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Vanadium oxide 3- methoxy salen, a synthetic biologically active complex against HeLa and McCoy cell lines

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ABSTRACT

Background: Vanadium is an essential dietary microelement that plays a key role in metabolic pathways and has anti-neoplastic effects. In this regard, vanadium oxide 3-methoxy salen complex was produced and its anticancer effects were evaluated against HeLa and McCoy cell lines.

Methods: Schiff bases produced from equivalents of Vanadyl acetylacetonate [VO(acac)2] in methanol were used to make a vanadium oxide 3-methoxy salen complex. Then, The antioxidant property of compound, cell viability and cytotoxicity assay, DNA fragmentation analysis and determination of the apoptosis pathway genes were evaluated.

Results: The result showed that the compound with an RC50 value of 126.3 μM demonstrated considerable free radical scavenging activity. The combination strongly suppressed the viability and proliferation of HeLa and McCoy cell lines in a dose-dependent manner, with IC50 values of 213 μM and 175 μM, respectively. When the viability and cytotoxicity values of the treated cells were compared, it was discovered that the cells had died of apoptosis, which was validated by DNA fragmentation analysis. Capspase 3, Bcl2 antagonist/killer, and Bcl2 associated X protein (Bax) gene expression levels all increased significantly in a quantitative investigation of apoptotic pathway genes, with 2-CT values of 2.36, 2.63, and 3.18, respectively.

Conclusion: In HeLa and McCoy malignant cell lines, lower quantities of the complex caused programmed cell death. This potential of complex can be used in cancer chemoprevention and cancer therapy.

Keywords: Apoptosis, Bax, Caspase, Salen Complex,Vanadium

INTRODUCTION:

Vanadium is an essential dietary microelement that, along with its chemical compounds is known to have wide physiological effects [1]. Vanadium not only plays a key role in the metabolic pathways of thyroid glands, bone growth, lipids, and carbohydrates but also shows in vitro and in vivo anti-neoplastic effects [2, 3]. Recent studies have demonstrated that vanadium and its derivatives could not only induce apoptosis and DNA cleavage, but also retard cell proliferation, reduce the incidence of mammary tumors, kill yeast cells, inhibit hepatoma cells, and improve glucose homeostasis by insulin-like effects [4-7].

Depending on the type of the complexes and/ or valence of the salts, vanadium and its derivatives have been reported as both preventive and anti-tumor agents, but some of their complexes or ions have shown carcinogenic effects through induction of retrotransposition [8-10]. However, there is little difference between the effective and toxic doses of the element. The surrounding ligands of the vanadium compounds may improve the adsorption of the compound into cells, and the anticancer effects of the compounds may ensue from the inhibition of protein tyrosine phosphatase or the activation of tyrosine phosphorylation [10]. However, some chemical conditions like lower pH of cytoplasm and/or the tissues immediately surrounding cancer cells can improve the cytotoxic effects of some vanadium complexes [5]. Recent studies have shown that the vanadium oxide 3- methoxy salen complex could serve as an anti-oxidative, anti-diabetic and anti-proliferative agent [11, 12]. Diabetic rats were given the methoxy VO-salen complex, which lowered their blood glucose levels [11]. According to recent research, vanadium may cause chromatin condensation and cell cycle arrest in treated cells, suggesting that it has the potential to become an anti-cancer treatment in the future [13].

Materials and Methods:

The vanadium oxide 3-methoxy salen complex was produced and tested as a physiologically active molecule against In HeLa and McCoy malignant cell lines in the current investigation.

Synthesis of Schiff base ligands

The Schiff base ligands were synthesized quantitatively by reacting meso-1,2-diphenyl-1,2-ethylenediamine and 1,2-ethylenediamine in ethanol with 2 equivamounts of salicylaldehyde, 3-methoxysalicylaldehyde, and 5-bromo-salicylaldehyde. A dropwise addition of diammine (1 mmol) in 40-50 mL ethanol was made to a vigorously agitated ethanolic solution of aldehyde (40 mL) (2 mmol). After that, the mixture was mixed and refluxed for 1 hour. The resulting yellow precipitate was collected by filtering, washed with ethanol, and dried in a desiccator after the mixture was cooled. The interaction of Schiff base ligands with equivamounts of vanadyl acetylacetonate [VO(acac)2] in methanol produced the whole vanadyl Schiff base complex [14].

