Research in Pharmaceutical Sciences, February 2015; 10(1): 68-74
Received: Feb 2014
Accepted: Apr 2014

School of Pharmacy & Pharmaceutical Sciences
Isfahan University of Medical Sciences

Original Article

Evaluation of protective effect of amifostine on dacarbazine induced genotoxicity

M. Etebari*, A. Jafarian-Dehkordi and V. Lame

Department of Pharmacology and Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

Abstract

Anticancer therapy with alkylating agents has been used for many years. Dacarbazine (DTIC) as an alkylating agent is used alone or in combination with other chemotherapy drugs. In order to inhibit the formation of secondary cancers resulting from chemotherapy with DTIC, preventional strategies is necessary. The present study was undertaken to evaluate the genoprotective effect of amifostine on the genotoxic effects of DTIC in cell culture condition. To determine the optimum genotoxic concentration of DTIC, HepG2 cells were incubated with various DTIC concentrations including 5, 10 and 20 µg/ml for 2 h and the genotoxic effects were evaluated by the comet assay. The result of this part of the study showed that incubation of HepG2 cells with DTIC at 5 µg/ml was sufficient to produce genotoxic effect. In order to determine the protective effects of amifostine on genotoxicity induced by DTIC, HepG2 cells were incubated with different concentrations of amifostine (2, 3 and 5 mg/ml) for 1 h which was followed by incubation with DTIC at 5 µg/ml for 2 h. One hour incubation of cells with different concentrations of amifostine before incubation with DITC indicated that at least 5 mg/ml concentration of amifostine can prevent genotoxic effects induced by DTIC on HepG2 cells under described condition. In conclusion amifostine could prevent DNA damage induced by DTIC on HepG2 cells.

Keywords: DNA damage; Comet assay; Dacarbazine; Amifostine

INTRODUCTION

Dacarbazine (DTIC) as an anticancer drug is used in combination with other chemotherapy drugs in the treatment of several cancer types such as Hodgkin's disease, malignant melanoma, soft tissue sarcoma, neuroblastoma and fibrosarcomas (1-7). Although the clear mechanism of action of this drug is not known, it seems to act as an alkylating agent (8-11). Liver has the key role in transformation of this prodrug to its reactive compound, methyl triazeno imidazole carboxamide (MTIC) which is able to attach an alkyl group to DNA. The repairing mechanisms of DNA could repair these kinds of defects by a repairing enzyme called O-6-methylguanine methyltransferase (MGMT). In the absence of active enzyme in repairing process, mutation which could be fatal to cells may occur (9). Several studies have shown that DTIC could act as a purine analog in order to interact with sulfhydryl groups in inhibition of DNA, RNA and protein synthesis (9,11). Distribution of this drug to different parts of the body, could affect normal cells and as a result numerous side effects such as nausea, vomiting, neutropenia, myelosuppression and alopecia could take place. Chemoprotective agents and symptomatic treatments are suggested to reduce these side effects. Development of secondary neoplasia as a result of chemotherapy especially with alkylating agents is common (12-15). Collins and coworkers reported an acute myeloid leukemia as a secondary cancer following treatment by DTIC (16). Amifostine, an organic thiophosphate, could protect normal cells against toxic effects of anticancer drugs and radiotherapy, while it's not effective on neoplastic cells. Amifostine as a prodrug is activated by membrane-bound alkaline

*Corresponding author: M. Etebari
Tel: 0098 31 37922634, Fax: 0098 31 36680011
Email: etebari@pharm.mui.ac.ir

phosphatase to its active metabolite WR-1065 (17-19) acting as a scavenger of oxygen free radicals which is able to bind to platinum and alkylating agents (20). Higher concentrations of alkaline phosphatase in normal cells and higher pH of normal tissues in comparison with cancerous cells lead to the selective uptake of WR-1065 by normal cells (19,21,22).

Several methods have been applied to evaluate the DNA damages (23,24). Comet assay, known as Single Cell Gel method (SCG) has been introduced as a micro electrophoresis method for direct observation of DNA damages. The mechanism by which comet assay detects DNA damage has been explained previously (25). The cells trapped in the agarose gel are lysed under the alkaline pH to release DNA from the cells. Under the effect of electrical flow in electrophoresis, the DNA molecules move toward anode to form the comets. The comet formation pattern is determined by the size of the DNA fragments and the number of broken ends (26). As the extent of the damage increases, the free DNA fragments contain longer tails. To perform this test, a suspension of the separated cells should be prepared. DNA damage should be assessed in the cells without giving them the opportunity to be exposed to any other genotoxic agents (27). Microscopic observation of DNA migration is possible using ethidium bromide staining and a fluorescent microscope (28).

