HAJIZADEH[1,3], G. BAKHSHI[4], M. MAHMOODI[5]

[1]Molecular Medicine Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[2]Pistachio Safety Research Center, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[3]Department of Clinical Biochemistry, Faculty of Medicine, Rafsanjan University of Medical Sciences, Rafsanjan, Iran
[4]Department of Veterinary Sciences, Islamic Azad University, Baft, Iran
[5]Department of Clinical Biochemistry, Afzalipoor Faculty of Medicine, Kerman University of Medical Sciences, Kerman, Iran

Abstract – *Objective:* *The anti-cancer effects of 4-bromo-2-(((5-chloro-2-hydroxyphenyl) imino) methyl) phenol ([IV(L)] complex) have been verified. The main mechanisms used by the [IV(L)] complex to induce apoptosis in cancer cells have yet to be clarified. This study has been designed to explore the effects of the [IV(L)] complex on the expression of apoptosis-related genes, including P53, caspase-8, bax, bcl-2, bim, P21, and bid, in human liver hepatocellular carcinoma (HepG2) cell lines and mouse fibroblast cells (L929), as normal cells.*

Materials and Methods: *The RPMI medium was used to culture L929 and HepG2 cells at the IC50 concentration of the [IV(L)] complex. The expression of some apoptosis-related genes (p53, caspase-8, bax, bcl-2, bim, P21, and bid) was evaluated before and after 48 h from treating the cells by the [IV(L)] complex at the IC50 concentration using the real-time PCR. Data analyzed via SPSS 18 (SPSS Inc., Chicago, IL, USA) software and* p*<0.05 was designated as the significant level.*

Results: *The [IV(L)] complex increased the mRNA expression levels of P53 and P21 genes in HepG2 (p< 0.05) cells. In addition, the expression levels of caspase-8 and bid decreased after treatment with the [IV(L)] complex (*p*=0.05), and the expression levels of bax and bim genes remained fixed in the HepG2-treated cells. In L929 cells, the mRNA expression levels of P53, caspase-8, bim, P21, and bid increased, with the expression level of the bax gene significantly decreased after 48 h from treatment with the [IV(L)] complex (*p*=0.001)*

Conclusions: *The Vanadium [IV(L)] complex induced apoptosis in HepG2 cells using the P53-P21 pathway-dependent method. In L929 cells, the mRNA expression levels of caspase-8 and bid were up-regulated in the treated cells. Therefore, it seems that apoptosis is triggered by both the P53-P21 pathway and the extrinsic apoptotic pathway in L929 cells.*

KEYWORDS: *Vanadium complex, HepG2 cells, L929 cells, Hepatocellular carcinoma, Apoptosis.*

LIST OF ABBREVIATIONS: *HCC: Hepatocellular carcinoma, Vanadium [IV(L)] complex: IV complex with 4-bromo-2-(((5-chloro-2-hydroxyphenyl) imino) methyl) phenol (L), FBS: Fetal Bovine Serum, EDTA: Ethylene Diamine Tetra Acetic Acid, MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyl Tetrazolium Bromide, L929: Mouse fibroblast cells, HepG2: Liver hepatocellular carcinoma.*