Synthesis of the [VO(3-methoxy-salen)]

In a hot methanolic solution (70 mL) of VO(acac)2 (1 mmol), 3-methoxy-salen (1 mmol) and pyridine (1.5 mL) were added, and the mixture was rapidly agitated for 90 minutes under reflux. Filtration was used to collect the green precipitate, which was then washed with ethanol and ether before being dried in the air. [VO(3-methoxy-salen)] yield: (%82). C18H18N2O5V anal. calcd: 54.96; H, 4.58; N, 7.12. C was 54.79, H was 4.31, and N was 7.43. 981 [(V=O)], 1621 [(C=N)] IR (KBr, cm-1) (Fig. 1).

DPPH radical scavenging activity

According to a previously reported approach, the complex's antioxidant property was assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging test [15]. The RC50 value was established as the minimum concentration of an agent (μM) necessary to reduce DPPH radicals by 50% in the experiment condition [16].

Cell line and culture conditions

The National Cell Bank of Iran provided the HeLa and McCoy cell lines. The cells were grown in 25 cm^2 culture flasks at 37 ºC in a humidified environment with 5% CO2 in RPMI 1640 (Gibco) media supplemented with fetal bovine serum (10%), penicillin (100U/ml),

and streptomycin (100g/ml). In the next experiments, all cells had a passage number of 3- 5.

Cell viability and cytotoxicity assay

The MTT colorimetric test and the trypan blue exclusion experiment were used to assess the VOMS complex's cell viability and antiproliferative properties [17, 18]. The cells were treated with concentrations of the complex of 75, 150, 300, 600, and 1200 M while in exponential development, and the results were recorded after 8 and 16 hours of incubation. The IC50 and CC50 values were established as the concentrations of a substance necessary to impede 50% of the cell growth and disrupt 50% of cell membrane, respectively [16].

DNA fragmentation analysis

A previously reported approach was used to assess DNA internucleosomal breakage as a crucial aspect of programmed cell death. Actinomycin D-treated cells were employed as an apoptosis-positive control [18].

Determination of the apoptosis pathway genes by SYBER Green quantitative real-time PCR

At first, 1 ml of TRIzol reagent (RNX, Cinnagen Co., Iran) per cell culture flask was used to homogenize the cells. Total RNAs were extracted from the cells using the manufacturer's recommended methodology, followed by an additional chloroform extraction step to achieve phase separation. The total RNAs were resuspended in DEPC-treated double-distilled water, and the final concentration was measured using a 260 nm absorbance measurement (A260). The quality of the RNA was determined using electrophoresis on a 1% agarose gel. In the presence of 0.01 M DTT, 0.5 mM each dNTP, 0.5 μg oligo-dT. Primer, 40 U RNase free ribonuclease inhibitor, and 200 U M MuLV reverse transcriptase, first-strand cDNA was synthesized from 2 μg of each RNA sample (Fermentas, Germany) [19]. The reverse transcriptase enzyme was omitted from one of the RT PCR processes, which was carried out in triplicate. This extra reaction was carried out to determine the amount of genomic DNA contamination in each sample. The quantitative RT-PCR (qPCR) was performed using the Real-time PCR SYBER GREEN I kit (Qiagen, USA) to amplify the caspase 3, Bcl2 antagonist/killer gene [20], in triplicate, the Bcl2 associated X protein (Bax) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) genes [21, 22]. The GAPDH primer was chosen with the help of the beacon designer program (Version 7.5) (Table 1).

The qPCR was performed on the Rotor-Gene 3000 in two phases, according to the supplier's instructions, and using first strand cDNA as a template (Corbett Research, Australia). The data were processed using the previously published $2^{-\Delta\Delta CT}$ approach [23]. [(Ct target - Ct GAPDH)time x – (Ct target - Ct GAPDH) time 0] was used to determine the $2^{-\Delta\Delta CT}$ value. and are expressed as gene expression levels that are related to one another.

Table 1. The oligonucleotides which were used for the quantitative analysis of the apoptosis pathway genes.