According to the wide application of DTIC in cancer treatment protocols and its serious side effects especially secondary cancers, seeking new strategies to prevent the side effects seems imperative. With regard to the preventative effects of amifostine on normal cells, this study was performed to evaluate the genoprotective and dose dependent effects of this drug on genotoxicity of DTIC on the metabolically competent human hepatoma cell line (HepG2 cells).

MATERIALS AND METHODS

Materials

DTIC and amifostine were respectively purchased from Medac Co. (Germany) and MedImmune Pharma BV. (Poland). Tris, Triton X-100, H_2O_2, NaCl, ethylenediaminetetraacetic acid (EDTA), NaOH and NaH_2PO_4 were procured from Merck Co. (Germany). Low melting point agarose (LMA), Na_2HPO_4, KCl and ethidium bromide obtained from Sigma Co. (USA). Normal melting point agarose (NMA) was supplied by Cinnagen Co. (Iran), RPMI-1640, FBS and antibiotic were purchased from PAA Co. (Australia). HepG2 cells obtained from Pasture Institute (Iran).

Cell culture

HepG2 cell line was cultured in RPMI medium (containing 10 % fetal bovine serum and 250 µl of penicillin/streptomycin to avoid the growth of undesirable and pathogenic bacterial microorganisms) and incubated under 5 percent CO_2 at 37 $^{\circ}$C in micro-filter plates. Cells were incubated with different concentrations of DTIC (5, 10 and 20 µg/ml) for 2 h to select the lowest genotoxic concentration of DTIC. In the next step HepG2 cell were incubated with the adopted DTIC concentration (5 µg/ml) for 2 h which was followed for further 1 h incubation in the presence of different concentrations of amifostine (2, 3 and 5 mg/ml). As described in our previous studies, the upper medium of each well was thrown away and wells were washed with PBS. Cells was dissociated from the culture surface using trypsin solution and then suspended in 1 ml fresh medium for the next stages of the comet assay (29,30).

Alkaline comet assay

The comet assay procedure has been described in our previous studies (29-32). Briefly, incubated cell suspensions (1×10^6 cells/ml) were mixed with 1% LMP agarose at 37 ºC,were placed on the precoated slides (1% NMP agarose), and covered by cover glasses for 5 min at 2-8 °C. The slides were incubated with lysis solution (pH=10.0) for 40 min and rinsed with distilled water to remove excess lysis solution. In the next step, slides were incubated with electrophoresis buffer (pH> 13.0) for 40 min. Electrophoresis was conducted for 40 min at 25 V with an electricity current adjusted to 300 mA. After

this stage, the slides were rinsed with distilled water to remove excess alkaline buffer and were placed in neutralization solution (pH=7.5) for 10 min. The slides were covered by sufficient dye solution (20 µg/ml ethidium bromide) for 5 min and washed with distillated water. Finally comets were visualized under × 400 magnification using fluorescence microscope with an excitation filter of 510-560 nm and barrier filter of 590 nm (23). All stages of comet assay were performed in dark conditions and all solutions were prepared freshly and used cool (29,30).

Statistical analysis

Tail moment (percentage of DNA in tail × tail length), tail length (the length of the comet tail), and percent of DNA in tail (percentage of colored spots in tail) are the most frequently used factors in the evaluation of DNA damages in the comet assay method. We used these factors for statistical analysis in this investigation (33,34).

One-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post hoc test was used to compare the results of the comet assay.

The *p*-values of 0.05 and less were considered as statistically significant.