Corresponding Author: Mehdi Mahmoodi, MD; e-mail: mahmoodies@yahoo.com

INTRODUCTION

Cancers are nowadays the main causes of morbidities and mortalities in both developed and developing countries[1]. Liver cancer is most common in less developed countries, as demonstrated by the fact that in 2012 as many as 83% of the estimated 782,000 new cases worldwide occurred in such areas. Liver cancer is the second leading cause of cancer-associated mortality worldwide[2]. Hepatocellular carcinoma (HCC) is the most prevalent type of cancer, ranked fifth among human cancers, with approximately 750,000 new cases arising universally each year[3]. HCC could be induced by several factors: well-known risk factors for liver cancer including hepatitis B virus (HBV), hepatitis C virus (HCV), aflatoxins, alcohol consumption, oral contraceptives, tobacco smoking, androgenic steroids, and diabetes mellitus are suspected risk factors[2,4]. Current knowledge regarding non-infectious occupational risk factors and liver cancer is inconclusive except for the well-established association between vinyl chloride monomer (VCM) and angiosarcoma of the liver (ASL). High liver cancer mortality has been described among heavy construction equipment operators, chimney sweepers, chemical workers, seamen, painters and exposure to solvent methylene chloride and textile machinery[4]. Even though some therapeutic approaches such as chemotherapy, radiotherapy, and immunotherapy are used to treat cancers [5], they are associated with various complications due to their effects on noncancerous normal cells[6-9]. Thus, finding new cancer treatment strategies is one of the major concerns of researchers to reduce complications[10-12]. HepG2 is a famous HCC cell line that is used by researchers to examine new treatment strategies *in vivo*[13]. Vanadium (IV) is a metal ion with its complex introduced as a candidate to treat cancers[14]. Due to the low side effects of Vanadium IV, as compare to other metal ions such as platinum[15], current studies are mainly focused on Vanadium complexes to probably develop a safe and effective drug. Previous studies[8] revealed that the IV complex with 4-bromo-2-(((5-chloro-2-hydroxyphenyl) imino) methyl) phenol (L), abbreviated as the [IV(L)] complex, induces apoptosis more in HepG2 cell lines than in L929 cells, as normal cell lines. It has been suggested that the IV complexes could overcome cancer cells by several mechanisms, including the upregulation of free radical reactions[16,17], the alteration of expression in molecules involved in apoptosis, such as phosphpo-p21-activated protein kinases (PAK), oinositide-3-kinase-protein kinase B/Akt (PI3K-PKB/Akt), cyclin-dependent kinase (CDK) 4, 6, and 7, death-associated protein kinase (DAPK), protein 2-alpha (AP2), Fas-associated protein with death domain (FADD), c-Jun N-terminal kinase (JNK), Caspases 3,6,7,10, and 11, as well as B-cell lymphoma-extra (Bcl-x)[18,19]. However, the effects of the [IV(L)] complex on the expression levels of p53, caspase-8, bax, bcl-2, bim, p21, and bid genes in HepG2 and L929 cells are yet to be clarified.

Therefore, based on our investigation that confirmed the cytotoxicity of the [IV(L)] complex more on HepG2 cells than L929 cells, the major goal of this study is to find the main genes targeted by the [IV(L)] complex to induce apoptosis in cancerous cells.

MATERIALS AND METHODS

Cell Lines and Cell Culture Conditions

Human liver cancerous cells (HepG2), and mouse fibroblast (L929) cell lines were purchased from the Pasteur Institute, Tehran, Iran. The cell lines were cultured in the Roswell Park Memorial Institute-1640 (RPMI-1640) culture medium under standard conditions as described in the previous investigation[16]. The determination of the inhibiting cell growth by 50% (IC50), the percentage of the survived cells using the MTT assay (Roche, Mannheim, Germany), the preparation of 4-bromo-2-(((5-chloro-2-hydroxyphenyl)imino)methyl)phenol (H2L), 2,2′-bipyridine)[4-bromo-2-((5-chloro-2-hydroxyphenylimino)methyl)phenol] oxido-vanadium(IV) [VOL(bipy)], and the FTIR spectral data were described in detail in the previous study[16]. Our research received the Ethics Committee approval by Rafsanjan University of Medical Sciences (RUMS) with code No. IR.RUMS.REC.1394.178. All the laboratory tests were performed at the Molecular Medicine Research Center and the Research Institute of Basic Medical Sciences of RUMS.

RNA Extraction and cDNA Synthesis

The total RNA of HepG2 and L929 cell lines was purified before and after 48 h of treatment with the [IV(L)] complex, using the RNX solution from Cinnaclon Company (Tehran, Iran). The cDNA was synthesized using commercial kits from Pars Tous Company (Mashhad, Iran). The total mRNA, oligo-dT, and RNase-free water were added to the tubes and incubated at 70°C and then 4°C for 10 and 4 min, respectively. The premix was then added and incubated at 40°C for 60 min. The next step was to incubate vials at 95°C for 10 min to inactivate the reverse transcriptase enzyme.

