E-ISSN: 2707-2835

P-ISSN: 2707-2827

IJPLS 2020; 1(2): 38-43

Received: 23-05-2020

Accepted: 26-06-2020

Deepanshi Tiwari

Research Scholar, Department
of Pharmacology, Advance
Institute of Biotech and
Paramedical Sciences, Uttar
Pradesh, India

Mamta Tiwari

Associated Professor,
Department of Pharmacology,
Advance Institute of Biotech
and Paramedical Sciences
Uttar Pradesh, India

Corresponding Author:

Deepanshi Tiwari

Research Scholar, Department
of Pharmacology, Advance
Institute of Biotech and
Paramedical Sciences, Uttar
Pradesh, India

Vincristine: Beyond on anticancer treatment

Deepanshi Tiwari and Mamta Tiwari

DOI: <https://doi.org/10.33545/27072827.2020.v1.i2a.17>

Abstract

Vincristine is a chemotherapeutic agent that is a component of many combination regimens for a variety of malignancies, including several common pediatric tumors. 78% of patients with advanced malignant disease were treated with vincristine, an alkaloid derived from *vinca rosea* Linn, 59% of these survived from the beginning of treatment and could be evaluated. Favourable responses were seen in patients with Hodgkin's disease, reticulum cell sarcoma, lymphosarcoma, carcinoma of the breast, acute leukemia and choriocarcinoma. Toxic include a high incidence of alopecia and neurologic complications. Vincristine treatment is limited by a progressive sensorimotor peripheral neuropathy. Vincristine-induced peripheral neuropathy (VIPN) is particularly challenging to detect and monitor in pediatric patients, in whom the side effect can diminish long term quality of life. Further research is needed to predict, prevent, and treat disease to maximize therapeutic benefit and avoid unnecessary toxicity from vincristine treatment.

Keywords: Vincristine, *Catharanthus rosea* Linn, hodking's disease, lymphosarcoma, vincristine induced peripheral neuropathy

Introduction

Vincristine is originally derived from the periwinkle plant *Catharanthus roseus*. Vincristine act as inhibitors during the metaphase of the cell cycle and by binding to the microtubules inhibit the development of the mitotic spindle ^[1]. The vinca alkaloids are important for being anticancer drugs like vincristine and vinblastine. The productivity of vinblastine and vincristine is very low in plants (0.001-0.0003%) resulting in their extraordinary high price ^[2]. The United States Food and Drug Administration (FDA) approved indications of vincristine are acute lymphocytic leukemia, lymphoid blast crisis of chronic myeloid leukemia and Hodgkin and Non-Hodgkin lymphoma ^[3]. Vincristine also has several off-label uses that include central nervous system (CNS) tumors, Ewing sarcoma, gestational trophoblastic tumors, multiple myeloma, ovarian cancer, primary CNS lymphoma, small cell lung cancer, and advanced thymoma in adult patients ^[4].

The productivity of vinblastine and vincristine is very low in plants (0.001-0.0003%) resulting in their extraordinary high price. Vinblastine is a dimeric indole alkaloid and is formed by coupling of vindoline and catharanthine catalysed by horseradish peroxidase¹. The yield of coupling products, (15' 20'- anhydro vinblastine) was reported very low (0.9%) ^[5]. Vinblastine is converted into vincristine by the oxidation of its methyl group. Most of the key enzymes of the indole alkaloid biosynthetic pathway have been isolated from seedlings and/or cell suspension cultures of the *C. roseus* 2 ^[6]. The cell cultures do not produce dimeric and monomeric indole alkaloids but catharanthine is produced in considerable amounts. Vincristine, vinblastine and vindoline were reported only in shoot cultures and differentiated tissues but not in roots ^[7]. Recently a stable, high producing and salt tolerant cell lines of *C. roseus* plant has been developed to achieve industrial production of the alkaloids. The major limitation of these drugs in cell cultures is their low yield. Particularly the improvement of catharanthine production in *C. roseus* cell cultures is of great interests for pharmacologists and chemists because catharanthine and vindoline can be coupled to form vinblastine in high yield, and vindoline is abundant in plants ^[8]. Elicitors can also modulate the production of these alkaloids as reported by Moreno *et al.* Involvement of metabolic engineering for alternative production methods was encouraged and made possible due to low vinblastine and vincristine contents in the plants ^[9, 10]. Engineering techniques like semi-synthesis, total chemical synthesis or even of chemical ^[11, 12] or enzymatic coupling ¹³ of commercial

available catharanthine a vindoline is useful plant.