RESULTS

Study of genotoxic effects of dacarbazine

The genotoxic effect of DTIC was tested on the basis of the previous studies (6,35,36). To determine the most appropriate genotoxic concentration of DTIC, HepG2 cells were incubated with 5, 10 and 20 µg/ml of DTIC for 2 h before starting the comet assay (Fig. 1). One-way ANOVA for the results of tail length showed that tail length was increased significantly (P<0.0001). Tukey's multiple comparison post hoc test indicated that the tail length was significantly (P<0.001) increased at all concentrations of DTIC examined as compared to negative control group (Fig. 1A). One-way ANOVA followed by Tukey's multiple comparison post hoc test indicated that at all concentrations of DTIC the percentage of DNA in the tail was significantly increased (P<0.001) compared to the control group (Fig. 1B).Statistical analysis also showed that the tail moment for all groups was increased significantly (P<0.0001) (Fig. 1C).

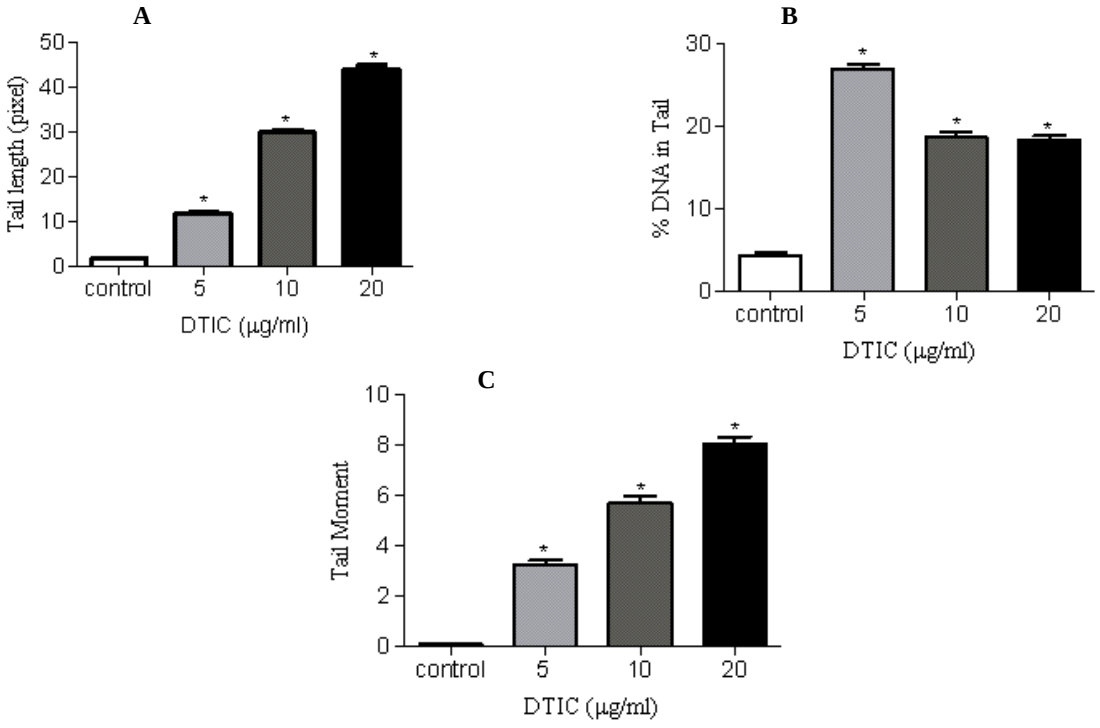

Fig. 1. Comparison of three studied factors in DTIC treated groups A; Tail length, B; % DNA in tail and C; Tail moment. Each graph has been represented as Mean ± SEM. The sign (*) shows significantly increased results (P<0.001) in compare with the control group.

Up to this point, the studies indicated that all studied factors including tail length, percent DNA in the tail, and tail moment were increased at lowest DITC concentration (5 µg/ml) amongst DITC concentrations studied. Thus this concentration was selected for induction of the DNA damage for assessing the protective effects of amifostine.

Study of genoprotective effects of amifostine

In order to determine the protective effects of amifostine on genotoxicity induced by DTIC, HepG2 cells were incubated with different concentrations of amifostine (2, 3 and 5 mg/ml) for 1 h followed by incubation with DTIC at 5 µg/ml for 2 additional h (Fig. 2). The ANOVA followed by Tukey's multiple comparison post hoc test showed that all amifostine concentrations which was then accompanied by DTIC (5 µg/ml) were able to inhibit the genotoxic effects of DTIC and decreased the tail length significantly (P<0.001) concentration dependently as compared with the DTIC group (Fig. 2A).

