International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2024.115.120

Research Article
A Comparative Study of Mitotic Index and Chromosome Aberrations in Vincristine and Doxorubicin-Treated Normal Female Mice

[1]Rana Almuhur, [1]Abdulrahim Aljamal, [2]Mahmoud Al Shawabkeh, [3]Fatima-Azzahra Delmani, [4]Taha Alqadi and [5]Alia Khwaldeh

[1]Department of Biological Sciences, Al al-Bayt University, Al-Mafraq, Jordan
[2]Department of Basic Dental Sciences, Faculty of Dentistry, Applied Science Private University, Amman 19328, Jordan
[3]Department of science, Faculty of Science, Jerash University, Amman-Irbid Highway, Jordan
[4]Department of Biology, Adham University College, Umm-Al-Qura University, Makkah 24382, Saudi Arabia
[5]Department of Medical Laboratory Sciences, Faculty of Allied Medical Sciences, Jadara University, Irbid, Jordan

Abstract

Background and Objective: Cancer is a broad term encompassing a group of diseases characterized by the uncontrolled growth and spread of abnormal cells. It can affect virtually any part of the body and can arise from various factors, including genetic mutations, environmental exposures, lifestyle choices and infections. This study aimed to evaluate the effects of two chemotherapy drugs, vincristine (VCR) and doxorubicin (DOXO), commonly used in cancer treatment, on various biological markers and chromosomal integrity in normal female mice. **Materials and Methods:** Forty white female mice were divided into four groups of 10 mice each: Group 1 was treated with 0.2 mL PBS, group 2 was treated with 0.04 mg/0.1 mL vincristine (VCR), group 3 was treated with 0. 06 mg/0.1 mL doxorubicin (DOXO) outside the untreated group, serum collected from treated animals was evaluated for total sialic acid, lactate dehydrogenase, creatine kinase enzymes, mitotic index and chromosomal aberration count. **Results:** Mice treated with VCR showed significantly increased CK levels in bone marrow cells while LDH levels remained unchanged. The TSA levels in serum and bone marrow homogenates were significantly reduced in the VCR and DOXO-treated groups (21 and 26-30%, respectively). The MI also decreased significantly in the treatment groups (VCR = 22%, DOXO = 30%). Chromosomal structural aberrations were also observed in the form of breaks, loops or other forms, although DOXO caused more aberrations. **Conclusion:** The VCR and DOXO were effective in reducing the severity of TSA and MI as well as changing chromosomal patterns. These results suggest that TSA can be used as an indicator of *in vivo* cellular response in cancer patients treated with chemotherapy.

Key words: Vincristine, doxorubicin, mitotic index, chromosomal aberrations, sialic acid

Citation: Almuhur, R., A. Aljamal, M. Al Shawabkeh, F.A. Delmani, T. Alqadi and A. Khwaldeh, 2024. A comparative study of mitotic index and chromosome aberrations in vincristine and doxorubicin-treated normal female mice. Int. J. Pharmacol., 20: 115-120.

Corresponding Author: Rana Almuhur, Department of Biological Sciences, Al Al-Bayt University, Al-Mafraq, Jordan