Fig 1: Vinca ^[13]

Vincristine is used in combination therapy to treat acute leukemias and lymphomas and constitutes an important component of the regime that has been so successful in treating childhood leukemias. Vinblastine is often used in combination to treat bladder and breast cancers and is an integral part of the curative treatment regime for Hodgkin's disease. Vinorelbine was approved for use in Europe (1991) and the U.S. (1995) for the treatment of non-small-cell lung cancer and vindesine has been approved for the treatment of melanoma ^[14]. Neurotoxicity (vincristine) or myelosuppression (vinblastine) are the main side effects of administration and neutropenia is the principal dose-limiting toxicity of the vinca alkaloids, but recovery occurs following treatment. However, the major limitation to the continued use of the vinca alkaloids is the emergence of drug resistance derived principally from over expression of phosphoglycoprotein (Pgp), an efflux pump that transports many of the major drugs out of the cell. In fact, vinblastine represents one of the most studied prototypical substrates for Pgp efflux responsible for multidrug resistance (MDR). Thus, in addition to identifying vinblastine and vincristine analogues that may address the current dose-limiting toxicities, the development of a modified vinca alkaloid that is not a substrate for Pgp efflux and is efficacious against MDR tumors would constitute a major advance ^[15, 16]. Additionally, the emerging evidence that the vinca alkaloids also possess antiangiogenic activity that may contribute to their *in vivo* antitumor activity, especially in combination with other drugs, may provide additional future clinical applications. Due to the pharmaceutical importance and low natural abundance of vinblastine and vincristine, *C. roseus* has become one of the most extensively studied medicinal plants serving as a model for biotechnological studies of plant secondary metabolism. Their biosynthesis involves the participation of at least 35 intermediates, 30 enzymes, 30 biosynthetic and 2 regulatory genes, and 7 intra- and intercellular compartments ^[9]. Presently, the clinical supplies of 1 and related drugs are derived from natural sources ^[17]. Fortunately, the doses are so small that the production amounts are manageable even with the trace natural abundance of 1 (0.01%) or 2 (0.0003%) in the source plants. Nonetheless, the effort required even for this limited quantity suggests that an efficient synthetic approach might provide a viable alternative. Even the development of

an effective coupling protocol starting with the more abundant naturally occurring (+)-catharanthine ^[18, 10] and vindoline, vinpocetine, vincamine ^[19] may supplant the direct use of plant produced vinblastine or vincristine. Interestingly, only *C. roseus* produces catharanthine and does so with an absolute configuration enantiomer with structurally related alkaloids also found in *C. roseus* and related alkaloids found in nature. More significantly, an effective synthetic approach would provide access to analogues that incorporate deep-seated structural changes that have not yet been explored ^[20]. Typically, it has been semi synthetic derivatives of the natural products that have been examined, restricting the structural sites and opportunities to improve on the properties of 1 or 2 ^[21].

Fig 2: Vincristine

Mechanism of action of vincristine

Vincristine acts by binding to tubulin and inhibiting the formation of microtubules. This inhibition causes mitosis to arrest at metaphase, through the disruption of mitotic spindle formation, especially during the M and S phases ^[22, 23]. Another mechanism of action of vincristine includes interfering with nucleic acid and protein synthesis by blocking glutamic acid utilization.

Metabolism

Vincristine is rapidly removed from the bloodstream and is tightly bound to tissues, but it poorly penetrates the blood-brain barrier. Vincristine undergoes extensive metabolism in the liver via CYP3A4 ^[24]. Patients with serum bilirubin levels >3 mg/dl will require dose adjustments; per the manufacturer's label, the dose in such patients should be 50% of the normally administered dose. Vincristine is excreted primarily in the feces.

Pharmacological activity of vincristine on Hodgkin's disease

Vincristine was found to be useful in the treatment of Hodgkin's disease reticulum cell sarcoma, lymphosarcoma, choriocarcinoma and to a minor degree in the treatment of some mammary tumours. It is generally ineffective in leukemias and in most carcinomas ^[25, 26]. Toxic effects include depression of erythropoiesis and granulopoiesis, frequent alopecia, occasional gastrointestinal toxicity and occasional neurological complications.

