eISSN: 09748369

Clastogenic effects of the anticancer drug Epirubicin on mouse bone marrow cells

Biology and Medicine

RS Pandit, RC Choudhury

Published: 6th Jan 2012

Volume 3, Issue 5, Page 43-49, 2011

www.biolmedonline.com

Clastogenic effects of the anticancer drug Epirubicin on mouse bone marrow cells

RS Pandit, RC Choudhury*
Department of Zoology, Berhampur University, Berhampur - 760 007, Odisha, India.

***Corresponding Author:** rameshchoudary@hotmail.com

Abstract
Epirubicin, a semi-synthetic anthracycline antibiotic, is widely prescribed singly or in combination therapy for the treatment of various types of cancers. However, the reported occurrence of second malignancy in epirubicin pre-treated cancer survivors has necessitated its cytogenotoxicity testing. Therefore, the clastogenic potential of epirubicin was assessed here from bone marrow cells of Swiss mice after single intraperitoneal administration of the drug. Each of the three tested doses of epirubicin (2, 4 and 6 mg kg^{-1} b. w.) induced significantly high percentages ($p \leq 0.01$) of aberrant metaphases and chromosomal aberrations (excluding gaps) at 24 h post-treatment and significant increase ($p \leq 0.05$) in the frequency of micronucleus in polychromatic erythrocytes at 30 h post-treatment. Thus, epirubicin was highly clastogenic to bone marrow cells of Swiss mice. Its interference in the activity of topoisomerase-II and its free radical generation potential were attributed to its clastogenicity. Such clastogenic effects of epirubicin might have been the cause of recurrence of second malignancy in post-chemotherapeutic cancer survivors.

Keywords: Epirubicin; Anthracycline antibiotic; Chromosome aberration; Micronucleus; Bone marrow cells; Swiss mice.

Introduction

Anthracycline-antibiotics are widely prescribed as antineoplastic agents for the successful treatment of various types of cancers, particularly leukaemia (Bhutani *et al*., 2002), solid tumours (Minotti *et al.*, 2004; Wasowska *et al.,* 2005) and breast cancer (Gianni *et al*., 2009). Epirubicin (EPI), a semi-synthetic anthracycline antibiotic, isolated from *Streptomyces peucetius var. caesius*, is the 4'-epimer of doxorubicin (DOX). The sugar moiety of EPI differs from the natural daunosamine sugar in steric configuration of the hydroxyl group bearing C-4 in inverted position, thereby forming the L-arabino configuration of EPI instead of the L-lyxo in DOX. The anthracycline ring of EPI is lipophilic and its saturated end of the ring system contains the hydroxyl group adjacent to the amino sugar by producing a hydrophilic centre. Both the acidic function in phenolic ring and basic function in amino group confer EPI as amphoteric (Plosker and Faulds, 1993; Minotti *et al*., 2004; Wasowska *et al*., 2005). In cancer chemotherapy, EPI is prescribed singly (Bastholt *et al*., 1996; Lopez *et al*., 2002; Barni *et al*., 2004) or as a major component in combination chemotherapy regimen (de Azambuja *et al*., 2009) for the effective treatment of various types of cancers. Moreover, dose-response relationship of EPI (Focan *et al*., 1993; Bastholt *et al*., 1996) with provision of its dose intensification (Lanchbury and Habboubi, 1993) has facilitated the treatment of different malignancies (The French Epirubicin Study Group, 2000; Lopez *et al.,* 2002).

However, treatments with EPI not only exhibit multiple side effects (Minotti *et al*., 2004) but also induce an increased risk of secondary leukaemia (Campone *et al*., 2005; Praga *et al*., 2005; Mays *et al*., 2010). These causes of concern necessitated the assessment of the cytogenotoxic potential of EPI. But, there is sporadic occurrences of such test reports (Othmann, 2000; Brumfield and Mackay, 2002; Lehman *et al*., 2003, Buschini *et al*., 2003; Sen *et al*., 2010), which have been carried out either *in vitro*, or in lower organisms *in vivo*, or after chronic exposures of EPI along with other drugs. The results of such studies are fragmentary, inadequate and inconclusive. Therefore, the present study has been undertaken to evaluate the clastogenic potential of the lone drug EPI from bone marrow cells of Swiss mice after a single intraperitoneal exposure of three different doses.