Real-Time PCR Analysis

The real-time PCR analysis was performed using a master mix from Pars Tous Company (Mashhad, Iran) by specific primers for P53, caspase-8, bax, bcl-2, bim, P21, and bid (Table 1). The real-time PCR protocol was

TABLE 1. Primer sequences used in the study (F, forward; R, reverse).

Gene	*Primer sequences (5'→3')*
bcl-2	F: CTTCTTTGAGTTCGGTGGGG R: AAATCAAACAGAGGCCGCAT
p53	F: TGAAGCTCCCAGAATGCCAG R: GCTGCCCTGGTAGGTTTTCT
Bim	F: GACAAGAATCCGACCAAATGGCAAA R: AAGGATCCATGAGAAATCCTTGTGG
Caspase-8	F: ATTAGGGACAGGAATGGAACAC R: GGAGAGGATACAGCAGATGAAG
bax	F: TGCCTCAGGATGCGTCCACCAA R: CCCCAGTTGAAGTTGCCGTCAG
Bid	F: CCTTGCTCCGTGATGTCTTTC R: TCCGTTCAGTCCATCCCATTT
puma	F: GACGACCTCAACGCACAGTA R: AGGAGTCCCATGATGAGATTG
P21	F: GGAGACTTCTCAGGGTCGAAAAC R: GGGCTTCCTCTTGGAGAAGATC
b-actin	F: GGGCATGGGTCAGAAGGATT R: CGCAGCTCATTGTAGAAGGT

run in a real-time PCR machine, i.e., the ABI Applied Biosystems (Foster City, CA, USA) by starting the incubation process at 95°C for 5 min followed by 45 cycles of 95°C (30 s), 58°C (30 s), and 72°C (30 s). β-actin was used as the housekeeping gene and the raw data were calculated using the $2^{\Delta\Delta ct}$ method.

Statistical Analysis

After the evaluation of the normal distribution of the data, the Student's *t*-test and one-way ANOVA under SPSS 18 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Two-tailed *(p*= 0.05) was considered as significant level.

RESULTS

Data analysis results revealed that P53 mRNA levels increased significantly in both HepG2 (3.04 ± 1.39 fold changes, p= 0.03) and L929 (771.62 ± 45.27 fold changes, p= 0.001) cells after 48 h of treatment by the [IV(L)] complex at the IC50 concentration, compared with HepG2 (1 ± 0.18 fold changes) and L929 (1 ± 0.05 fold changes) cells before the vanadium complex treatment, respectively.

Besides, statistical analysis results revealed that after treatment by the [IV(L)] complex, the expression level of the caspase-8 gene decreased significantly in HepG2 (p< 0.05) cells, while it increased in L929 (p< 0.001) cells.

Treatment with the [IV(L)] complex led to a significant decrease in mRNA levels of bax in L929 cells (p< 0.001), while mRNA levels did not change significantly in HepG2 cells (p> 0.05).

Besides, Bcl-2 mRNA levels decreased 3 times in HepG2 cell lines (p= 0.05), while the levels remained fixed in L929 cell lines.

Although the expression levels of bim did not change in HepG2 cells after treatment with the [IV(L)] complex (p> 0.05), the values increased significantly in L929 cells (p= 0.001).

The results also demonstrated that the mRNA expression levels of P21 increased significantly in both HepG2 (p= 0.05) and L929 cells (p= 0.001) after 48 h from treatment with the [IV(L)] complex.

The treatment of cells with the [IV(L)] complex led to a significant increase in the mRNA expression levels of the bid gene in L929 cells (p= 0.001). However, the mRNA expression levels of the bid gene in treated HepG2 cells decreased significantly (p= 0.05) compared with the untreated cells.

Table 2 illustrates the data on the relative expression levels of all genes studied in both HepG2 and L929 cells before and after the treatment of the cells with the [IV(L)] complex at the IC-50 concentration in 48 h. Figure 1 illustrates the effects of the [IV(L)] complex on the expression levels of P53, caspase-8, bax, bcl-2, bim, P21, and bid in HepG2 and L929 cells.