Gene	Primer sequence (5'→3')	Amplification Size (bp)	Refrences
caspase3 Forward Revers	AGAACTGGACTGTGGCATTGAG GCTTGTCGGCATACTGTTTCAG	191	(21)
bak Forward Revers	GAACAGGAGGCTGAAGGGGT TCAGGCCATGCTGGTAGACG	307	(21)
bax Forward Revers	TGCTTCAGGGTTTCATCCAG GGCGGCAATCATCCTCTG	170	(22)
GAPDH Forward Revers	AGAACATCATCCCTGCCTCTACTG CCTCCGACGCCTGCTTCAC	192	This work

Results:

Synthesis of the complex

The interaction between tetradentate ligands and VO produced the required oxovanadium(IV) complex (acac) [24]. The complex had a decent yield. The IR spectra of VO(3-methoxy-Salen)] displays a V=O stretching vibration at 981 cm-1, indicating that the compound is monomeric. The complex's C=N stretching vibrations were centered at 1612 cm-1. The production of the VO-schiff base complex was also validated by elemental analysis (Figure 1).

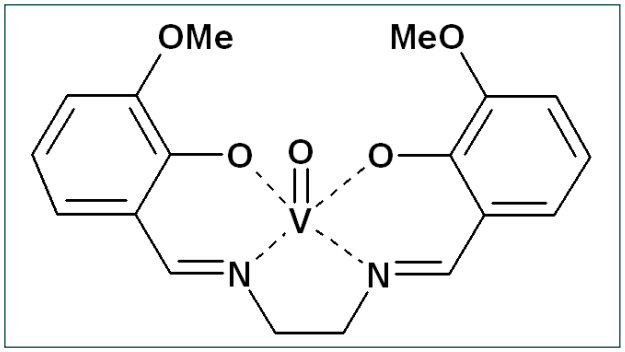

Figure 1. The structure VOS complex

Antioxidant Activity:

The antioxidant properties of the vanadium oxide 3-methoxy salen complex were assessed using the DPPH test after it was produced and purified. With an RC50 value of 126.3 μM, substantial DPPH radical scavenging activity was observed. The complex's antioxidant activity increased in a dose-dependent way.

Cytotoxic and viability assay

For 8 and 16 hours, log-phase monolayer cells were treated with the complex at concentrations of 40, 80, 160, 320, and 640 μM. After 8 hours of treatment, the vitality of McCoy and HeLa cells was suppressed with IC50 values of 175 μM and 213 μM, respectively. With increasing treatment time, the IC50 value decreased significantly. The inhibitory potential of the complex is dependent on the kind of treated cells, according to a comparison of the viabilities of the treated cells. The McCoy cells were more susceptible to the complex's cytotoxic action than the HeLa cells (Table 2).

Until ~200 μM of the complex, the percentage viability of the cells vs. the concentrations of the complex showed a significant linear relationship. The linear curve reached a plateau at concentrations larger than ~200 μM, and the viability was unaffected by increasing the complex concentration (Figure 2).