The percentage of DNA in the tail and the tail moment (P<0.001) decreased significantly at 3 and 5 mg/ml of amifostine, while at the concentration of 2 mg/ml of amifostine the percentage of DNA in the tail decreased less proportionally than other two concentrations (P<0.05) as compared with the DTIC group (Fig. 2B and 2C).

Different concentrations of amifostine (2, 3 and 5 mg/ml) tested by comet assay method after 1 h incubation did not show genotoxic effect on HepG2 cells.

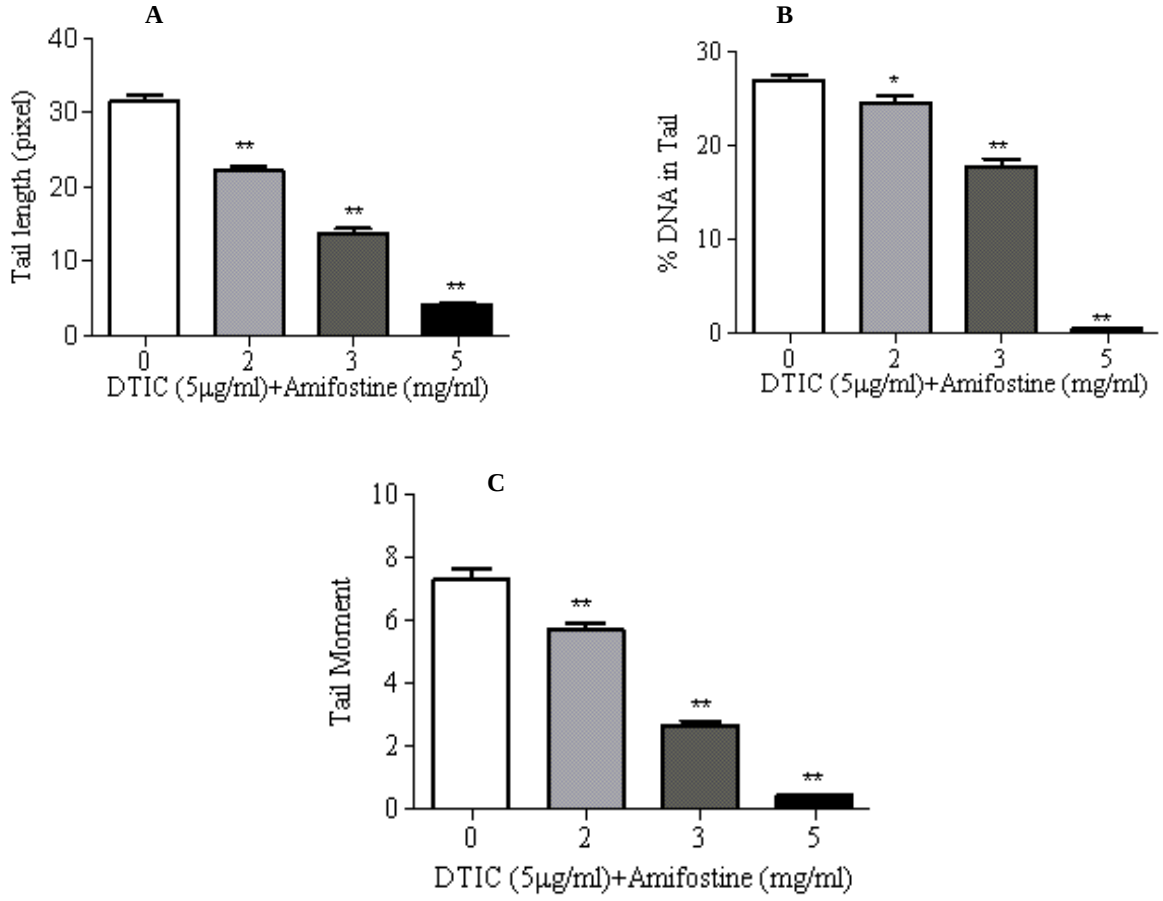

Fig. 2. Comparison of three studied factors in amifostine plus DTIC treated groups A; Tail length, B; % DNA in tail and C; Tail moment. Each graph has been represented as Mean ± SEM. The sign (**) and (*) show significantly decreased results (respectively P<0.001 and P<0.05) in compare with the DTIC group.