DISCUSSION

It has been reported that HCC is a prevalent type of cancer in developed and developing countries[1,2]. Although HCC has drug-resistant attributes in some cases[20-22], our previous study[16] revealed that [IV(L)] the complex treatment led to strong apoptosis in HepG2 cell lines, compared with L929 cells as nor-

TABLE 2. Relative expression of P53, caspase-8, Bax, Bcl-2, Bim, P21, and Bid in HepG2 and L929 cell lines before and after 48 h from treatment with the [IV(L)] complex. Data analysis revealed that the [IV(L)] complex treatment led to a significant change in all of the genes evaluated both in HepG2 and L929 cell lines (Except bax and bim for HepG2 cell lines).

	Target gene	*Before treatment*	*After treatment*	*p-value*
P53	HepG2	1 ± 0.18	3.04 ± 1.39	$p< 0.05$
	L929	1 ± 0.05	771.62 ± 45.27	$p< 0.001$
Caspase-8	HepG2	1 ± 0.23	0.19 ± 0.04	$p< 0.05$
	L929	1 ± 0.08	328.81 ± 93.59	$p< 0.001$
Bax	HepG2	1 ± 0.20	0.68 ± 0.01	$p> 0.05$
	L929	1 ± 0.27	0.008 ± 0.01	$p< 0.001$
Bcl-2	HepG2	1 ± 0.37	0.35 ± 0.20	$p< 0.05$
	L929	1 ± 0.49	1.37 ± 0.9	$p> 0.05$
Bim	HepG2	1 ± 0.47	1.60 ± 0.4	$p> 0.05$
	L929	1 ± 0.07	1994.98 ± 582.31	$p< 0.001$
P21	HepG2	1 ± 0.48	6.67 ± 0.65	$p< 0.05$
	L929	1 ± 0.58	2265.79 ± 167.53	$p< 0.001$
Bid	HepG2	1 ± 1.29	0.08 ± 0.10	$p< 0.05$
	L929	1 ± 0.87	1792.66 ± 490.32	$p< 0.001$

mal cells. However, the main mechanisms that lead to apoptosis in HepG2 cells via the [IV(L)] complex are yet to be determined. Thus, the current study was designed to explore the effects of the [IV(L)] complex treatment on the expression levels of pro/anti-apoptotic genes, including P53, caspase-8, bax, bcl-2, bim, P21, and bid. The results demonstrated that the expression levels of all pro-apoptotic genes increased significantly in L929 cells, except for the bax gene that was down-regulated after 24 h from treatment by the [IV(L)] complex. Although HepG2 cells showed similar patterns in terms of the expression levels of P53 and P21 genes, the expression levels of bax and bim remained unchanged (Figure 2 and Table 2). Besides, the mRNA expression levels of caspase-8 and bid genes were down-regulated after treatment by the [IV(L)] complex in HepG2 cells, while the genes were significantly up-regulated in L929 cells. Thus, it can be concluded that the [IV(L)] complex induces apoptosis in HepG2 cells via the P53-P21 signaling pathway, while this complex in L929 cells may trigger apoptosis via both the P53-P21 signaling pathway and the extrinsic apoptotic pathway (caspase-8/bid). However, our previous study [16] revealed that the early apoptosis rate was significantly higher in HepG2 cell lines than in L929 cell lines. Due to the fact that normal cells receive normal positive feedbacks from intracellular signaling pathways, it may be hypothesized that, although the mRNA levels of pro-apoptotic genes increased in normal cells, the positive feedback of anti-apoptotic genes might have regulated the effects of the [IV(L)] complex and inhibited apoptosis. Quite notably, although the pro-apoptotic gene, i.e., bax, did not increase in HepG2 and L929 cells, it appears that the asymmetrical expression levels of pro/anti-apoptotic genes in cancerous cells, i.e., HepG2, led to more apoptosis in HepG2 cells than in L929 cells. Besides, bax levels decreased in both cell lines. Due to the fact that the bax gene expression level is regulated by P53 and P53, it induces apoptosis via several mechanisms, including the up-regulation of the bax gene; thus, it may be implied that the [IV(L)] complex induces apoptosis in both cell lines independently of the bax gene. Besides, due to the significantly increased expression level of P53, this gene uses other mechanisms other than bax to induce apoptosis. The data revealed that bcl-2 was down-regulated in HepG2 cells, while it was unchanged in L929 cells. Hence, it can be concluded that the increased ratio of bax/bcl-2 in HepG2 cells, yet not in L929 cells, might have led to the activation of apoptosis via the intrinsic pathway. The up-regulation of caspase-8 and bid in L929 cells, yet not in HepG2 cells, indicates the activation of apoptosis via the extrinsic pathway. Furthermore, the fixedness of the expression levels of bim, as well as the down-regulation of bid in HepG2 cells imply that these genes are not involved in the induction of apoptosis in HepG2 cells. Nair et al[14] demonstrated that the nicotinoyl hydrazone component of Vanadium up-regulated p53 in SiHa and HeLa cancerous cell lines, and subsequently increased apoptosis in them. Thus, it appears that P53 is the main target of the [IV(L)] complex to up-regulate and induce apoptosis in HepG2 cell lines. In addition, the results demonstrated that P21 had the highest rate of the expression increase among the pro-apoptotic genes. Zhang et al[23] reported that vanadium induced apoptosis in C141 tumor cell lines by the up-regulation of P21 and P53 in a dependent manner. All in all, the [IV(L)] complex is an important chemical agent