When the IC50 and CC50 values were compared, it was

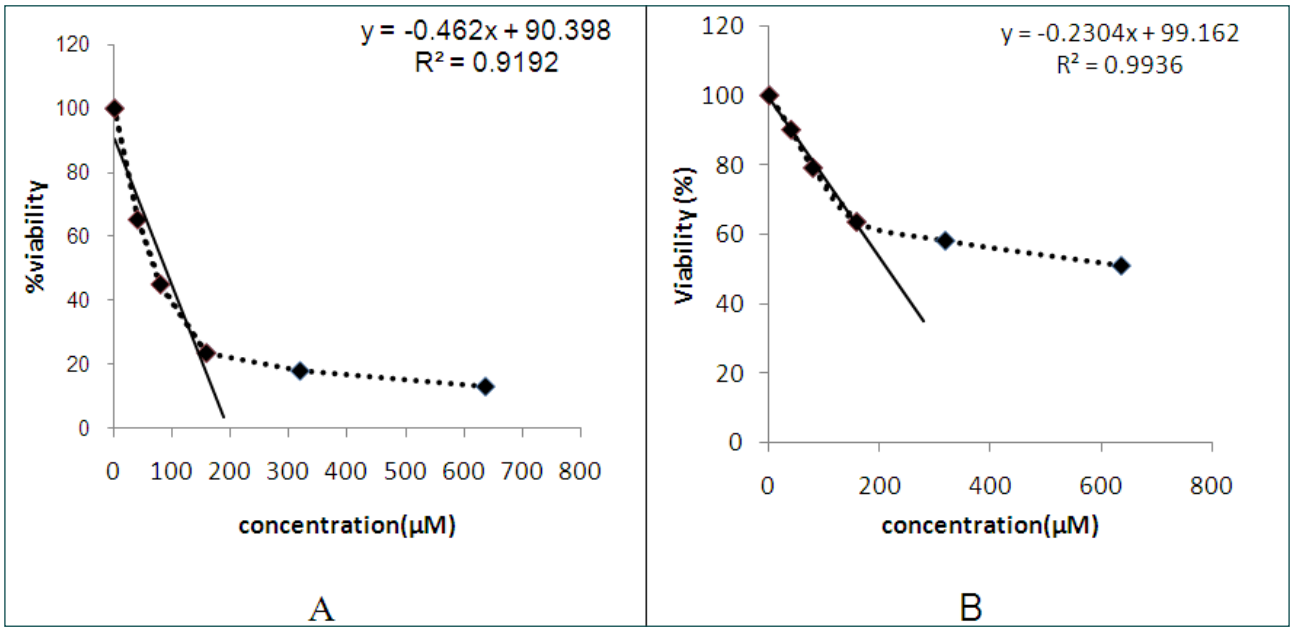

Figure 2. The effects of vanadium oxide 3-methoxy salen complex on the McCoy and HeLa cells. Figures A and B show the linear dependency of the viability vs. the concentrations in the McCoy and HeLa Cells after treatment.

Table 2. The antiproliferative effect of vanadium oxide 3- methoxy salen against the cancerous cell lines.

	8- our treatment			16-hour treatment		
Cell line	IC50 (µM)	Viability at 40 µM (%)	Viability at 160 µM (%)	IC50 (µM)	Viability at 40 µM (%)	Viability at 160 µM (%)
McCoy	175	90	57	89	72	20
Hela	213	90	53	175	88	49.2

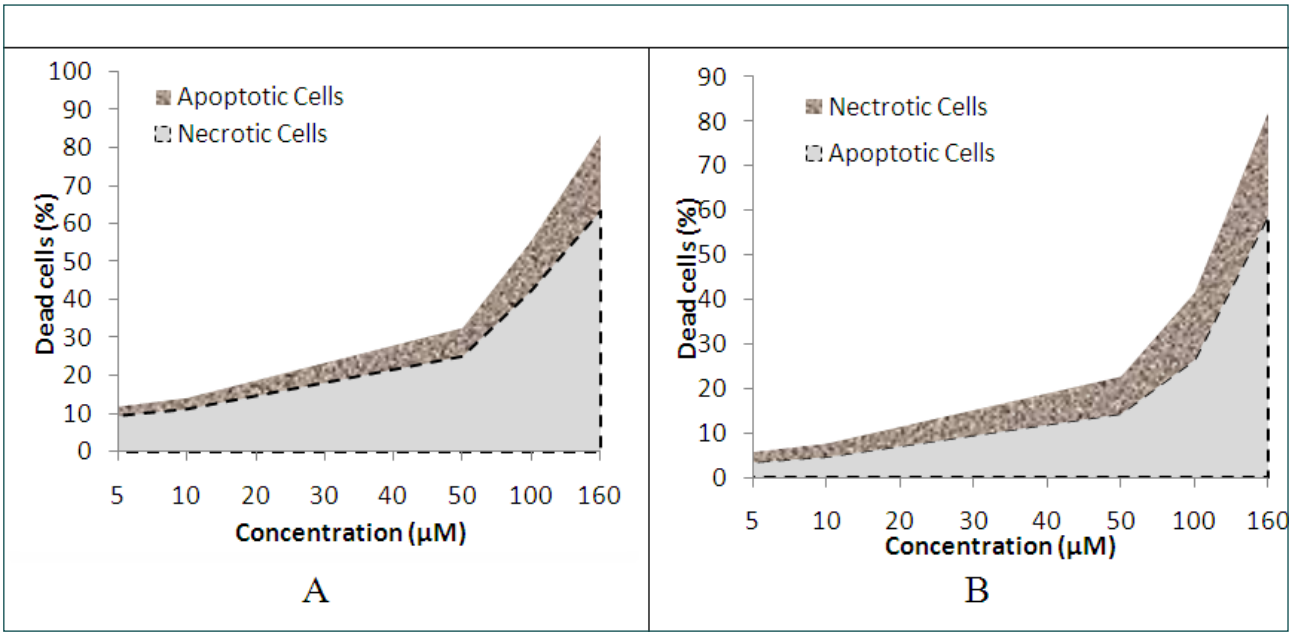

Figure 3. Comparison between the IC50 and CC50 values of the vanadium oxide 3-methoxy salen complex against the McCOy (A) and HeLa (B) cells.