Antiviral effect

The simplex herpes virus (type I) with a cytopathogenicity effect at 0.8 µg/mL. Catharoseumine, a monoterpenoid indole alkaloid, has a unique peroxy bridge, which was identified as a potential inhibitor against falcipain-2 protozoa parasites (causes of malaria), showing an IC₅₀ value of 4.06 µM. Vinblastine and vincristine showed an antiparasitic effect against Trypanosoma that causes trypanosomiasis in humans, inhibiting its mitosis and affecting its cellular shape in a dose-dependent manner [27]. The use of 15 µM of vinblastine and 50 µM of vincristine inhibited cellular division and cytokinesis, and affected cellular morphology, while the effect of 3 µM of vinblastine and 10 µM of vincristine inhibited cytokinesis without affecting cell cycle progression [28, 29].

Antimicrobial activity

Vincristine was tested against microorganisms such as Pseudomonas, Salmonella, Staphylococcus and thus, these extracts show promissory effects as prophylactic agents in the treatment of many diseases. Ramya et al. evaluated the *in vitro* antibacterial activity through the use of crude extracts of *Catharanthus*. The results indicated that leaf extracts showed a higher antibacterial activity than the extracts prepared from other parts of the *Catharanthus* plant. Thus, the aqueous extracts of leaves, stems, roots and flowers showed low microorganism growth [26, 30] tested leaf extracts of *Catharanthus var. rosea*, which showed an excellent activity against Aspergillus. Stem extracts of var. alba showed a maximum inhibitory activity against Bacillus while the flowers of *Catharanthus var. rosea* showed a higher activity against Bacillus in the methanolic extract [31]. The MIC (Minimal Inhibitory Concentration) against the tested microorganisms was in the range of 100–20 mg/mL. In a different study, foliar acetonic, ethanolic, and chloroformic extracts were tested against pathogenic microorganisms to determine its antimicrobial potential [32]. The ethanolic extract showed the maximum antibacterial activity when compared to the acetonic and chloroformic extracts, in such a way that Staphylococcus was the most susceptible bacteria, followed by Escherichia, Pseudomonas and Streptococcus [33].

Anti-inflammatory activity

Several factors have been suggested as possible predictors for VIN. A study from 2010 aimed to identify predictors for chemotherapy-induced peripheral neuropathy, including vincristine, in 52 patients [34]. It was discovered that the number of chemotherapy cycles was a predictor of VIN, whereas age and co-administration with non-steroidal anti-inflammatory drugs did not correlate with neuropathy incidence and severity. Consistently, Anghelescu *et al.* observed no difference in age between ALL patients with or without neuropathy development [35]. No differences in sex, BMI group, initial leukocyte count, ALL immune phenotype, DNA index, or different genetic translocations were noted. The only significant clinical predictive variable observed was white non-Hispanic race [36].

Genotoxicity

Previous studies have shown that Vinca alkaloids have the potential to induce genotoxic effects in different biological systems. The VCR and VBL have been shown to increase the frequency of micronuclei in experimental animals and in

cultured human lymphocytes [37–40]. In addition, they have also been shown to cause chromosomal mutations *in vivo* and in cultured cancer cells [43, 45]. In cultured human lymphocytes, VRB and VCR increased the rate of micronucleus formation. In Drosophila, VCR and VBL induced a significant genotoxic effect as measured using wing somatic mutation and the recombination test [46]. However, some other studies have shown lack of mutagenic effect for Vinca alkaloids *in vivo* and in cultured cells [47–50]. Thus, the genotoxicity of Vinca alkaloids is still controversial. In addition, oxidative DNA damage induced by these compounds has still not been investigated

Ocular toxicity

Vincristine-induced ocular toxicity may be manifested as cranial nerve palsy, optic neuropathy/atrophy, or cortical blindness [51]. Depolarization of neurotubules by vincristine results in neurofibrillary degeneration and impairment of axonal transport [52]. Blindness may occur as the result of optic nerve ischemia, primary toxic axonal injury to the retinal nerve fiber layer, or disruption of microtubule polymerization [53]. The latter was also found to be associated with impairment of axoplasmic flow and loss of neurosynaptic activity in visual cells, resulting in night blindness.

Production of cell culture and tissue culture

The great pharmacological importance of the terpenoid indole alkaloids vincristine and vinblastine, associated to its low content in plants (approximately 0.0005% of dry weight), *in vitro* tissue and cell cultures, will permit the stimulation of intense research regarding the biosynthesis pathways of terpenoid indole alkaloids yet unknown through *in vitro* culture studies under biotic or abiotic elicitation strategies with the objective of increasing the production of *C. roseus* alkaloids [54, 55].