Materials and Methods

Test animal

Swiss albino mice (*Mus musculus*), initially procured from M/S Ghosh Enterprise, Kolkata, India, were reared in the animal house of the

department in hygienic condition. The animal house was maintained at an optimal temperature of 23 ± 2^0 C with a 12 h light/dark cycle. The mice were provided with balanced diet and drinking water *ad libitum.* Healthy mice of approximately 20g b. w. each were selected for experiments from among the reared stock of mice.

Test chemicals

Alrubicin® (Epirubicin hydrochloride injection), manufactured by Alkem Laborataries Ltd., Mumbai, India, was used as the test chemical. It is a semi-synthetic anthracycline cytotoxic antibiotic. Ledoxan (Cyclophosphamide injection), manufactured by Dabur Pharma, New Delhi, India, was used as the positive control chemical. Sodium Chloride (NaCl) was used as the negative control.

Dose of the chemicals and route of administration

EPI is generally prescribed in combination with other drugs, such as cyclophosphamide + epirubicin + 5-fluorouracil (CEF). The FDA approved doses are: (1) CEF 120 (with EPI @ $60mg/m^2$) i. v. on days 1, 8 repeated every 28 days for six cycles, or (2) CEF 100 (with EPI @ $100mg/m^2$) on day 1 and repeated 21 days for six cycles. Therefore, in accordance with human therapeutic dose, three different doses of EPI (2, 4 and 6 mg/kg b. w.) were selected for testing on mice by taking the relative b. w. and surface area of mouse to that of an adult person into considerations. Cyclophosphamide (CY) 40 mg/kg b. w. of mice was selected as the dose of positive control chemical and 0.9% sodium chloride @ 10ml/kg b. w. was used as the negative control. Dilution of the chemicals was made in such a way that the volume of each treatment was maintained to 1ml/100g b. w. of mice. The tests were undertaken after single intraperitoneal treatment of the chemical to mice for one cell cycle duration which is suitable for the assessment of clastogenicity.

Endpoints studied

1. Mitotic metaphase chromosome aberration (CA) study from bone marrow cells at 24h post-treatment.
2. Micronucleus Test (MNT) from polychromatic erythrocytes (PCEs) at 30h post-treatment.

Experimental protocol

Sixty healthy mice (30 females and 30 males), approximately 20g b. w. each, were selected and randomly divided into five groups of 12 mice (6 females and 6 males) each. One group, the negative control group, was treated with 0.9% sodium chloride @ 1 ml/100g b. w. Another group, the positive control group, was treated with CY@ 40mg/kg b. w. Rest three groups were treated with EPI @ 2, 4 or 6 mg/kg b. w. At 24h post-treatment, six mice (3 females and 3 males) from each of the treated groups were employed in CA study and the rest six mice of each group (3 females and 3 males) were utilized in MNT at 30h post-treatment, which is required for enucleation of the main nucleus and formation of PCE.

Procedure

Slides for mitotic metaphase CA study from bone marrow cells at 24h post-treatment were prepared following the colchicine- sodium citrate hypotonic- methanol, glacial acetic acid- flame drying- Giemsa technique of (Choudhury *et al*., 2000). More than 150 well spread metaphases were scanned from each animal under binocular research microscope. Aberrant metaphases and different CAs in them were recorded. Percentages of aberrant metaphases (including metaphases with only gaps) and aberrations (excluding gaps) per 100 metaphases were calculated. Each pulverized metaphase was equated to 10 aberrations in calculation.

Slides for MNT were prepared from bone marrow cells at 30h post-treatment following the simple procedure of (Choudhury *et al*., 2000). About 2000 blue tinged PCEs were scanned from each animal under binocular research microscope and the MN in them were recorded. MN per 1000 PCEs was calculated.