discovered that cell death may be classified as necrosis as well as apoptosis. More than 35% and 20% of the HeLa and McCoy cells, respectively, were presumably experiencing apoptotic cell death at a concentration of 160 µM (Figure 3).

Analysis of Apoptosis

The treated cells were tested for DNA fragmentation, which is a sign of apoptosis. The degree of fragmentation rose in a dose-dependent manner, and fragmentation in the treated cells was more efficient than in the positive controls (figure 4).

In HeLa cells treated with 160 M, qPCR of genes implicated in the apoptotic pathway was done. The $2^{-\Delta\Delta CT}$ values for the cp3, bac, and bax genes were 2.36, 2.63, and 3.18, respectively, when compared to GAPDH (Table 3).

Discussion:

Vanadium is not carcinogenic, but its presence in cancer cells causes changes in the expression of p53 and Bax, as well as the downregulation of Bcl2 proteins and antiproliferative activity, due to interactions with several critical enzymatic processes. In vitro and in vivo research have shown that vanadium has anti-carcinogenic and anticancer properties in various forms [25].

Results of antioxidant evaluation demonstrated that the overproduction of reactive oxygen species, which causes oxidative stress, adds to an overburdening of the cellular defense system, which may end in cell death [26, 27]. According to the previous reports, the significant radical scavenging activity of the methoxy VO-salen is attributed to its vanadium elements which can reduce the cytotoxic effect of H2O2 [12].

Table 3. The Relative quantitation results of the apoptosis pathway genes (t: treatment; c: control).

bax^t	bax^c	bak^t	bak^c	$cp3^t$	$cp3^c$	$GAPDH^t$	$GAPDH^c$	
18.09	18.67	22.21	22.32	22.82	22.97	18.34	17.25	Ct
-1.67		-1.4		-1.24				ΔΔCt
3.18		2.63		2.36				$2^{-\Delta\Delta Ct}$
1.7	0.24	cc	3.3	0.23	0.9	1.03	1.3	SD

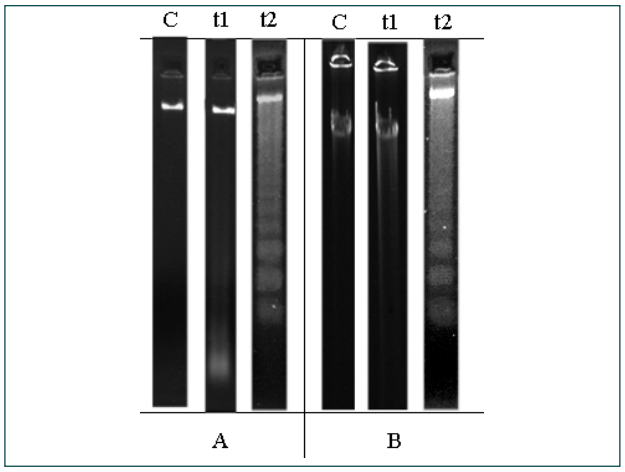

Figure 4. The vanadium oxide 3-methoxy salen complex-induced DNA fragmentation in the McCOy (A) and HeLa (B) Cell lines. C-) Negative control, t1 and t2) Treatment with 50 μM and 160 μM of the vanadium oxide 3-methoxy salen complex.

The IC50 values of vanadium salts have previously been reported to range from 27 to 47 μM [28]. It is obvious, however, that the kind of ligands used may impact the cytotoxicity of vanadium complexes, and these results demonstrated a low amount of cytotoxicity.