L-ASP, and dexamethasone against acute lymphoblastic leukemia is enhanced by the BH3-mimetic ABT-737

Several multiagent regimens, including the combination of vincristine, prednisone, and L-ASP, are reported to provide complete response (CR) rates of approximately 40% in multiple-relapse patients [6]. L-ASP, which depletes asparagine and glutamine in leukemic cells [11], is a critical component of therapy for childhood ALL. As a single agent, L-ASP induced complete remissions in 40% to 60% of patients with ALL, and in combination with vincristine and prednisone is associated with an initial remission rate of 95%. 12,13 Both *in vitro* and *in vivo* resistance to L-ASP has been associated with poor long-term outcome [56]. In addition, relapsed patients with greater asparagine depletion on day 14 of re-induction were more likely to achieve a second remission in the context of 6-drug therapy [57]. However, expression of asparagine synthetase (AS), which may oppose the action of L-ASP by re-synthesis of asparagine, has varied widely in clinical ALL samples, but a relationship of AS levels to drug resistance has not been reported [58, 59]. ABT-737 is a small molecule that binds to and inhibits the Bcl-2 family anti-apoptotic proteins Bcl-XL, Bcl-2, and Bcl-w. Similar to the BH3-only “sensitizing” protein Bad, ABT-737 does not directly activate Bax or Bak or induce cytochrome c release. Instead, ABT-737 binds to multidomain antiapoptotic Bcl-2 family proteins, preventing them from sequestering proapoptotic BH3-only

proteins.^{18,19} Overexpression of anti-apoptotic Bcl-2 (Bcl-2 and Bcl-XL) family proteins has been observed in acute myeloid leukemia (AML) ^[20-22], ALL ^[23, 24], and other cancers ^[60]. Bcl-XL overexpression has been reported as an independent predictor of poor event-free survival (EFS) in pediatric ALL ^[61]. Effective pharmacologic inhibition of the Bcl-2 family of proteins could lower the apoptotic threshold in leukemia cells, resulting in synergy with other chemotherapeutic agents, including drugs commonly used for remission induction in primary and relapsed leukemia, such as vincristine, glucocorticoids, and L-ASP. Using both *in vitro* and *in vivo* models of ALL, we investigated the potential for synergistic activity of ABT-737 in combination with vincristine, L-ASP, and dexamethasone (VXL), drugs commonly used.

Anti-tumour activity

Different percentages of the crude methanolic extracts have been found to show significant anticancer activity against several cell types under *in vitro* conditions and with a high activity against multidrug-resistant tumor types ^[62]. On the other hand, Ruskin and Aruna showed that the ethanolic extract of vincristine has *in vivo* antitumor activity in the Ehrlich carcinoma tumor model, while the *in vitro* study of the ethanolic extract showed significant antitumor activity

Antileukemic activity

Vincristine is employed to treat lymphocytic acute leukemia (the most frequent malign homeopathy in childhood), of which several chromosomic alterations with prognostic importance are known. Among them there are the translocation ^[4, 11] and the translocation ^[9, 22] which are indicators of a bad prognosis, while hyperdiploidy is associated with a good prognosis ^[63, 64] and it attacks lymphomas including solid tumours in children.

Antioxidant enzymatic activity

An experiment with different concentrations of sodium chloride in two varieties of *Catharanthus* (*var. alba* and *rosea*) was carried out ^[65]. It was found that the enzymatic activity of the superoxide dismutase increased, at levels of 50 mM of sodium chloride, which helps to raise the levels of this enzyme with antioxidant value ^[66].

Hematologic activity

Overall survival for adult hematologic cancer patients has improved during the past decades due to new treatment options, and more than 80% of children with acute lymphoblastic leukemia (ALL) are now long-term survivors ^[67]. This therapeutic success, however, comes with the cost of more people experiencing early and late-onset adverse effects, consequently affecting the recovering patient's QOL, which is especially important in children with a long expected lifespan after treatment. Although the intensity of the symptoms may not be extensive, the inconvenience is not correlated, and QOL can be greatly impaired ^[68]. Given the increasing numbers of cancer survivors, the clinical significance of chemotherapy-induced neuropathy is increasing; consequently, clinical and molecular risk predictors, prevention and treatment options, and measuring methods are urgently warranted ^[69]. In this paper, we systematically review parameters related to vincristine-induced neurotoxicity in hematologic patients ^[70].