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The potency of chemotherapeutic drugs to control uncontrolled growth of tumor cells is variable, including alkylating agents, antimicrotubule agents and topoisomerase inhibitors. Alkylating agents can alkylate proteins, RNA and DNA, cross-link with double-stranded DNA, causing breaks and leading to apoptosis. Therefore, alkylating agents are cell cycle independent[1]. Anti-microtubule agents are plant derivatives that block microtubule function and prevent cell division. These structures are dynamic and their assembly and disassembly are correlated with specific phases of the cell cycle[2]. Vincristine drug (VCR) is a vinca alkaloid gained from *Catharanthus roseus*[3]. It is also known as leurocristine, used in the treatment of several types of cancer, including acute lymphocytic leukemia, acute myeloid leukemia, Hodgkin lymphoma, neuroblastoma and small cell lung cancer[4]. It is included in the World Health Organization's 2019 list of essential medicines and has been approved by the Food and Drug Administration since 1963 under the trade name Oncovin[5]. Reports have confirmed its ability to inhibit leukocyte production and maturation[6]. However, one of the disadvantages is that it affects all types of rapidly dividing cells and therefore very specific drug delivery is essential[7]. Doxorubicin (DOXO), chemically known as 14-hydroxy daunorubicin, is a chemotherapy agent extracted from *Streptomyces peucetius*[8] and sold under the trade name Adriamycin. It is on the World Health Organization's List of Essential Medicines, Geneva 2019, 2021 and on the UK National Medicines Formulary: BNF 69, 2015. The drug is used to treat many types of cancer, including breast cancer, brain cancer, lymphoma and acute lymphocytic leukemia[9,10]. Some side effects have been associated with treatment with this medication, including hair loss, bone marrow suppression, vomiting, skin rash, stomatitis, allergic reactions, anaphylactic shock, heart damage, urine color and cardiomyopathy[11].

The assessment of tumor initiation and progression has improved significantly to date with the identification of hundreds of tumor markers[12]. These substances can be produced by the tumor itself or the body in response to a benign condition[12]. Measuring tumor marker level before starting cancer treatment allows doctors to determine the extent of the disease before treatment and, when measured during treatment, to monitor the patient's response to treatment. If tumor markers decrease to normal levels after treatment, this may be an indication of a positive response to cancer treatment[12,13]. In this study, Lactate Dehydrogenase (LDH)[14], creatine kinase (CK) (National Cancer Institute, 2000) and total sialic acid (TSA)[15] were measured and used as cell biomarkers for injury.

The purpose of this study was to evaluate the effects of two commonly used chemotherapy drugs, VCR and DOXO, on LDH, CK, Sialic acid (SA), chromosomal structural abnormalities (CSA) and mitotic index (MI) cells in blood serum and bone marrow cells in a normal female mouse model.

This is a novel study to evaluate the comparative effects of DOXO and VCR on CSA and MI compared with TSA levels in normal female rats.

MATERIALS AND METHODS

Study area: This study was done in Al-Mafraq City in Jordan starting from April, 2023 and finished in September, 2023.

Animals: Forty mice were procured from the Department of Medical Technology, Al-Ahliyya Amman University, Jordan. Then divided into 4 groups, each group of 10 mice: Control mice group 1 was not treated, group 2 was treated with PBS, group 3 was treated with 0. 04 mg/0.1 mL VCR and group 4 was treated with 0.06 mg/0.1 mL DOXO.

Animals were treated orally once daily for 10 days and then sacrificed by cervical dislocation.

Blood was collected in non-heparinized tubes to coagulate and then centrifuged at 1200 rpm for 10 min and collected serum was stored at -20°C until further analysis.

Harvesting bone marrow cells: Bone marrow cell suspensions were collected from mouse femurs, removed and cleared from the muscle and then injected with PBS (pH 7.2) into one end of the bone. The cell suspension was collected into a sterile test tube and centrifuged at 2000 rpm for 10 min. The supernatant was discarded and the pelleted cells were stored at -20°C until further evaluation.

Biochemical analysis: Serum collected from treated animals was evaluated for LD[16] and CK[17] enzymes based on spectrophotometric methods. Quantitative determination of TSA by colorimetric method at 549 nm wavelength is performed using the SIGMA-Aldrich (MAK314) kit.

Mitotic index (MI) and chromosomal aberration count (CAC): The cell suspension obtained in a sterile culture medium tube was spread on a glass slide and stained with Giemsa dye

for 2-3 min, washed with Sorensen's buffer and allowed to air dry at room temperature. Slides were analyzed under a compound light microscope Olympus microscopes made in Japan with an oil immersion lens (100×) to determine mitotic index and chromosomal aberrations.