The predominant mechanism of cell death, according to DNA fragmentation experiments, was dose-dependent activation of apoptosis. The DNA fragmentation caused by vanadium compounds, as previously documented, might cause the cell-cycle arrest and/or cytotoxicity through nucleosomal fragmentation. Through DNA breakage and fragmentation, as well as plasma membrane lipoperoxidation, the chemicals may cause the cell-cycle arrest and/or cytotoxicity. After interacting with the nucleotide phosphate groups in DNA, vanadium compounds may have antiproliferative effects [29, 30]. These findings suggest that in the complex-treated cells, programmed cell death was effectively triggered. Caspase 3 is a key enzyme in the apoptotic pathway's execution stage, causing internucleosomal DNA cleavage, chromatin condensation, and the activation of other proteases [31].

The therapy with vanadium generates considerable chromatin condensation and cell cycle arrest, which results in apoptosis. Vanadium has the potential to be turned into an anti-cancer medication in the future, according to apoptosis-based studies. In the treated cells, the expression of the bax gene was much higher than that of the other genes, according to the findings. The translocation of Bax from the cytosol to the mitochondrial intermembrane contact sites causes mitochondrial permeability transition, loss of mitochondrial potential, the release of cytochrome C, activation of caspases, and DNA breakage, all of which lead to apoptosis [32, 33]. Vanadium's anticancer effects were discovered to be mediated via the inhibition of cellular tyrosine phosphatases and/or the activation of tyrosine phosphorylases, which resulted in the induction of death in numerous cell lines [29]. Overexpression of the protein tyrosine phosphatase triggered the caspase pathway directly and caused p53-independent apoptosis, according to previous research [34]. The vanadium oxide 3-methoxy salen compound then activates the protein tyrosine phosphatase, causing apoptosis. Although SH2 domain-containing protein tyrosine phosphatase is predominantly a positive regulator of cell growth and development, it also plays a negative role in IFN-induced growth inhibition. IFN- also caused a greater degree of caspase expression [35].

In conclusion, our results showed that lower quantities of the complex in malignant cell lines by antiproliferative activity caused programmed cell death. This potential complex can be used in cancer chemoprevention and cancer therapy.

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REFERENCES

1. Kanna PS, Mahendrakumar CB, Indira BN, Srivastawa S, Kalaiselvi K, Elayaraja T, et al. Chemopreventive effects of vanadium toward 1,2-dimethylhydrazine-induced genotoxicity and preneoplastic lesions in rat colon. Environ Mol Mutagen. 2004;44(2):113-8. PubMed PMID: 15278915. Epub 2004/07/28. eng.
2. Abdolmaleki A, Zahri S. Comparison of toxicity and teratogenic effects of salen and vo-salen on chicken embryo. Drug and Chemical Toxicology. 2016;39(3):344-9.
3. Samanta S, Swamy V, Suresh D, Rajkumar M, Rana B, Rana A, et al. Protective effects of vanadium against DMH-induced genotoxicity and carcinogenesis in rat colon: removal of O(6)-methylguanine DNA adducts, p53 expression, inducible nitric oxide synthase downregulation and apoptotic induction. Mutat Res. 2008 Feb 29;650(2):123-31. PubMed PMID: 18155637. Epub 2007/12/25. eng.
4. Mehdi MZ, Srivastava AK. Organo-vanadium compounds are potent activators of the protein kinase B signaling pathway and protein tyrosine phosphorylation: mechanism of insulinomimesis. Arch Biochem Biophys. 2005 Aug 15;440(2):158-64. PubMed PMID: 16055077. Epub 2005/08/02. eng.
5. Piatkowski J, Podsiadly H, Bukietynska K. The effect of V(III)-adenine complex on yeast as a model of eukaryotic cells. J Biochem. 2007 Apr;141(4):545-52. PubMed PMID: 17317691. Epub 2007/02/24. eng.
6. Kulkarni A, Patil SA, Badami PS. Synthesis, characterization, DNA cleavage and in vitro antimicrobial studies of La(III), Th(IV) and VO(IV) complexes with Schiff bases of coumarin derivatives. Eur J Med Chem. 2009 Jul;44(7):2904-12. PubMed PMID: 19155104. Epub 2009/01/22. eng.
7. Ray RS, Ghosh B, Rana A, Chatterjee M. Suppression of cell proliferation, induction of apoptosis and cell cycle arrest: Chemopreventive activity of vanadium in vivo and in vitro. International Journal of Cancer. 2006;120:13-23.
8. Noutsopoulos D, Markopoulos G, Koliou M, Dova L, Vartholomatos G, Kolettas E, et al. Vanadium induces VL30 retrotransposition at an unusually high level: a possible carcinogenesis mechanism. J Mol Biol. 2007 Nov 16;374(1):80-90. PubMed PMID: 17920077. Epub 2007/10/09. eng.
9. Zhang Z, Huang C, Li J, Leonard SS, Lanciotti R, Butterworth L, et al. Vanadate-induced cell growth regulation and the role of reactive oxygen species. Arch Biochem Biophys. 2001 Aug 15;392(2):311-20. PubMed PMID: 11488607. Epub 2001/08/08. eng.
10. Faneca H, Figueiredo VA, Tomaz I, Goncalves G, Avecilla F, Pedroso de Lima MC, et al. Vanadium compounds as therapeutic agents: some chemical and biochemical studies. J Inorg Biochem. 2009 Apr;103(4):601-8. PubMed PMID: 19110313. Epub 2008/12/27. eng.
11. Roy S, Mondru AK, Dontamalla SK, Vaddepalli RP, Sannigrahi S, Veerareddy PR. Methoxy VO-salen stimulates pancreatic beta cell survival by upregulation of eNOS and downregulation of apoptosis in STZ-induced diabetic rats. Biological trace element research. 2011 Dec;144(1-3):1095-111. PubMed PMID: 21748304. Epub 2011/07/13. eng.
12. Mohammadi M, Yazdanparast R. Methoxy VO-salen complex: in vitro antioxidant activity, cytotoxicity evaluation and protective effect on CCl4-induced oxidative stress in rats. Food Chem Toxicol. 2009 Apr;47(4):716-21. PubMed PMID: 19152825. Epub 2009/01/21. eng.
13. Ray RS, Rana B, Swami B, Venu V, Chatterjee M. Vanadium mediated apoptosis and cell cycle arrest in MCF7 cell line. Chem Biol Interact. 2006 Nov 7;163(3):239-47. PubMed PMID: 16970931. Epub 2006/09/15. eng.
14. Boghaei DM, Bezaatpour A, Behzad M. Synthesis, characterization and catalyticactivity of novelmonomeric and polymeric vanadyl Schiffbase complex-