Statistical analysis

Averages with standard deviations were calculated from the recorded data at the two different endpoints of different groups of mice, and were pooled together for the male and female mice of each group separately. The calculated average data of different endpoints for the positive control group and that of the EPI-treated groups were compared with the respective data of the negative control groups of mice. Statistical significance of the differences among the respective pooled data at different levels ($p \leq 0.05$ or $p \leq 0.01$) were ascertained from the tables of (Kastenbaum and Bowman, 1970) which were prepared specifically for mutation studies.

Results

The percentages of aberrant metaphases in the female and male mice of the negative control group were 2.57± 0.66 and 3.01±0.47, and that of the positive control group were 63.33±0.35 and 51.29±0.68 respectively. The induced percentages of aberrant metaphases by the three different doses of EPI (2, 4 or 6 mg/kg b. w.) in the female and male mice were 12.44±2.14 and 22.22±8.49, 19.99±2.90 and 24.66±4.66, and 25.33±6.96 and 29.77±5.09, respectively. The increased percentages of aberrant metaphases in the positive control group and in the EPI-treated groups of mice were significantly higher ($p \leq 0.05$ or $p \leq 0.01$) than that of the respective negative control group of mice (Table 1).

Average CAs (excluding gaps) per 100 metaphases in female and male mice of the negative control group were 0.64±0.03 and 0.33±0.29, and that of the positive control group were 110.65±4.35 and 81.31±3.35 respectively. EPI 2, 4 and 6 mg/kg-induced CAs (excluding gaps) per hundred metaphases in female and male groups of mice were 33.99±8.96 and 29.55±7.89, 40.88±3.67 and 55.99±1.15, and 72.88±10.66 and 70.22±11.49, respectively. The positive control chemical and all the three different doses of EPI-induced CAs were increased significantly ($p \leq 0.01$) from that of the respective negative control mice (Table 1).

Table 1: Cyclophosphamide (CY)- and Epirubicin (EPI)–induced chromosomal aberrations (CAs) in bone marrow cells of mice at 24 h post-treatment.

Chemicals	Dose (mg Kg^{-1} body weight)	Number of mice treated & gender	Number of metaphases examined	Number of aberrant metaphases	Type and number of CAs							Total CAs (excluding gaps)	Average percentage of aberrant metaphases ±SD	Average CAs (excluding gaps) per 100 metaphases ±SD
					Chromatid type		Chromosome type		Frag	Min	Pul			
					Gap	Brk	Gap	Brk						
0.9% NaCl (Negative control)	10 (ml kg^{-1})	3f	465	12	10	--	2	--	3	--	--	3	2.57±0.66	0.64±0.03
		3m	596	18	15	1	2	--	1	--	--	2	3.01±0.47	0.33±0.29
EPI	2	3f	450	56	12	20	3	--	13	5	11	153	12.44±2.14*	33.99±8.96**
		3m	450	110	11	90	4	--	37	3	--	133	22.22±8.49**	29.55±7.89**
	4	3f	450	90	19	48	2	--	20	13	9	214	19.99±2.90**	40.88±3.67**
		3m	450	111	13	128	1	--	32	5	11	282	24.66±4.66**	55.99±1.15**
	6	3f	500	129	24	115	--	--	19	12	16	338	25.33±6.96**	72.88±10.66**
		3m	450	134	13	145	1	--	17	17	12	316	29.77±5.09**	70.22±11.49**
CY(Positive control)	40	3f	377	239	56	75	15	20	205	48	--	416	63.33±0.35**	110.65±4.35**
		3m	427	219	87	104	22	60	158	37	--	348	51.29±0.68**	81.31±3.35**

SD - standard deviation, Frag - fragment, Min - minute, Pul - pulverization, Brk - break, f - female, m - male, (*) p 0.05, (**) p 0.01