DISCUSSION

The results of this study indicated that concentrations of 5, 10 and 20 µg/ml of DTIC are genotoxic on HepG2 cells after incubation for 2 h. The concentration 5 µg/ml was recognized as the lowest concentration of DTIC being genotoxic on HepG2 cells under the condition already described. All three concentrations of amifostine (2, 3 and 5 mg/ml) were found to protect the genotoxic effects of DTIC on HepG2 cells. alkylating agents such as DTIC are used as a chemotherapeutic agent in the treatment of various cancers (37-39). The most important side effect of these classes of drugs is apparition of secondary neoplasia or cancers in additional sites (12-15). Alkylating agents substitute alkyl groups on the DNA leading to the damage of DNA, breaking labile bonds of DNA, formation of micronucleus, and finally leading to chromosomal breaking and genome instability.

These damages lead to the inhibition of biosynthesis pathways, cell cycle arrest, teratogenicity and apoptosis (35,40). DTIC may inhibit DNA and RNA synthesis by acting as a purine analogue. This drug is biotransformed to MTIC by demethylation in the liver and then to diazomethane, which attacks to the nucleophilic groups on DNA (8,35,40,41). Amifostine is approved by FDA to be used for reducing the side effects of cisplatin in patients with advanced ovarian cancer (42). Nowadays, amifostine known as a selective cytoprotective agent of normal tissues against the toxicity of chemotherapy and radiotherapy which acts as a scavenger of free radicals and conjugate of electrophilic substances (17, 43-46). WR-2721, a prodrug, which is dephosphorylated by *alkaline phosphatase* can activate free thiol metabolites in the tissues. The selective protection of non-malignant tissues is believed to be due to the higher *alkaline phosphatase* activity in normal tissues, different mechanisms of amifostine uptake, and different membrane-bound alkaline phosphatase concentrations (40,41, 47-50). The genotoxic effects of doxorubicin after 3, 6 and 9 h exposure to 10 µg/ml doxorubicin have been previously reported. The results of this study showed time dependent genotoxicity of doxorubicin (51). Buschini and coworkers determined cytoprotective effect of amifostine (0, 50 and 100 µg/ml) on bleomycin genotoxicity by the comet assay. In this study, amifostine could reduce bleomycin genotoxic effects (52). In another study, amifostine was shown to have selective protection against melphalan-induced DNA damage in normal and tumoral cells (53). Amifostine was also proven to be an effective cytoprotector against the toxic effects of cisplatin (54). Blasiak and coworkers evaluated cytoprotective effects of vitamin C and E and amifostine on idarubicin genotoxicity on lymphocyte cells. Vitamin C and amifostine (14 mM) reduced DNA damage induced by idarubicin while vitamin E increased DNA damage of idarubicin (55).

CONCLUSION

The result of our investigation showed that lowest concentration of DTIC (5 µg/ml) could be genotoxic HepG2 cells incubated for 2 h. One-hour incubation of cells with different concentrations of amifostine before incubation with DTIC (5 µg/ml) indicated that studied concentrations of amifostine are able to prevent genotoxic effects of DTIC on HepG2 cells. It can be concluded that amifostine could prevent genotoxic effect of DTIC on HepG2 cells and could be suggested to be included in the chemotherapy protocols containing DTIC in order to prevent formation of secondary cancers.

ACKNOWLEDGMENTS

The content of this paper is extracted from the Pharm.D thesis NO. 391441 submitted by V. Lame which was financially supported by the Research Department of Isfahan University of Medical Sciences, Isfahan, I.R. Iran.

REFERENCES

1. Yi JH, Yi SY, Lee HR, Lee SI, Kim JH, Park KW, *et al*. Dacarbazine-based chemotherapy as first-line treatment in noncutaneous metastatic melanoma: multicenter, retrospective analysis in Asia. Melanoma Research. 2011;21:223-227.