Mitotic index (MI) is defined as the ratio between the number of mitotic cells and interphase nuclei in 100 cells applied to the equation[18]:

$$\text{Mitotic index (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells}} \times 100$$

Chromosomal aberrations were assessed according to Sharma and Sharma[19]. Around 100 metaphase cells were analyzed per treatment to score chromosomal changes.

Statistical analysis: Collected data were analyzed by two way ANOVA and SPSS, version 22. Results are regarded as statistically significant at a probability value $p<0.05$.

Ethical approval: The experimental protocol underwent approval by the Animal Ethical Committee within the Department of Biological Sciences at Al-Bayt University in Mafraq, Jordan. Animals were handled and cared for in compliance with CPCSEA regulations before the experiment. Acute toxicity was assessed following the guidelines set by the OECD.

RESULTS

The results in Table 1 show serum LDH and CK enzyme levels after treatment with VCR or DOXO. Both chemotherapy drugs (VCR and DOXO) significantly reduced the amount of LD enzyme compared to the normal group or the PBS group. The CK enzyme concentration increased significantly (50%) in the VCR group (463±7.74 to 692±11.47) but decreased insignificantly (7%) in the DOXO group.

The TSA values in serum and bone marrow cells were shown in Table 2. The values show that VCR and DOXO significantly reduced serum TSA concentrations (from 475 to 375 (21%) and from 475 to 350 (26%), respectively) and from 10.95 to 8.60 (20%) and bone marrow depression from 10.95 to 7.05 (30%).

It was also found that the mean MI of bone marrow cells was significantly reduced in the VCR and DOXO-treated groups. The VCR resulted in a 22% reduction (from 2.3 to 0.8) while DOXO resulted in a 30% reduction (from 2.3 to 0.6) (Table 3).

Study of Giemsa-stained chromosomes from chemotherapy-treated mice revealed distinctive abnormalities comparable to control mice.

Chromosomal aberrations in response to VCR and DOXO included breaks (7 and 9%, respectively), ring chromosome formation (1% each) and other structure formation (13 and 20%, respectively (Table 4). Additionally, total chromosomal aberrations (TCA) recorded compared to VCR and DOXO were 21 and 30%, respectively.

Table 1: Measurement of LDH and CK in serum of female mice treated with VCR and DOXO chemotherapeutic drugs

Group	LDH level (n = 5)	CK level (n = 5)
Normal[a]	92.43±1.53	461.8±33.5*[c]
PBS (control)[b]	93.00± 0.58	463±7.74*[c]
VCR 0.04 mg/0.1 mL[c]	88.00±1.15	692±5.47 11.47*[abd]
DOXO 0.06 mg/0.1 mL[d]	88.00±1.73	427±5.74*[c]

DOXO: Doxorubicin, LDL: Lactate Dehydrogenase, CK: Creatine kinase, Data are presented as Mean±Standard deviation, [a]Normal, [b]PBS (control), [c]VCR 0.04 mg/0.1mL, [d]DOXO 0.06 mg/0.1 mL and statistically significance level at $p<0.05$ and *$p<0.05$

Table 2: Measurement of total sialic acid (TSA) in sera and bone marrow cells of female mice treated with VCR and DOXO chemotherapeutic drugs

Group	TSA (Blood sera) (n = 5)	TSA (Bone marrow homogenate) (n = 5)
Normal (untreated control)[a]	462.5	10.10±0.15
PBS treated (control)[b]	475	10.95±0.30
VCR treated (0.04 mg/0.1 mL)[c]	375**	8.60±0.37*
DOXO treated (0.06 mg/0.1 mL)[d]	350**	7.05±0.28**

TSA: Total sialic acid, Data are shown as Mean±Standard deviation and significance level $p<0.05$, *$p<0.05$ and **$p<0.001$

Table 3: Mitotic index (MI) of bone marrow cell in different groups of female mice treated with VCR and DOXO chemotherapy drugs