Average MN per thousand PCEs in the female and male mice of the negative control and positive control groups were 1.99±0.77 and 3.66±0.83, and 17.15±1.05 and 29.85±4.02, respectively. Increase in the induction of MN in the positive control group of mice was statistically highly significant ($p \leq 0.01$). EPI 2, 4 and 6 mg/kg-induced average MN per 1000 PCEs in the female and male mice were 9.06±0.61 and 8.35±0.84, 9.35±1.63 and 8.57±2.04, and 9.87±0.65 and 9.85±1.15, respectively. All the three doses of EPI-induced MN were increased from that of the respective negative control group of mice. Such increases in MN frequency in EPI 2 and 4 mg/Kg treated female mice are statistically significant ($p \leq 0.05$), when compared to that of the female mice of the negative control group. Although such increases in the EPI-induced MN in rest groups of mice are statistically not significant, they are almost on the borderline (Table 2).

Table 2: Cyclophosphamide (CY)- and Epirubicin (EPI)-induced MN in PCEs of mice at 30h post-treatment.

Chemicals	Dose (mg kg^{-1} body weight)	Number of mice treated & gender	Number of PCEs observed	Number of PCEs with MN	Total number of MN	Average MN per 1000 PCEs ± SD
0.9% NaCl (Negative control)	10 (ml kg^{-1})	3f	6228	12	12	1.99±0.77
		3m	6365	22	23	3.66±0.83
EPI	2	3f	6292	55	57	9.06±0.61*
		3m	6439	44	54	8.35±0.84
	4	3f	6337	55	59	9.35±1.63*
		3m	7035	59	60	8.57±2.04
	6	3f	6994	52	69	9.87±0.65
		3m	6495	51	64	9.85±1.15
CY (Positive control)	40	3f	10841	171	193	17.15±1.05**
		3m	6066	155	177	29.85±4.02**

SD - standard deviation, PCEs - polychromatic erythrocytes, MN - micronucleus, f - female, m - male, (*) $p \leq 0.05$, (**) $p \leq 0.01$

Discussion

CY, a covalent DNA-binding agent, has reportedly induced CAs, MN, sister chromatid exchanges, heritable translocations, specific locus mutations and dominant lethal mutations in both mammalian and non-mammalian test systems, which have been updated for a review (Anderson *et al*., 1995). Moreover, its use as positive control in cytogenotoxicity tests has been recommended (Krisna *et al*., 1995). Induction of significantly ($p \leq 0.01$) high percentages of aberrant metaphases, CAs (excluding gaps) per hundred metaphases and MN per thousand PCEs by CY 40 mg/kg b. w. of mice , in the present study (Table 1 and 2), are in complete agreement with its earlier reported clastogenicity in mouse bone marrow.

Induction of significantly high percentages of aberrant metaphases ($p \leq 0.05$ or $p \leq 0.01$) and CAs (excluding gaps) per 100 metaphases ($p \leq 0.01$) by all the three tested doses of EPI (2, 4 and 6 mg/kg) in bone marrow cells of Swiss mice, when compared to that of the negative control mice, indicated that EPI was highly clastogenic to mouse bone marrow cells. EPI-induced structural chromosomal aberrations were mostly chromatid breaks, fragments, minutes and a few pulverized metaphases (Table 1). Both, the percentages of aberrant metaphases and CAs were found increased along with the increase in the doses of EPI. This dose-dependent increase in the clastogenicity of EPI, in the present study, is in complete agreement with that of Othman (2000), reported on Chinese hamster cell line *in vitro.* EPI-induced clastogenicity was found higher in male groups of mice than the respective female mice in all the three doses, except in the induction of CAs by EPI 6 mg/kg (Table 1). EPI-induced MN in all the groups of mice treated with the different doses of EPI, were remarkably increased from that of the negative control mice (Table 2). Such increases are statistically significant ($p \leq 0.05$) in the female mice that received EPI 2 and 4 mg/kg. However, in the rest groups of EPI-treated female and male mice, such increases are in the borderline. As micronuclei are generated out of the aberrated chromosomes, this increased induction of MN in all the EPI treated groups of mice strongly supported the contention that EPI was highly clastogenic.