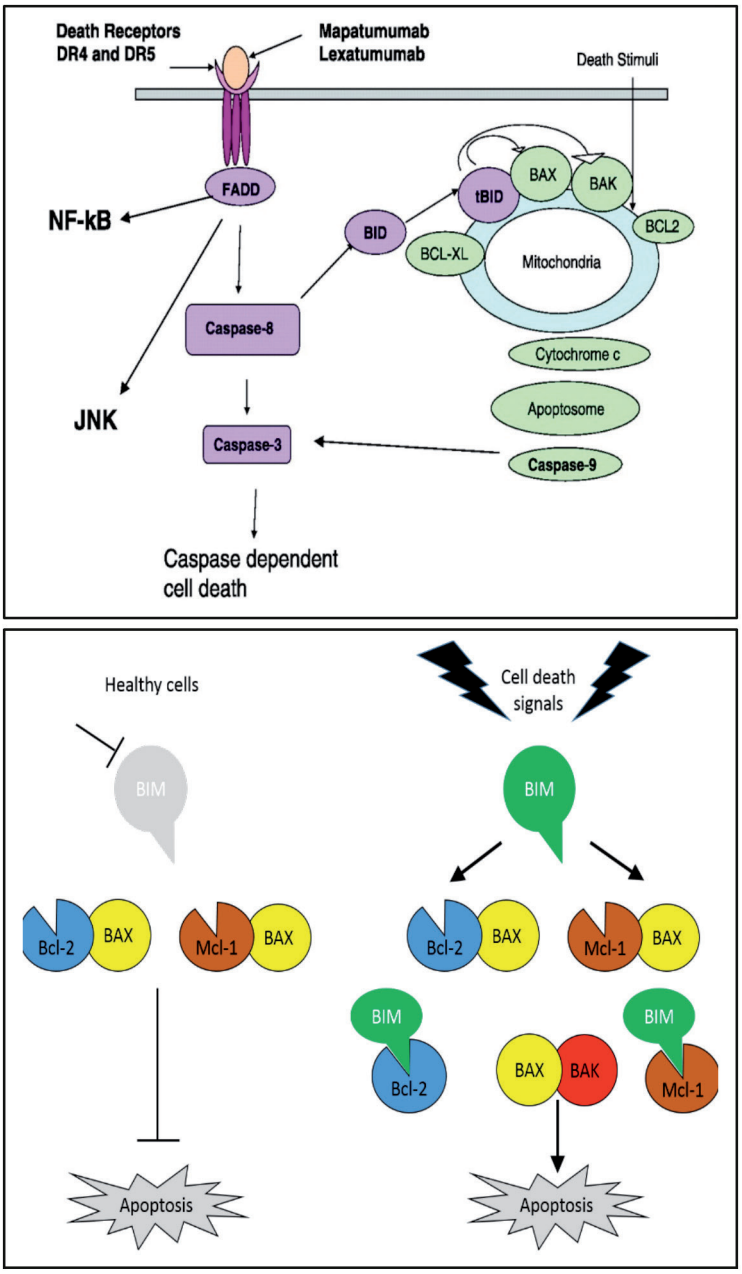

Fig. 1. The overview of apoptotic pathways: the death receptor and mitochondrial pathways. The schematic representation of apoptotic events. The two main apoptosis pathways are the extrinsic and intrinsic pathway, and the perforin/granzyme pathway.