Group	MI
Normal[a] (n = 5)	2.30±0.12
VCR 0.04 mg/0.1 mL (n = 5)	0.80±0.20**[aa]
DOXO 0.06 mg/0.1 mL (n = 5)	0.60±0.19**[a]

MI: Mitotic index, Data are presented as Mean±Standard deviation, [a]Normal and significance level $p<0.05$, *$p<0.05$ and **$p<0.001$

Table 4: Chromosomal aberration of Bone marrow cell in different groups of female mice treated with VCR and DOXO chemotherapy drugs

		Chromosomal aberrations			
Group	Number of cells	Break	Ring	OSA	TCA (%)
Normal	100	0	0	0	0
VCR 0.04 mg/0.1mL	100	7	1	13	21
DOXO 0.06 mg/0.1mL	100	9	1	20	30

OSA: Other structural abnormalities and TCA: Total chromosomes aberrations observed

DISCUSSION

The lactate dehydrogenase level in the chemotherapy-treated mice group (VCR and DOXO) was not far different from the control group, suggesting that VCR and DOXO have no role in activating metabolic pathways based on the release of LDH. The LDH is the enzyme that catalyzes the conversion of pyruvate to lactate and vice versa, as it converts NAD^+ to NADH in both directions of the reaction. It is found in most living cells and is widely expressed in tissues. It is released when tissue is damaged and is therefore used as a common marker of injury and disease[14]. The LDH is correlated with tumor initiation and metabolism.

Cancer cells undergo a Warburg effect[20], in which the majority of their stored glucose is converted to lactate, favoring fermentative glycolysis, even under fully aerobic conditions. This leads to faster cell growth and multiplication than energy production alone[20-23]. Therefore, LDH is often used as a non-specific tumor marker[20-22].

The significant increase in CK levels in VCR-treated mice, but not in the DOXO group, nor in the control group, suggests a role for VCR in inducing cell damage. The most common side effects of vincristine treatment that have been reported include sensory problems, hair loss, constipation, difficulty walking, headaches, lung damage and low white blood cell counts[4]. Reports have confirmed its ability to inhibit leukocyte production and maturation[6]. However, one of the disadvantages is that it affects all types of rapidly dividing cells and therefore very specific drug delivery is essential[7]. Elevated blood CK can indicate several tissue damages, such as rhabdomyolysis, myocardial infarction, myocarditis, endocrine disorders and musculoskeletal diseases[24].

The TSA levels were significantly reduced in mice treated with VCR and DOXO chemotherapy. This decrease may be due to drug interference with RNA synthesis or protein synthesis[25], which would affect serum concentrations of glycoproteins and one component, sialic acid. The reduction in TSA observed after DOXO treatment was consistent with the results of a study Koyama *et al.*[26], which showed that actinomycin inhibits oligosaccharide and glycoprotein synthesis. The decrease in TSA levels after DOXO treatment may be due to its negative effect on sialyltransferase activity, which inhibits AS and prevents the release of sialic acid from cells[27]. The TSA may be a potential biomarker of tumors because levels are elevated in patients with certain types of cancer[28,29] and decrease after treatment with chemotherapy[30]. Other studies have shown that TSA returns to normal levels after chemotherapy treatment[29,31] and thus this biomarker may be useful in assessing response to chemotherapy[32].

Studies of the integrity, structure and karyotype of nuclear chromosomes are important aspects of cancer research[33]. Present study showed chromosomal changes, including breakage and ring formation, after mice were treated with VCR or DOXO compared with controls. Other cytogenetic studies in mice after chemotherapy treatment showed an increase in chromosomal numerical and structural abnormalities[30].