EPI was reportedly mutagenic to *Salmonella typhimurium*, causing frame-shift and transition mutations (Brumfield and Mackay, 2002), and cytotoxic to *Saccharomyces cerevisiae* (Buschini *et al*., 2003). It caused somatic mutations and homologous recombinations in *Drosophila melanogaster* (Lehman *et al*., 2003), damaged DNA in CBA mice (Garaj-Vrhovac *et al*., 2003) and in the peripheral blood lymphocytes of patients under

therapy (Barni *et al*., 2004; Sánchez-Suárez *et al*., 2008), and cytotoxic to breast cancer cell lines (Azab *et al*., 2005). However, there is a striking increase in the use of EPI, singly or in combination chemotherapy, for the successful treatment of sarcomas, particularly breast cancers. On the other hand, there is the risk of secondary leukaemia in cancer survivors pre-treated with EPI (Campone *et al*., 2005; Praga *et al*., 2005; Mays *et al*., 2010).

Although the prime objective of cancer chemotherapy is to kill the cancerous cells, their selective killing without affecting the non-cancerous cells is not feasible, as cancer chemotherapeutics are mostly not target specific, and moreover, they are cytogenotoxic (Choudhury *et al*., 2000; Palo *et al*., 2009). These drugs have been designed to affect and interfere in the genetic system of the cancerous cells, thereby killing the cells through various mechanisms. As EPI is clastogenic, during its systemic treatment to cancer patients, the non-cancerous cells of the patients exposed to the drug are also equally affected along with the cancerous cells. Non-cancerous cells with such gross effects on their genome might be eliminated and some of them with tolerable effects might be sustained. Since chromosomal aberrations are related to incidence of cancer (Rossner *et al*., 2005; Boffetta *et al*., 2007; Bonassi *et al*., 2008; Mays *et al*., 2010), induction of secondary leukaemia and other secondary cancers in post-EPI treated cancer survivors is, most plausibly, due to the accumulation of clastogenic effects of EPI on non-cancerous cells of cancer patients during chemotherapy. Therefore, assessment of the detail cytogenotoxic potential of EPI is essential to make it target specific and/or to modulate its effects (Azab *et al*., 2005).

Cytogenotoxicity of anthracycline antibiotics has been attributed to the inhibition of topoisomerase II (topo-II) activity and to the production of free radicals. These drugs disrupt the topo-II mediated DNA cleavage and religation reactions resulting in the formation of stabilized cleavage complexes leading to DNA strand breaks, major deletions and illegitimate recombinational events during DNA replication (Gewirtz, 1999). These drugs also reportedly induce intracellular production of free radicals, like superoxides, hydrogen peroxides and hydroxyl radicals (Doroshow, 1983), leading to oxidative DNA damages and other lesions on DNA (Anderson and Berger, 1994; Olinski *et al*., 1997). Therefore, use of antioxidant(s) along with EPI treatment might be useful in modulating its cytogenotoxicity.

Acknowledgement

The authors thankfully acknowledge the Head, Department of Zoology, Berhampur University, Odisha, India for providing the necessary laboratory facilities to undertake this work. Mr. R. S. Pandit acknowledges the Berhampur University for the award of Research Fellowship and other financial assistance to carry out this work.

References

Anderson D, Bishop JB, Garner RC, Ostrosky-Wegman P, Selby PB, 1995. Cyclophosphamide: Review of its mutagenicity for an assessment of potential germ cell risks. Mutation Research, 330(1-2):115-181.

Anderson RD, Berger NA, 1994. Mutagenicity and carcinogenicity of topoisomerase-interactive agents. Mutation Research, 309: 109-142.

Azab SS, El-Demerdash E, Abdel-Naim AB, Youssef E, El-Sharkawy N, Osman AM, 2005. Modulation of epirubicin cytotoxicity by tamoxifen in human breast cancer cell lines. Biochemical Pharmacology, 70:725-732.