2. Batty N, Hagemeister FB, Feng L, Romaguera JE, Rodriguez MA, McLaughlin P, *et al*. Doxorubicin, bleomycin, vinblastine and dacarbazine chemotherapy with interferon for advanced stage classic Hodgkin lymphoma: a 10-year follow-up study. Leuk.Lymphoma. 2012;53:801-806.
3. Minuk LA, Monkman K, Chin-Yee IH, Lazo-Langner A, Bhagirath V, Chin-Yee BH, *et al*. Treatment of Hodgkin lymphoma with adriamycin, bleomycin, vinblastine and dacarbazine without routine granulocyte-colony stimulating factor support does not increase the risk of febrile neutropenia: a prospective cohort study. Leuk. Lymphoma. 2012;53:57-63.
4. Wilson KS, Robert C, Thomas L, Bondarenko I. Ipilimumab plus dacarbazine in melanoma. N Engl J Med. 2011;365:1256-1258.
5. Walter T, Bruneton D, Cassier PA, Hervieu V, Pilleul F, Scoazec JY, *et al*. Evaluation of the combination 5-fluorouracil, dacarbazine, and epirubicin in patients with advanced well-differentiated neuroendocrine tumors. Clin. Colorectal Cancer. 2010;9:248-254.
6. Kumar SG, Narayana K, Bairy K, D'Souza UJ, Samuel VP, Gopalakrishna K. Dacarbazine induces genotoxic and cytotoxic germ cell damage with concomitant decrease in testosterone and increase in lactate dehydrogenase concentration in the testis. Mutat. Res. 2006;607:240-252.
7. Al-Hawary B, Al-Saleh A. Cytogenetic effects of dacarbazine on mouse bone marrow cells in vivo. Mutat. Res. 1989;223:259-266.
8. Psaroudi MC, Kyrtopoulos SA. Toxicity, mutation frequency and mutation spectrum induced by dacarbazine in CHO cells expressing different levels of methylguanine-DNA methyltransferase. Mutat. Res. 2000;447:257-265.
9. Sanada M, Hidaka M, Takagi Y, Takano TY, Nakatsu Y, Tsuzuki T, *et al*. Modes of actions of two types of anti-neoplastic drugs, dacarbazine and ACNU, to induce apoptosis. Carcinogenesis. 2007;28:2657-2663.
10. Pourahmad J, Kobarfard F, Amirmostofian M. Lysosomal Oxidative Stress Cytotoxicity Induced by Dacarbazine and It's Pyridine Derivative in Hepatocytes. IJPS. 2006;2:195-202.
11. Pourahmad J, Amirmostofian M, Kobarfard F, Shahraki J. Biological reactive intermediates that mediate dacarbazine cytotoxicity. Cancer Chemother. Pharmacol. 2009;65:89-96.
12. Carey RW, Kunz VS. Acute non-lymphocytic leukemia (ANLL) following treatment with dacarbazine for malignant melanoma. Am.J.Hematol. 1987;25:119-121.
13. Lev DC, Onn A, Melinkova VO, Miller C, Stone V, Ruiz M, *et al*. Exposure of melanoma cells to dacarbazine results in enhanced tumor growth and metastasis in vivo. J.Clin.Oncol. 2004;22:2092-2100.
14. Horiguchi M, Kim J, Matsunaga N, Kaji H, Egawa T, Makino K, *et al*. Glucocorticoid-dependent expression of O6-methylguanine-DNA methyltransferase gene modulates dacarbazine-induced hepatotoxicity in mice. J.Pharmacol.Exp.Ther. 2010;333:782-787.