Conclusion

The vincristine is anticancer drugs that act by binding to intracellular tubulin. In tumour cells, vincristine and vinblastine inhibit the DNA repair and the RNA synthesis mechanisms, blocking the DNA-dependent RNA polymerase ^[71, 72]. *C. roseus* is an important medicinal plant with several applications in pharmaceutical and industrial products. In the present, vincristine is alkaloids for the treatment of childhood leukemia and Hodgkin lymphoma. Production rate of vincristine in *C. roseus* is very low, its extraction costly, and too inefficient to be industrialized ^[73]. The semi-synthesis also faces many obstacles because of the necessary presence of precursors and intermediaries ^[74]. The great pharmacological importance of the terpenoid indole alkaloids vincristine, associated to its low content in plants (approximately 0.0005% of dry weight), *in vitro* tissue and cell cultures, will permit the stimulation of intense research regarding the biosynthesis pathways of terpenoid indole alkaloids yet unknown through *in vitro* culture studies ^[75]. Here, I conclude that vincristine has wide variety of pharmacological activity including cancer, further, studies, experiment and research give more pharmacological activity of vincristine.

Acknowledgement

Primarily I would thank God for being able to complete this review with success. Then I would like to thank my co-guide Mrs. Mamta Tiwari and all the author of this review, whose valuable guidance has been helped me to patch his review and make it full proof.

Conflict of interest

None.

Contribution

All authors participated equally.

Reference

1. Zhou X, Xu Z, Li A, Zhang Z, Xu S. Double-sides sticking mechanism of vinblastine interacting with α,β -tubulin to get activity against cancer cells. J Biomol Struct Dyn 2019;37(15):4080-4091.
2. Pernot B, Gyan E, Maillot F, Hodges P, Ertault M, Ferreira-Maldent N. Lymphomas diagnosed in an internal medicine department compared to lymphomas diagnosed in other departments: Clinical and outcome differences. Medicine (Baltimore) 2018;97(47):e13228.
3. Schiller GJ, Damon LE, Coutre SE, Hsu P, Bhat G, Douer D. High-Dose Vincristine Sulfate Liposome Injection, for Advanced, Relapsed, or Refractory Philadelphia Chromosome-Negative Acute Lymphoblastic Leukemia in an Adolescent and Young Adult Subgroup of a Phase 2 Clinical Trial. J Adolesc Young Adult Oncol 2018;7(5):546-552.
4. Martino E, Casamassima G, Castiglione S, Cellupica E, Pantalone S, Papagni F, Rui M, Siciliano AM, Collina S. Vinca alkaloids and analogues as anti-cancer agents: Looking back, peering ahead. Bioorg Med Chem Lett 2018;28(17):2816-2826.
5. Goodbody A, Endo A, Vukovic TJ, Misawa M. The coupling of catharanthine and vindoline to form 3', 4'-anhydrovinblastine by haemoproteins and haemin. Planta Med 1988;54:210-214.