Barni S, Piazza E, Frontini L, Visini M, Ardizzoia A, Grassi MM, *et al.*, 2004. Single-agent epirubicin as primary chemotherapy in T2–T3, N0–N2, M0 breast carcinoma: 6-year follow-up. Oncology, 67:40-47.

Bastholt L, Dalmark M, Gjedde SB, Pfeiffer P, Pedersen D, Sandberg E, *et al.*, 1996. Dose-response relationship of epirubicin in the treatment of postmenopausal patients with metastatic breast cancer: a randomized study of epirubicin at four different dose levels performed by the Danish Breast Cancer Cooperative Group. Journal of Clinical Oncology, 14:1146-1155.

Bhutani M, Kumar L, Vora A, Bhardwaj N, Pathak AK, Singh R, *et al.*, 2002. Randomized study comparing 4-Epi-doxorubicin (Epirubicin) versus doxorubicin as a part of induction treatment in adult acute lymphoblastic leukemia. American Journal of Hematology, 71:241–247.

Boffetta P, van der Hel O, Norppa H, Fabianova E, Fucic A, Gundy S, *et al.*, 2007. Chromosomal aberrations and cancer risk: results of a cohort study from Central Europe. American Journal of Epidemiology, 165:36–43.

Bonassi S , Norppa H, Ceppi M, Strömberg U, Vermeulen R, Znaor A, *et al.*, 2008. Chromosomal aberration frequency in lymphocytes predicts the risk of cancer: results from a pooled cohort study of 22358 subjects in 11 countries. Carcinogenesis, 29:1178–1183.

Brumfield JM, Mackay WJ, 2002. Mutagenic activity of idarubicin and epirubicin in the bacterium Salmonella typhimurium. The Texas Journal of Science, 54: 249-260.

Buschini A, Poli P, Rossi C, 2003. Saccharomyces cerevisiae as an eukaryotic cell model to assess cytotoxicity and genotoxicity of three anthraquinones. Mutagenesis, 18:25-36.

Campone M, Roche H, Kerbrat P, Bonneterre J, Romestaing P, Fargeot P, *et al.*, 2005. Secondary leukemia after epirubicin-based adjuvant chemotherapy in operable breast cancer patients: 16 years experience of the French Adjuvant Study Group. Annals of Oncology, 16:1343–1351.

Choudhury RC, Ghosh SK, Palo AK, 2000. Cytogenetic toxicity of methotrexate in mouse bone marrow. Environmental Toxicology and Pharmacology, 8:191-196.

de Azambuja E, Paesmans M, Beauduin M, Vindevoghel A, Cornez N, Finet C, *et al.*, 2009. Long-term benefit of high-dose epirubicin in adjuvant chemotherapy for node-positive breast cancer: 15-year efficacy results of the Belgian multicentre study. Journal of Clinical Oncology, 27:720-725.

Doroshow JH, 1983. Anthracycline antibiotic-stimulated superoxide, hydrogen peroxide, and hydroxyl radical production by NADH dehydrogenase. Cancer Research, 43:4543-4551.

Focan C, Andrien JM, Closon MT, Dicato M, Driesschaert P, Focan-Henrard D, *et al.*, 1993. Dose-response relationship of epirubicin-based first-line chemotherapy for advanced breast cancer: a prospective randomized trial. Journal of Clinical Oncology, 11:1253-1263.

Garaj-Vrhovac V, Horvat-Knežević A, Kopjar N, Bašić I, 2003. Assessment of DNA damage in leucocytes of CBA mice induced by epirubicin. Meeting Report: From Hazard to Risk, 33rd Annual Meeting of European Environmental Mutagen Society, UK, 24-28. August 2003.

Gewirtz DA, 1999. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. Biochemical Pharmacology, 57:727-741.

Gianni L, Norton L, Wolmark N, Suter TM, Bonadonna G, Hortobagyi GN, 2009. Role of anthracyclines in the treatment of early breast cancer. Journal of Clinical Oncology, 27:4798-4808.