15. Khan F, Sherwani AF, Afzal M. Analysis of genotoxic damage induced by dacarbazine: an in vitro study. Toxin Rev. 2010;29:130-136.
16. Collins CM, Morgan DS, Mosse C, Sosman J. Dacarbazine induced acute myeloid leukemia in melanoma. Melanoma Res. 2009;19:337-340.
17. Błasiak J, Gloc E, Młynarski W, Drzewoski J, Skórski T. Amifostine differentially modulates DNA damage evoked by idarubicin in normal and leukemic cells. Leuk.Res. 2002;26:1093-1096.
18. Merlin J-L, Marchal S, Ramacci C, Berlion M, Poullain M-G. Enhancement of fotemustine (Muphoran) cytotoxicity by amifostine in malignant melanoma cell lines. Anti-Cancer Drug. 2002;13:141-147.
19. Blasiak J, Gloc E, Pertynski T, Drzewoski J. DNA damage and repair in BCR/ABL-expressing cells after combined action of idarubicin, STI571 and amifostine. Anti-Cancer Drug. 2002;13:1055-1060.
20. Marzatico F, Porta C, Moroni M, Bertorelli L, Borasio E, Finotti N, *et al*. In vitro antioxidant properties of amifostine (WR-2721, Ethyol). Cancer Chemoth.Pharmacol. 2000;45:172-176.
21. Müller A-C, Pigorsch S, Beyer C, Lautenschläger C, Dunst J. Radioprotective effects of amifostine in vitro and in vivo measured with the comet assay. Strahlenther.Onkol. 2004;180:517-525.
22. Dedieu S, Canron X, Rezvani HR, Bouchecareilh M, Mazurier F, Sinisi R, *et al*. The cytoprotective drug amifostine modifies both expression and activity of the pro-angiogenic factor VEGF-A. BMC.Med. 2010;8:19.
23. Mozaffarieh M, Schoetzau A, Sauter M, Grieshaber M, Orgül S, Golubnitschaja O, *et al*. Comet assay analysis of single–stranded DNA breaks in circulating leukocytes of glaucoma patients. Molecular vision. 2008;14:1584-1588.
24. Jena G, Kaul C, Ramarao P. Genotoxicity testing, a regulatory requirement for drug discovery and development: Impact of ICH guidelines. Indian J.Pharmacol. 2002;34:86-99.
25. Sardas S. Genotoxicity tests and their use in occupational toxicology as biomarkers. Indoor Built Environ. 2005;14:521-525.
26. Rivero M, Vázquez-Gundın F, Goyanes V, Campos A, Blasco M, Gosálvez J, *et al*. High frequency of constitutive alkali-labile sites in mouse major satellite DNA, detected by DNA breakage detection-fluorescence in situ hybridization. Mutat.Res. 2001;483:43-50.
27. De Meo M, Laget M, Castegnaro M, Dumenil G. Genotoxic activity of potassium permanganate in acidic solutions. Mutat.Res. 1991;260:295-306.
28. Olive P, Banath J, Fjell C. DNA strand breakage and DNA structure influence staining with propidium iodide using the alkaline comet assay. Cytometry. 1994;16:305-312.
29. Etebari M, Zolfaghari B, Jafarian-Dehkordi A, Rakian R. Evaluation of DNA damage of hydro-