6. Kutchan M. Alkaloid biosynthesis-The basis for metabolic engineering of medicinal plants. *The Plant Cell* 1995;7:1059-1070.
7. De Luca V, Fernandez JA, Campbell D, Kurz WGW. Developmental regulation of enzymes of indole alkaloid biosynthesis in *Catharanthus roseus*. *Plant Physiol* 1988;86:447-450
8. Goodbody A, Endo T, Misawa M. Alkaloid production in root and shoot cultures of *Catharanthus roseus*. *Plant Med* 1987;53:479-482.
9. Neuss N, Neuss MN. In *The Alkaloids*; Brossi, A., Suffness, M., Eds.; Academic: San Diego 1990;37:229.
10. Noble RL, Beer CT, Cutts JH, Ann NY. *Acad. Sci.* 1958, 76, 882. (b) Noble, R. L. *Lloydia* 1964;27:280.
11. Mangeney P, Andriamialisoa RZ, Lallemand JY, Langlois N, Langlois Y, Potier P. *Tetrahedron* 1979;35:2175.
12. Andriamialisoa RZ, Langlois N, Langlois Y, Potier P. *Tetrahedron* 1980;36:3053.
13. Anubhav Dubey, Deepanshi Tiwari, Kshama Srivastava, Om Prakash, Rohit Kushwaha. A discussion on vinca plant. *J Pharmacogn Phytochem* 2020;9(5):27-31
14. Fahy J, Dulfos A, Ribet JP, Jacquesy JC, Berrier C, Jouannetaud MP, Zunino FJ. *Am. Chem. Soc* 1997;119:8576.
15. Hill BT, Fiebig HH, Waud WR, Poupon MF, Colpaert F, Kruczynski A. *Eur. J Cancer* 1999;35:512.
16. Hill SA, Lonergan SJ, Denekamp J, Chaplin DJ. *Eur. J Cancer* 1993;29:1320.
17. Baguley BC, Holdaway KM, Thomsen LL, Zhuang L, Zwi LJ. *Eur. J Cancer* 1991;27:482.
18. Vacca A, Iurlaro M, Ribatti D, Minschetti M, Nico B, Ria R, Pellegrino A, Dammacco F. *Blood* 1999;94:4143.
19. Dubey Anubhav, Tiwari M, Singh Yatendra, Kumar N, Srivastava K. Investigation of anti-Pyretic activity of vinpocetine in wistar rat, *International Journal of Pharmaceutical Research* 2020;12(2):1901-1906.
20. Dubey A, Kumar R, Kumar S, Kumar N, Mishra A, Singh Y, Tiwari M. Review on Vinpocetine, *Int. J of Pharm. & Life Sci* 2020;11(5):6590-6597.
21. Misawa M, Goodbody AE. Production of antitumor compounds by plant cell cultures. In: Dicosmo, F; Misawa, M., eds. *Plant cell culture and secondary metabolism toward industrial application*. New York: CRC Press 1996, P123-138.
22. Moreno PRH, Heijden VR, Verpoote R. Cell and tissue cultures of *Catharanthus roseus*: a literature survey. *Plant cell, tissue and organ culture* 1995;42:1-7.
23. Munier F, Perentes E, Herbort CP, Uffer S, Biollaz J. Selective loss of optic nerve beta tubulin in vincristine-induced blindness. *Am J Med* 1992;93(2):232-234.
24. Rosenthal S, Kaufman S. Vincristine neurotoxicity. *Ann Intern Med* 1974;80(6):733-737.
25. Byrd RL, Rohrbach TM, Raney RB Jr, Norris DG. Transient cortical blindness secondary to vincristine therapy in childhood malignancies. *Cancer* 1981;47(1):37-40.
26. Rippas H, Carr RE, Siegel IM, Greenstein VC. Functional abnormalities in vincristine-induced night blindness. *Invest Ophthalmol Vis Sci* 1984;25(7):787-794.