Kastenbaum MA, Bowman KO, 1970. Tables for determining the statistical significance of mutation frequencies. Mutation Research, 9:527-549.

Krishna G, Petrere J, Anderson J, Theiss J, 1995. Use of cyclophosphamide as a positive control in dominant lethal and micronucleus assays. Mutation Research, 335:331-337.

Lanchbury AP, Habboubi N, 1993. Epirubicin and doxorubicin: a comparison of their characteristics, therapeutic activity and toxicity. Cancer Treatment Review, 19:197-228.

Lehmann M, Franco A, Vilar KP, Reguly ML, de Andrade HHR, 2003. Doxorubicin and two of its analogues are preferential inducers of homologous recombination compared with mutational events in somatic cells of Drosophila melanogaster. Mutation Research, 539:167-175.

Lopez M, Vici P, Lauro LD, Carpano S, 2002. Increasing single epirubicin doses in advanced soft tissue sarcomas. Journal of Clinical Oncology, 20:1329-1334.

Mays AN, Osheroff N, Xiao Y, Wiemels JL, Felix CA, Byl JAW, *et al.*, 2010. Evidence for direct involvement of epirubicin in the formation of chromosomal translocations in t (15;17) therapy-related acute promyelocytic leukemia. Blood, 115:326-330.

Minotti G, Menna P, Salvatorelli E, Cairo G, Gianni L, 2004. Anthracyclines: molecular advances and pharmacologic developments in antitumor activity and cardiotoxicity. Pharmacological Reviews, 56:185-229.

Olinsiki R, Jaruga P, Foksinski M, Bialkowski K, Tujakowski, J, 1997. Epirubicin-induced oxidative DNA damage and evidence for its repair in lymphocytes of cancer patients who are undergoing chemotherapy. Molecular Pharmacology, 52:882-885.

Othman ES, 2000. Cytogenetic effect of the anticancer drug epirubicin on Chinese hamster cell line in vitro. Mutation Research, 469:109-115.

Palo AK, Sahoo D, Choudhury RC, 2009. Cytosine arabinoside-induced cytogenotoxicity in bone marrow and spermatogonial cells of mice and its potential transmission through the male germline. Mutation Research, 673:29-36.

Plosker GL, Faulds D, 1993. Epirubicin. A review of its pharmaco-dynamics and pharmacokinetics

properties and therapeutic use in cancer chemotherapy. Drug, 45:788-856.

Praga C, Bergh J, Bliss J, Bonneterre J, Cesana B, Coombes RC, *et al.*, 2005. Risk of acute myeloid leukemia and myelodysplastic syndrome in trials of adjuvant epirubicin for early breast cancer: correlation with doses of epirubicin and cyclophosphamide. Journal of Clinical Oncology, 23:4179-4191.

Rossner P, Boffetta P, Ceppi M, Bonassi S, Smerhovsky Z, Landa K, *et al.*, 2005. Chromosomal aberrations in lymphocytes of healthy subjects and risk of cancer. Environmental Health Perspectives, 113:517-520.

Sánchez-Suárez P, Ostrosky-Wegman P, Gallegos-Hernández F, Peñarroja-Flores R, Toledo-García J, Bravo JL, *et al.*, 2008. DNA damage in peripheral blood lymphocytes in patients during combined chemotherapy for breast cancer. Mutation Research, 640:8-15.

Sen AK, Karakas E, Bilaloglu R, 2010. Genotoxic effect of epirubicin in mouse bone marrow in vivo. Zeitschrift für Naturforschung C, 65c:211-217.

The French Epirubicin Study Group, 2000. Epirubicin-based chemotherapy in metastatic breast cancer patients: Role of dose-intensity and duration of treatment. Journal of Clinical Oncology, 18:3115-3124.

Wasowska M, Oszczapowicz I, Wietrzyk J, Opolski A, Madej J, Dzimira S, *et al.*, 2005. Influence of the structure of new anthracycline antibiotics on their biological properties. Anticancer Research, 25:2043-2048.