that induces apoptosis in HepG2 cells by increasing the expression levels of two important pro-apoptotic genes, namely P53 and P21, in both HepG2 and L929 cells. Moreover, the complex induces apoptosis significantly in HepG2 and L929 cells via intrinsic and extrinsic pathways, respectively. Due to the fact that the intrinsic pathway is mainly the safe and plausible mechanism to fight cancers[17], it appears that the [IV(L)] complex can be considered as an important drug that needs to be evaluated through *in vivo* studies.

Besides, it has been documented that sodium orthovanadate, another component of vanadium, inhibits the proliferation of human HCC cells by increasing autophagy[24]. Since autophagy is a mechanism that is different from apoptosis, it could be hypothesized that the [IV(L)] complex could suppress the proliferation of HepG2 cells by integrating apoptosis and autophagy. This is a condition that needs to be explored through further studies. Considering the strengths of this work in synthesizing and examining a new compound (Transitional Metal complex) in the prevention of liver cancer. Some restrictions were also encountered by the researcher, including the financial limitations of the study on the number of additional genes and different cell lines, as well as the time limit for testing on cancerous tissues and animals.

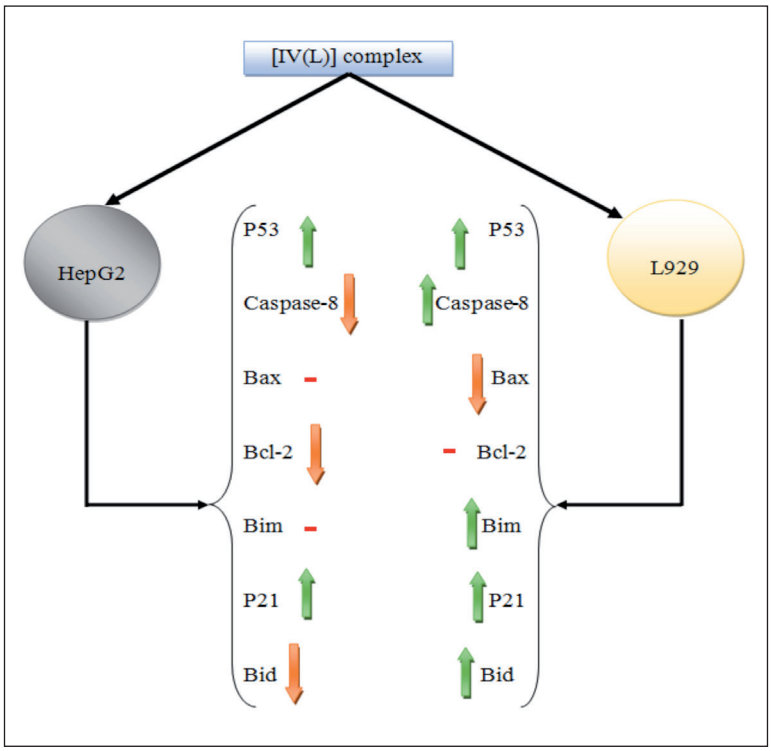

Fig. 2. The effects of the [IV(L)] complex on the expression levels of p53, caspase-8, bax, bcl-2, bim, p21, and bid in HepG2 and L929 cells. The figure illustrates that the [IV(L)] complex has increased p53, caspase-8, bim, p21, and bid significantly in L929 cells, and p53 and 21 in HepG2 cells. It has also decreased bax in L929 cells, and bax, bcl-2, and bid in HepG2 cells, while bcl-2 and bim have remained unchanged in the L929 and HepG2 cells, respectively. The results demonstrated that the bax/bcl-2 ratio increased and decreased, in HepG2 cells and L929 cells, respectively.