The VCR is known to interfere with the cell cycle in S and G1 phases, causing abnormalities[34], due to affected nucleoproteins[35]. Vincristine binds to tubulin protein and participates in tubulin dimerization and microtubule formation. This causes defects in chromosome segregation during cell division[5] and leads to apoptosis[36]. Some antitumor chemotherapeutic drugs with antibiotic effects, such as actinomycin D, can cause chromosomal abnormalities in hamster lymphocyte cultures through the induction of breakage chromosomes, bicentric chromosomes and chromosome exchange[37]. Doxorubicin interacts with DNA, causing inhibition of macromolecule biosynthesis[38]. It also inhibits the progression of topoisomerase II activity in unwinding DNA supercoils during transcription[39]. By stabilizing topoisomerase II which partially intercalates between DNA base pairs after breaking the DNA strand for replication, shutting down the integral enzyme prevents DNA release and stops replication[38]. Its cytotoxicity may also be due to its role in the production of free radicals, especially the quinone type[39]. Cells exposed to DOXO were found to have several deficiencies in the cellular response to DNA damage, such as epigenetic and transcriptional dysregulation[40] after histone export of transcriptionally active chromatin[40].

CONCLUSION

The study's findings on LDH, CK, TSA and chromosomal alterations post-VCR and DOXO treatments offer potential implications for clinical assessments, treatment response evaluations and advancements in understanding drug-induced cytotoxic effects. Further research validating these biomarkers and elucidating underlying mechanisms is essential for optimizing cancer therapies and improving patient outcomes.

SIGNIFICANCE STATEMENT

The investigation into vincristine (VCR) and doxorubicin (DOXO) chemotherapy effects on female mice revealed significant findings: VCR increased bone marrow CK levels, while both drugs reduced total sialic acid (TSA), indicating cellular changes, decreased mitotic index (MI), slowing cell division crucial in cancer treatment. Genetic effects: DOXO caused more chromosomal aberrations, highlighting potential differences in their genotoxic impact. These results provide vital insights into how VCR and DOXO affect cellular and genetic levels, paving the way for potential clinical applications in cancer treatment and monitoring patient response.

ACKNOWLEDGMENT

The authors thank all staff in the medical laboratory for their analysis and support during the study.