alcoholic and aqueous extract of Echium amoenum and Nardostachys jatamansi. J Res Med Sci. 2012;17:782-785.
30. Etebari M, Ghannadi A, Jafarian-Dehkordi A, Ahmadi F. Genotoxicity evaluation of aqueous extracts of Cotoneaster discolor and Alhagi pseudalhagi by comet assay. J Res Med Sci. 2012;17:S238-S242.
31. Etebari M, Sajjadi SE, Jafarian-Dehkordi A, Panahi M. Antigenotoxic Effects of Methanolic and Aqueous Extracts of Kelussia Odoratissima Mozaffarian against Damage Induced by Methyl Methanesulfonate. J.Isfahan Med.Sch. 2013;30:2062-2071.
32. Tavakoli M, Bateni E, Rismanchian M, Fathi M, Doostmohammadi A, Rabiei A, *et al*. Genotoxicity effects of nano bioactive glass and Novabone bioglass on gingival fibroblasts using single cell gel electrophoresis (comet assay): An in vitro study. Den.Res.J. 2012;9:314-320.
33. Belpaeme K, Cooreman K, Kirsch-Volders M. Development and validation of the in vivo alkaline comet assay for detecting genomic damage in marine flatfish. Mutat.Res. 1998;415:167-184.
34. Tice R, Vazquez M. Protocol for the application of the pH> 13 alkaline single cell gel (SCG) assay to the detection of DNA damage in mammalian cells. Sigma (x-100). 1998;503:465-8353.
35. Braybrooke JP, Houlbrook S, Crawley JE, Propper DJ, O'Byrne KJ, Stratford IJ, *et al*. Evaluation of the alkaline comet assay and urinary 3-methyladenine excretion for monitoring DNA damage in melanoma patients treated with dacarbazine and tamoxifen. Cancer Chemother.Pharmacol. 2000;45:111-119.
36. Souliotis VL, Valavanis C, Boussiotis VA, Pangalis GA, Kyrtopoulos SA. Comparative study of the formation and repair of O6-methylguanine in humans and rodents treated with dacarbazine. Carcinogenesis. 1996;17:725-732.
37. Greene MH, Boice Jr JD, Greer BE, Blessing JA, Dembo AJ. Acute nonlymphocytic leukemia after therapy with alkylating agents for ovarian cancer: a study of five randomized clinical trials. N.Engl.J.Med. 1982;307:1416-1421.
38. Esteller M, Garcia-Foncillas J, Andion E, Goodman SN, Hidalgo OF, Vanaclocha V, *et al*. Inactivation of the DNA-repair gene MGMT and the clinical response of gliomas to alkylating agents. N.Engl.J.Med. 2000;343:1350-1354.
39. Schabel Jr F, Trader M, Laster Jr W, Corbett T, Griswold Jr D. cis-Dichlorodiammineplatinum (II): combination chemotherapy and cross-resistance studies with tumors of mice. Cancer Treat.Rep. 1979;63:1459-1473.
40. Grochova D, Smardova J. The antimutagenic and cytoprotective effects of amifostine: the role of p53. J Appl Biomed. 2007;5:171-178.
41. Feng M, Smith DE, Normolle DP, Knol JA, Pan CC, Ben-Josef E, *et al*. A phase I clinical and pharmacology study using amifostine as a radioprotector in dose-escalated whole liver radiation therapy. Int.J.Radiat.Oncol.Biol.Phys. 2012;83:1441-1447.
42. Cassatt DR, Fazenbaker CA, Bachy CM, Kifle G, McCarthy MP. Amifostine (ETHYOL) protects rats from mucositis resulting from fractionated or hyperfractionated radiation exposure. Int.J.Radiat.Oncol.Biol.Phys. 2005;61:901-907.
43. Glover D, Fox KR, Weiler C, Kligerman MM, Turrisi A, Glick JH. Clinical trials of WR-2721 prior to alkylating agent chemotherapy and radiotherapy. Pharmacol.Ther. 1988;39:3-7.
44. Smoluk GD, Fahey RC, Calabro-Jones PM, Aguilera JA, Ward JF. Radioprotection of cells in culture by WR-2721 and derivatives: form of the drug responsible for protection. Cancer Res. 1988;48:3641-3647.
45. Clark LS, Albertini RJ, Nicklas J. The aminothiol WR-1065 protects T lymphocytes from ionizing radiation-induced deletions of the HPRT gene. Cancer Epidemiol. Biomarkers Prev. 1997;6:1033-1037.
46. Durand R. Radioprotection by WR-2721 in vitro at low oxygen tensions: implications for its mechanisms of action. Br.J.cancer. 1983;47:387-392.
47. McCumber LM. The potential influence of cell protectors for dose escalation in cancer therapy: an analysis of amifostine. Med.Dosim. 2004;29:139-143.
48. Fuchs-Tarlovsky V. Role of antioxidants in cancer therapy. Nutrition. 2012.
49. Foster-Nora JA, Siden R. Amifostine for protection from antineoplastic drug toxicity. Am.J.Health Sys.Pharm. 1997;54:787-800.
50. Brizel DM. Pharmacologic approaches to radiation protection. J.Clin.Oncol. 2007;25:4084-4089.
51. Husseini GA, El-Fayoumi RI, O'Neill KL, Rapoport NY, Pitt WG. DNA damage induced by micellar-delivered doxorubicin and ultrasound: comet assay study. Cancer Lett. 2000;154:211-216.
52. Buschini A, Alessandrini C, Martino A, Pasini L, Rizzoli V, Carlo-Stella C, *et al*. Bleomycin genotoxicity and amifostine (WR-2721) cell protection in normal leukocytes vs. K562 tumoral cells. Biochem.Pharmacol. 2002;63:967-975.
53. Buschini A, Anceschi E, Carlo-Stella C, Regazzi E, Rizzoli V, Poli P, *et al*. Amifostine (WR-2721) selective protection against melphalan genotoxicity. Leukemia. 2000;14:1642-1651.
54. Santini V. Amifostine: chemotherapeutic and radiotherapeutic protective effects. Expert Opin.Pharmacother. 2001;2:479-489.
55. Błasiak J, Gloc E, Woźniak K, Młynarski W, Stolarska M, Skórski T, *et al*. Genotoxicity of idarubicin and its modulation by vitamins C and E and amifostine. Chem.Biol.Interact. 2002;140:1-18.