27. Tiburi M, Reguly ML, Schwartzmann G, Cunha KS, Lehmann M, Rodrigues de Andrade HH. Comparative genotoxic effect of vincristine, vinblastine, and vinorelbine in somatic cells of *Drosophila melanogaster*. *Mutat Res* 2002;519(1-2):141-149.
28. Gundy S, Baki M, Bodrogi I. Vinblastine, cisplatin and bleomycin (VPB) adjuvant therapy does not induce dose-dependent damage in human chromosomes. *Neoplasma* 1989;36(4):457-464.
29. Morgan WF, Crossen PE. A comparison of induced sister chromatid exchange levels in Chinese hamster ovary cells and cultured human lymphocytes. *Environ Mutagen* 1982;4(1):65-71.
30. The Effect of Vincalukoblastine on Dividing Cells in Vise. *Cancer Res* 1961;21:168.
31. Ernwi J, DuaTni P, JR. *Colchicine in Agriculture, Medicine, Biology, and Chemistry*. Ames, Iowa: The Iowa State College Press 1955.
32. Gonzalez-Cid M, Cuello MT, Larripa I. Mitotic arrest and anaphase aberrations induced by vinorelbine in hamster cells *in vitro*. *Anticancer Drugs* 1997;8(5):529-532.
33. Alzoubi K, Khabour O, Khader M, Mhaidat N, Al-Azzam S. Evaluation of vitamin B12 effects on DNA damage induced by paclitaxel. *Drug Chem Toxicol* 2014;37(3):276-280.
34. Alzoubi K, Khabour O, Hussain N, Al-Azzam S, Mhaidat M. Evaluation of vitamin B12 effects on DNA damage induced by pioglitazone. *Mutat Res* 2012;748(1-2):48-51.
35. Alsatari ES, Azab M, Khabour OF, Alzoubi KH, Sadiq MF. Assessment of DNA damage using chromosomal aberrations assay in lymphocytes of waterpipe smokers. *Int J Occup Med Environ Health* 2012;25(3):218-224.
36. Khabour OF, Alsatari ES, Azab M, Alzoubi KH, Sadiq MF. Assessment of genotoxicity of waterpipe and cigarette smoking in lymphocytes using the sister-chromatid exchange assay: A comparative study. *Environ Mol Mutagen* 2011;52(3):224-228.
37. Alzoubi KH, Khabour OF, Jaber AG, Al-Azzam SI, Mhaidat NM, Masadeh MM. Tempol prevents genotoxicity induced by vorinostat: role of oxidative DNA damage. *Cytotechnology* 2014;66(3):449-455.
38. Fraunfelder FT, Meyer SM. Ocular toxicity of antineoplastic agents. *Ophthalmology* 1983;90(1):1-3.
39. Albert DM, Wong VG, Henderson ES. Ocular complications of vincristine therapy. *Arch Ophthalmol* 1967;78(6):709-713.
40. Schochet SS, Lampert PW, Earle KM. Neuronal changes induced by intrathecal vincristine sulphate. *J Neuropathol Exp Neurol* 1968;27(4):645-658.
41. Bargallo N, Burrell M, Berenguer J, Cofan F, Bunesch L, Mercader JM. Cortical laminar necrosis caused by immunosuppressive therapy and chemotherapy. *Am J Neuroradiol* 2000;21(3):479-484.
42. Nelson RL. The comparative clinical pharmacology and pharmacokinetics of vindesine, vincristine and vinblastine in human patients with cancer. *Med Pediatr Oncol* 1982;10(2):115-127.
43. Potarazu SV. Ischemic optic neuropathy: models for mechanism of disease. *Clin neurosci* 1997;4(5):264-269.