CONCLUSIONS

Finally the results of this study as primary work on a new complex of Vanadium ([IV(L)]) complex showed that this complex can induce apoptosis in HepG2 cells using the P53-P21 pathway-dependent method. In L929 cells, the mRNA expression levels of caspase-8 and bid were upregulated in the treated cells. Therefore, it seems that apoptosis was triggered by both the P53-P21 pathway and the extrinsic apoptotic pathway in L929 cells. Since this complex did not show many adverse effects on normal cells, it can be suggested as a potent compound. After carrying out the necessary clinical trials, it may also be used as an anticancer drug.

Acknowledgments:

This article is an excerpt from Hamid Bakhshialiabad's Ph.D. thesis at the Molecular Medicine Research Center, Research Institute of Basic Medical Sciences, Rafsanjan University of Medical Sciences (RUMS), Rafsanjan, Iran. Authors thank the Molecular Medicine Research Center staffs, the Research Institute of Basic Medical Sciences and Rafsanjan University of Medical Sciences, for their assistance.

Funding:

This study has been financially supported by RUMS, under the Grant No. 1396.3.2-449 and the Ethical Code of IR.RUMS.REC.1396.50, at the Rafsanjan University of Medical Sciences.

Ethical approval:

This article includes no study on human participants or animals as performed by any of the authors.

Informed consent:

This article involves no studies on human participants.

Conflict of Interest:

The authors declare that they have no conflict of interests.

REFERENCES

1. Tervonen H, Foliaki S, Bray F, Roder D. Cancer epidemiology in the small nations of Pacific Islands. Cancer Epidemiol. 2017; t 50(Pt B): 184-192.
2. Ho DW, Lo RC, Chan LK, Ng IO. Molecular pathogenesis of hepatocellular carcinoma. Liver Cancer 2016; 5: 290-302.
3. Prada-Arismendy J, Arroyave JC, Röthlisberger S. Molecular biomarkers in acute myeloid leukemia. Blood Rev 2017; 31: 63-76.
4. Ledda C, Loreto C, Zammit C, Marconi A, Fago L, Matera S, Costanzo V, Fuccio Sanzà G, Palmucci S, Ferrante M, Costa C, Fenga C, Biondi A, Pomara C, Rapisarda V. Non infective occupational risk factors for hepatocellular carcinoma: a review (review). Mol Med Rep 2017; 15: 511-533.
5. Obeid JM, Kunk PR, Zaydfudim VM, Bullock TN, Slingluff CL Jr, Rahma OE. Immunotherapy for hepatocellular carcinoma patients: is it ready for prime time? Cancer Immunol Immunother 2018; 67: 161-174.
6. Le Grazie M, Biagini MR, Tarocchi M, Polvani S, Galli A. Chemotherapy for hepatocellular carcinoma: the present and the future. World J Hepatol 2017; 9: 907-920.