es. Journal of Molecular Catalysis A: Chemical. 2006;245(1-2):12-6.
15. Takao T, Watanabe N, Yagi I, Sakata K. A simple screening method for antioxidants and isolation of several antioxidants produced by marine bacteria from fish and shellfish. Bioscience Biotechnology and Biochemistry. 1994;58:1780-3.
16. Zahri S, Razavi SM, Moatamed Z. Antioxidant activity and cytotoxic effect of aviprin and aviprin-3"-O-D-glucopyranoside on LNCaP and HeLa cell lines. Nat Prod Res. 2011 Jun 30. PubMed PMID: 21714729. Epub 2011/07/01. Eng.
17. Zhang Q, Wu J, Hu Z, Li D. Induction of HL-60 apoptosis by ethyl acetate extract of Cordyceps sinensis fungal mycelium. Life Sci. 2004 Oct 29;75(24):2911-9. PubMed PMID: 15454342. Epub 2004/09/30. eng.
18. Zahri S, S. M. Razavi, F. H. Niri, S. Mohammadi. Induction of programmed cell death by Prangos uloptera, a medicinal plant. Biol Res. 2009;42(4):517-22. PubMed PMID: 20140307. Epub 2010/02/09. eng.
19. Zahri S, Zamani MR, Motallebi M, Sadeghi M. Cloning and characterization of cbhII gene from Trichoderma parceramosum and its expression in Pichia pastoris. Iranian Journal of Biotechnology. 2005;3(4):204-15.
20. Szymanski FM, Bakon L, Grabowski M, Piatkowski R, Filipiak KJ, Rdzanek A, et al. Previously undiagnosed congenitally corrected transposition of the great arteries in a 51-year-old woman with chronic heart failure symptoms. Int J Cardiol. 2007 Apr 4;116(3):e111-3. PubMed PMID: 17126929. Epub 2006/11/28. eng.
21. Ory K, Lebeau J, Levalois C, Bishay K, Fouchet P, Allemand I, et al. Apoptosis inhibition mediated by medroxyprogesterone acetate treatment of breast cancer cell lines. Breast Cancer Res Treat. 2001 Aug;68(3):187-98. PubMed PMID: 11727956. Epub 2001/12/01. eng.
22. Nagy B, Lundan T, Larramendy ML, Aalto Y, Zhu Y, Niini T, et al. Abnormal expression of apoptosis-related genes in haematological malignancies: overexpression of MYC is poor prognostic sign in mantle cell lymphoma. Br J Haematol. 2003 Feb;120(3):434-41. PubMed PMID: 12580957. Epub 2003/02/13. eng.
23. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods. 2001 Dec;25(4):402-8. PubMed PMID: 11846609. Epub 2002/02/16. eng.
24. Tsuchida E, Yamamoto K, Oyaizu K, Iwasaki N, Anson FC. Electrochemical Investigations of the Complexes Resulting from the Acid-Promoted Deoxygenation and Dimerization of (N,N'-Ethylenebis(salicylideneaminato))oxovanadium(IV). Inorganic chemistry. 1994;33 (6):1056–63.
25. Novotny L, Kombian SB. Vanadium: possible use in cancer chemoprevention and therapy. Journal of Cancer Research Updates. 2014;3(2):97-102.
26. Butterfield DA, Kanski J. Brain protein oxidation in age-related neurodegenerative disorders that are associated with aggregated proteins. Mech Ageing Dev. 2001 Jul 15;122(9):945-62. PubMed PMID: 11348660. Epub 2001/05/12. eng.
27. Rauscher FM, Sanders RA, Watkins JB, 3rd. Effects of isoeugenol on oxidative stress pathways in normal and streptozotocin-induced diabetic rats. J Biochem Mol Toxicol. 2001;15(3):159-64. PubMed PMID: 11424226. Epub 2001/06/26. eng.
28. Dabros W, Adamczyk A, Ciurkot K, Kordowiak AM. Vanadium compounds affect growth and morphology of human rhabdomyosarcoma cell line. Polish journal of pathology : official journal of the Polish Society of Pathologists. 2011 Dec;62(4):262-8. PubMed PMID: 22246913. Epub 2012/01/17. eng.
29. Evangelou AM. Vanadium in cancer treatment. Crit Rev Oncol Hematol. 2002 Jun;42(3):249-65. PubMed PMID: 12050018. Epub 2002/06/07. eng.
30. Sankar Ray R, Roy S, Ghosh S, Kumar M, Chatterjee M. Suppression of cell proliferation, DNA protein cross-links, and induction of apoptosis by vanadium in chemical rat mammary carcinogenesis. Biochimica et biophysica acta. 2004 Nov 18;1675(1-3):165-73. PubMed PMID: 15535980. Epub 2004/11/13. eng.
31. Abu-Qare AW, Abou-Donia MB. Biomarkers of apop-