44. Dunker S, Hsu HY, Sebag J, Sadun AA. Perioperative risk factors for posterior ischemic optic neuropathy. *J Am Coll Surg* 2002;194(6):705-710.
45. Hayreh SS. Ischemic Optic Neuropathy. *Prog Retin Eye Res* 2009;28(1):34-62.
46. Buono LM, Foroozan R. Perioperative posterior ischemic optic neuropathy: review of the literature. *Surv Ophthalmol* 2005;50(1):15-26.
47. Ozcelik B, Kartal M, Orhan I. Cytotoxicity, antiviral and antimicrobial activities of alkaloids, flavonoids, and phenolics acids. *Pharmaceutical Biology* 2011;49:396-402.
48. Ruskin RS, Aruna SR. *In-vitro* and *in-vivo* antitumor activity of *Catharanthus roseus*. *International Research Journal of Pharmaceutical and Applied Sciences* 2014;4:1-4
49. Kyakulaga A, Hassan AT, Brenda VP. *In vivo* antidiarrheal activity of the ethanolic leaf extract of *Catharanthus roseus* Linn. (Apocynaceae) in Wistar rats. *African Journal of Pharmacy and Pharmacology* 2011;5:1797-1800.
50. Kumari K, Gupta S. Antifungal properties of leaf extract of *Catharanthus roseus* L (g.) Don. *American Journal of Phytomedicine and Clinical Therapeutics* 2013;1:698-705.
51. Ramya S, Govindaraji V, Navaneetha KK, Jayakumararaj R. *In vitro* evaluation of antibacterial activity using crude extracts of *Catharanthus roseus* L. (G.) Don. *Ethnobotanical Leaflets* 2008;12:1067-1072.
52. Tjalma W, Weyler J, Goovaerts G *et al.* Prognostic value of bcl-2 expression in patients with operable carcinoma of the uterine cervix. *J Clin Pathol* 1997;50:33-36.
53. Gruhn B, Hongeng S, Yi H *et al.* Minimal residual disease after intensive induction therapy in childhood acute lymphoblastic leukemia predicts outcome. *Leukemia* 1998;12:675-681.
54. Sheard MA, Kang MH, Cabral D *et al.* Bone marrow-level oxygen tension enables enhanced and sustained growth of 3 new pediatric acute lymphoblastic leukemia cell lines [abstract]. *Proc Am Assoc Cancer Res* 2007;48:1808.
55. O'Donnell PH, Guo WX, Reynolds CP, Maurer BJ. N-(4-hydroxyphenyl) retinamide increases ceramide and is cytotoxic to acute lymphoblastic leukemia cell lines, but not to non-malignant lymphocytes. *Leukemia* 2002;16:902-910.
56. Stong RC, Korsmeyer SJ, Parkin JL, Arthur DC, Kersey JH. Human acute leukemia cell line with the t (4; 11) chromosomal rearrangement exhibits B lineage and monocytic characteristics. *Blood* 1985;65:21-31.
57. Brown JM, Lemmon MJ. Tumor hypoxia can be exploited to preferentially sensitize tumors to fractionated irradiation. *Int J Radiat Oncol Biol Phys* 1991;20:457-461.
58. Grigoryan R, Keshelava N, Anderson C, Reynolds CP. *In vitro* testing of chemosensitivity in physiological hypoxia. *Methods Mol Med* 2005;110:87-100.
59. Masters JR, Thomson JA, Daly-Burns B *et al.* Short tandem repeat profiling provides an international reference standard for human cell lines. *Proc Natl Acad Sci USA* 2001;98:8012-8017.
60. Keshelava N, Frgala T, Krejsa J, Kalous O, Reynolds CP. DIMSCAN: a microcomputer fluorescence-based cytotoxicity assay for preclinical testing of combination chemotherapy. *Methods Mol Med* 2005;110:139-153.
61. Frgala T, Kalous O, Proffitt RT, Reynolds CP. A novel cytotoxicity assay with a 4 log dynamic range that identifies synergistic drug combinations. *Mol Cancer Ther* 2007;6:886-897.
62. Bedikian AY, Vardeleon A, Smith T, Campbell S, Namdari R. Pharmacokinetics and urinary excretion of vincristine sulfate liposomes injection in metastatic melanoma patients. *J Clin Pharmacol* 2006;46:727-737.
63. Boos J. Pharmacokinetics and drug monitoring of L-asparaginase treatment. *Int J Clin Pharmacol Ther* 1997;35:96-98.
64. Brady ME, Sartiano GP, Rosenblum SL, Zaglama NE, Bauguess CT. The pharmacokinetics of single high doses of dexamethasone in cancer patients. *Eur J Clin Pharmacol* 1987;32:593-596.
65. Hawkins DS, Park JR, Thomson BG *et al.* Asparaginase pharmacokinetics after intensive polyethylene glycol-conjugated L-asparaginase therapy for children with relapsed acute lymphoblastic leukemia. *Clin Cancer Res* 2004;10:5335-5341.
66. Nelson RL. The comparative clinical pharmacology and pharmacokinetics of vindesine, vincristine, and vinblastine in human patients with cancer. *Med Pediatr Oncol* 1982;10:115-127.
67. Diaz GD, Li Q, Dashwood RH. Caspase-8 and apoptosis-inducing factor mediate a cytochrome c-independent pathway of apoptosis in human colon cancer cells induced by the dietary phytochemical chlorophyllin. *Cancer Res* 2003;63:1254-1261.
68. Liem NL, Papa RA, Milross CG *et al.* Characterization of childhood acute lymphoblastic leukemia xenograft models for the preclinical evaluation of new therapies. *Blood*. 2004;103:3905-3914.
69. Chou TC, Talalay P. Generalized equations for the analysis of inhibitions of Michaelis-Menten and higher-order kinetic systems with two or more mutually exclusive and nonexclusive inhibitors. *Eur J Biochem*. 1981;115:207-216.
70. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations [abstract]. *Am Stat Assoc J* 1958;53:457-481.
71. Datta SR, Katsov A, Hu L *et al.* 14-3-3 proteins and survival kinases cooperate to inactivate BAD by BH3 domain phosphorylation. *Mol Cell* 2000;6:41-51.
72. Zha H, Reed JC. Heterodimerization-independent functions of cell death regulatory proteins Bax and Bcl-2 in yeast and mammalian cells. *J Biol Chem* 1997;272:31482-31488.
73. Soysal T, Ferhanoglu B, Bilir M, Akman N. Oculomotor nerve palsy associated with vincristine treatment. *Acta Haematol* 1993;90(4):209-210.
74. Lash SC, Williams CPR, Marsh CS, Crithchley C, Hodgkins PR, MacKie EJ. Acute sixth-nerve palsy after vincristine therapy. *J AAPOS* 2004;8(1):67-68.
75. Toker E, Yenice O, Ogut MS. Isolated abducens nerve palsy induced by vincristine therapy. *J AAPOS* 2004;8(1):69-71.