REFERENCES

1. Malhotra, V. and M.C. Perry, 2003. Classical chemotherapy: Mechanisms, toxicities and the therapeutc window. Cancer Biol. Ther., 2: 1-3.
2. Rowinsky, E.K. and R.C. Donehower, 1991. The clinical pharmacology and use of antimicrotubule agents in cancer chemotherapeutics. Pharmacol. Ther., 52: 35-84.
3. Ravina, E., and H. Kubinyi, 2011. The Evolution of Drug Discovery: From Traditional Medicines to Modern Drugs. John Wiley & Sons, Hoboken, New Jersey, ISBN: 9783527326693, Pages: 528.
4. Schiller, G.J., L.E. Damon, W. Stock, S.E. Coutre, P. Hsu, L. Prasad and D. Douer, 2015. Marqibo®, vincristine sulfate liposome injection, for the treatment of advanced, relapsed or refractory philadelphia chromosome-negative (Ph-) acute lymphoblastic leukemia (ALL) in an adolescent young adult (AYA) population. Blood, Vol. 126. 10.1182/blood.V126.23.1291.1291.
5. Avendaño, C. and J.C. Menéndez, 2015. Anticancer Drugs Targeting Tubulin and Microtubules. In: Medicinal Chemistry of Anticancer Drugs, Avendaño, C. and J.C. Menéndez (Eds.), Elsevier, Amsterdam, Netherlands, ISBN: 9780444626493, pp: 359-390.
6. Silverman, J.A. and S.R. Deitcher, 2013. Marqibo® (vincristine sulfate liposome injection) improves the pharmacokinetics and pharmacodynamics of vincristine. Cancer Chemother. Pharmacol., 71: 555-564.
7. Morris, P.G. and M.N. Fornier, 2008. Microtubule active agents: Beyond the taxane frontier. Clin. Cancer Res., 14: 7167-7172.
8. Ajaykumar, C., 2020. Overview on the Side Effects of Doxorubicin. In: Advances in Precision Medicine Oncology, Arnouk, H. and B. Hassan (Eds.), IntechOpen, London, United Kingdom, ISBN-13: 978-1-83968-868-3, Pages: 260.
9. Rivankar, S., 2014. An overview of doxorubicin formulations in cancer therapy. J. Cancer Res. Ther., 10: 853-858.
10. Peter, S., S. Alven, R.B. Maseko and B.A. Aderibigbe, 2022. Doxorubicin-based hybrid compounds as potential anticancer agents: A review. Molecules, Vol. 27. 10.3390/molecules27144478.
11. Chatterjee, K., J. Zhang, N. Honbo and J.S. Karliner, 2010. Doxorubicin cardiomyopathy. Cardiology, 115: 155-162.
12. Sharma, S., 2009. Tumor markers in clinical practice: General principles and guidelines. Indian J. Med. Paediatr. Oncol., 30: 1-8.
13. Basuyau, J.P., M. Leroy and P. Brunelle, 2001. Determination of tumor markers in serum. Pitfalls and good practice. Clin. Chem. Lab. Med., 39: 1227-1233.
14. Parhamifar, L., H. Andersen and S.M. Moghimi, 2013. Lactate Dehydrogenase Assay for Assessment of Polycation Cytotoxicity. In: Nanotechnology for Nucleic Acid Delivery: Methods and Protocols, Ogris, M. and D. Oupicky (Eds.), Humana Press, Totowa, New Jersey, ISBN: 978-1-62703-140-0, pp: 13-22.
15. Fuster, M.M. and J.D. Esko, 2005. The sweet and sour of cancer: Glycans as novel therapeutic targets. Nat. Rev. Cancer, 5: 526-542.
16. Shah, F.Y., D.C. Shetty, G. Kaur, S. Gupta, A. Pathak and V. Aggarwal, 2023. Serum and saliva LDH levels in patients with habit, oral potentially malignant disorders (OPMDs), and oral squamous cell carcinoma (OSCC): A spectrophotometry study. J. Pharm. BioAllied Sci., 15: S881-S884.
17. Faulker, W.K. and S. Meites, 1982. Selected Methods for the Small Clinical Chemistry Laboratory. American Association for Clinical Chemistry, Incorporated, Washington, DC, ISBN: 9780915274130, Pages: 398.
18. Wulffraat, N.M., F.C. de Waal, I.H. Stamhuis, G.J. Broekema and A.H. Loonen, 1985. Bone marrow mitotic index: A methodological study. Acta Haematol., 73: 89-92.
19. Sharma, A.K. and A. Sharma, 1980. Chromosome Techniques: Theory and Practice. 3rd Edn., Butterworth-Heinemann, Oxford, United States, ISBN: 978-0-408-70942-2, Pages: 711.