tosis: release of cytochrome c, activation of caspase-3, induction of 8-hydroxy-2'-deoxyguanosine, increased 3-nitrotyrosine, and alteration of p53 gene. Journal of Toxicology and Environmental Health, Part B Critical Reviews. 2001 Jul-Sep;4(3):313-32. PubMed PMID: 11503418. Epub 2001/08/16. eng.

32. Bedner E, Li X, Kunicki J, Darzynkiewicz Z. Translocation of Bax to mitochondria during apoptosis measured by laser scanning cytometry. Cytometry. 2000 Oct 1;41(2):83-8. PubMed PMID: 11002262. Epub 2000/09/26. eng.
33. Jia L, Patwari Y, Srinivasula SM, Newland AC, Fernandes-Alnemri T, Alnemri ES, et al. Bax translocation is crucial for the sensitivity of leukaemic cells to etoposide-induced apoptosis. Oncogene. 2001 Aug 9;20(35):4817-26. PubMed PMID: 11521193. Epub 2001/08/25. eng.
34. Weng LP, Yuan J, Yu Q. Overexpression of the transmembrane tyrosine phosphatase LAR activates the caspase pathway and induces apoptosis. Current biology : CB. 1998 Feb 26;8(5):247-56. PubMed PMID: 9501065. Epub 1998/04/16. eng.
35. You M, Yu DH, Feng GS. Shp-2 tyrosine phosphatase functions as a negative regulator of the interferon-stimulated Jak/STAT pathway. Molecular and cellular biology. 1999 Mar;19(3):2416-24. PubMed PMID: 10022928. Pubmed Central PMCID: 84034. Epub 1999/02/18. eng.