7. Ceylan C, Kantar M, Tuna A, Ertam I, Aksoylar S, Günaydın A, Çetingül N.Cutaneous side effects of chemotherapy in pediatric oncology patients. Cutis 2015; 95: 11-6.
8. Bakhshi Aliabad H, Khanamani Falahati-Pour S, Ahmadirad H, Mohamadi M, Hajizadeh MR, Mahmoodi M. Vanadium complex: an appropriate candidate for killing hepatocellular carcinoma cancerous cells. Biometals 2018; 31: 981-990.
9. Hosseini FS, Falahati-Pour SK, Hajizadeh MR, Khoshdel A, Mirzaei MR, Ahmadirad H, Behroozi R, Jafari N, Mahmoodi M. Persian shallot, allium hirtifolium boiss, induced apoptosis in human hepatocellular carcinoma cells. Cytotechnology 2017; 69: 551-563.
10. Sheikhrezaei Z, Heydari P, Farsinezhad A, Fatemi A, Khanamani Falahati-Pour S, Darakhshan S, Noroozi Karimabad M, Darekordi A, Khorramdelazad H, Hassanshahi G. A new indole derivative decreased SALL4 gene expression in acute promyelocytic leukemia cell line (NB4). Iran Biomed J 2018; 22: 99-106.
11. Karimabad MN, Mahmoodi M, Jafarzadeh A, Darehkordi A, Hajizadeh MR, Khorramdelazad H, Falahati-Pour SK, Hassanshahi G. The novel Indole-3-formaldehyde (2-AITFEI-3-F) is involved in processes of apoptosis induction? Life Sci 2017; 15: 31-44.
12. Ramezani M, Ramezani M, Hassanshahi G, Mahmoodi M, Zainodini N, Darekordi A, Falahati-Pour SK, Mirzaei MR. Does the novel class of (2R, 4S)-N-(2, 5-difluorophenyl)-4-hydroxy-1-(2, 2, 2-trifluoroacetyl) pyrrolidine-2-carboxamide's have any effect on cell viability and apoptosis of human hepatocellular carcinoma cells? Int J Cancer Manag 2017; 10: e8413.
13. Han W, Hou G, Liu L. Polyphyllin I (PPI) increased the sensitivity of hepatocellular carcinoma HepG2 cells to chemotherapy. Int J Clin Exp Med 2015; 15: 20664-20669.
14. Nair RS, Kuriakose M, Somasundaram V, Shenoi V, Kurup MR, Srinivas P. The molecular response of vanadium complexes of nicotinoyl hydrazone in cervical cancers--a possible interference with HPV oncogenic markers. Life Sci 2014; 116: 90-97.
15. Leon IE, Cadavid-Vargas JF, Di Virgilio AL, Etcheverry SB. Vanadium, ruthenium and copper compounds: a new class of nonplatinum metallodrugs with anticancer activity. Curr Med Chem 2017; 24: 112-148.
16. Rezaei A, Falahati-Pour SK, Mohammadizadeh F, Hajizadeh MR, Mirzaei MR, Khoshdel A, Fahmidehkar MA, Mahmoodi M. Effect of a Copper (II) complex on the induction of apoptosis in human hepatocellular carcinoma cells. Asian Pac J Cancer Prev 2018; 19: 2877-2884.
17. Wang Q, Liu TT, Fu Y, Wang K, Yang XG. Vanadium compounds discriminate hepatoma and normal hepatic cells by differential regulation of reactive oxygen species. J Biol Inorg Chem 2010; 15: 1087-1097.
18. León IE, Díez P, Baran EJ, Etcheverry SB, Fuentes M. Decoding the anticancer activity of VO-clioquinol compound: the mechanism of action and cell death pathways in human osteosarcoma cells. Metallomics 2017; 19; 9: 891-901.
19. León IE, Díez P, Etcheverry SB, Fuentes M. Deciphering the effect of an oxovanadium(iv) complex with the flavonoid chrysin (VOChrys) on intracellular cell signalling pathways in an osteosarcoma cell line. Metallomics 2016; 8: 739-749.
20. Galun D, Srdic-Rajic T, Bogdanovic A, Loncar Z, Zuvela M. Targeted therapy and personalized medicine in hepatocellular carcinoma: drug resistance, mechanisms, and treatment strategies. J Hepatocell Carcinoma 2017; 11: 93-103.
21. Dong J, Zhai B, Sun W, Hu F, Cheng H, Xu J. Activation of phosphatidylinositol 3-kinase/AKT/snail signaling pathway contributes to epithelial-mesenchymal transition-induced multi-drug resistance to sorafenib in hepatocellular carcinoma cells. PLoS One 2017; 12: e0185088.
22. Guo YL, Kong QS, Liu HS, Tan WB. Drug resistance effects of ribosomal protein L24 overexpression in hepatocellular carcinoma HepG2 cells. Asian Pac J Cancer Prev 2014; 15: 9853-9857.
23. Zhang Z, Huang C, Li J, Shi X. Vanadate-induced cell growth arrest is p53-dependent through activation of p21 in C141 cells. J Inorg Biochem 2002; 89: 142-148.
24. Wu Y, Ma Y, Xu Z, Wang D, Zhao B, Pan H, Wang J, Xu D, Zhao X, Pan S, Liu L, Dai W, Jiang H. Sodium orthovanadate inhibits growth of human hepatocellular carcinoma cells in vitro and in an orthotopic model in vivo. Cancer Lett 2014; 351: 108-116.