20. Warburg, O., 1956. On the origin of cancer cells. Science, 123: 309-314.
21. Kim, H.S., H.E. Lee, H.K. Yang and W.H. Kim, 2014. High lactate dehydrogenase 5 expression correlates with high tumoral and stromal vascular endothelial growth factor expression in gastric cancer. Pathobiology, 81: 78-85.
22. Xu, H.N., S. Kadlececk, H. Profka, J.D. Glickson, R. Rizi and L.Z. Li, 2014. Is higher lactate an indicator of tumor metastatic risk? a pilot MRS study using hyperpolarized ^{13}C-pyruvate. Acad. Radiol., 21: 223-231.
23. O'Dwyer, A.M. and N.P. Sheppard, 1993. The role of creatine kinase in the diagnosis of neuroleptic malignant syndrome. Psychol. Med., 23: 323-326.
24. Hekimsoy, Z. and I.K. Oktem, 2005. Serum creatine kinase levels in overt and subclinical hypothyroidism. Endocr. Res., 31: 171-175.
25. Sun, Y., Y. Liu, X. Ma and H. Hu, 2021. The influence of cell cycle regulation on chemotherapy. Int. J. Mol. Sci., Vol. 22. 10.3390/ijms22136923.
26. Koyama, H., D. Ayusawa, M. Okawa, A. Takatsuki and G. Tamura, 1982. Tunicamycin-resistant mutations in mouse FM3A cells. Mutat. Res. Fundam. Mol. Mech. Mutagen., 96: 243-258.
27. Natoni, A., R. Bohara, A. Pandit and M. O'Dwyer, 2019. Targeted approaches to inhibit sialylation of multiple myeloma in the bone marrow microenvironment. Front. Bioeng. Biotechnol., Vol. 7. 10.3389/fbioe.2019.00252.
28. Maubach, P.A., B. Emmerich, A. Willer, A. Ogilvie and J. Rastetter, 1984. Urinary glycoproteins in acute leukemias: A 41000 dalton glycoprotein follows the kinetic of cytoreduction. Blut, 48: 243-246.
29. Berghuis, A.Y., J.F.A. Pijnenborg, T.J. Boltje and J.M.A. Pijnenborg, 2022. Sialic acids in gynecological cancer development and progression: Impact on diagnosis and treatmenty. Int. J. Cancer, 150: 678-687.
30. Lipton, A., H.A. Harvey, S. Delong, J. Allegra, D. White, M. Allegra and E.A. Davidson, 1979. Glycoproteins and human cancer: 1. Circulating levels in cancer serum. Cancer, 43: 1766-1771.
31. Greffard, A., N. Trabelsi, H. Terzidis, J. Bignon, M.C. Jaurand and Y. Pilatte, 1997. Inhibition of acid sialidase by inorganic sulfate. Biochim. Biophys. Gen. Subj., 1334: 140-148.
32. Dong, X., X. Xu, F. Han, X. Ping, X. Yuang and B. Lin, 2001. Determination of sialic acids in the serum of cancer patients by capillary electrophoresis. Electrophoresis, 22: 2231-2235.
33. Miller, O.J. and E. Therman, 2001. Human Chromosomes. Springer Science & Business Media, Berlin/Heidelberg, Germany, ISBN: 9780387950464, Pages: 501.
34. Delgado, M., R.R. Rainwater, B. Heflin, A. Urbaniak and K. Butler *et al*., 2022. Primary acute lymphoblastic leukemia cells are susceptible to microtubule depolymerization in G1 and M phases through distinct cell death pathways. J. Biol. Chem., Vol. 298. 10.1016/j.jbc.2022.101939.
35. Kanda, N. and H. Kato, 1979. *In vivo* sister chromatid exchange in cells of various organs of the mouse. Chromosoma, 74: 299-305.
36. Jordan, M.A., 2002. Mechanism of action of antitumor drugs that interact with microtubules and tubulin. Curr. Med. Chem. Anti-Cancer Agents, 2: 1-17.
37. Cherry, L.M. and T.C. Hsu, 1982. Restitution of chromatid and isochromatid breaks induced in the G_2 phase by actinomycin D. Environ. Mutagen., 4: 259-265.
38. Tacar, O., P. Sriamornsak and C.R. Dass, 2013. Doxorubicin: An update on anticancer molecular action, toxicity and novel drug delivery systems. J. Pharm. Pharmacol., 65: 157-170.
39. Pommier, Y., E. Leo, H. Zhang and C. Marchand, 2010. DNA topoisomerases and their poisoning by anticancer and antibacterial drugs. Chem. Biol., 17: 421-433.
40. Pang, B., X. Qiao, L. Janssen, A. Velds and T. Groothuis *et al*., 2013. Drug-induced histone eviction from open chromatin contributes to the chemotherapeutic effects of doxorubicin. Nat. Commun., Vol. 4. 10.1038/ncomms2921.