World Applied Sciences Journal 24 (7): 879-884, 2013
ISSN 1818-4952

DOI: 10.5829/idosi.wasj.2013.24.07.13231

Cytogentic Effects of *Aloe vera* (L.) Leaves Gel Extract on Mice Bone Marrow Cells

[1]*Suliman S. Abdelrawaf,* [2]*Hussain A. Mohowes Al-Saady and* [1]*Idress Hamad Attitalla*
[1]Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda, Libya
[2]Department of Biology, College of Science, University of Misan, Misan, Iraq

Submitted: Jun 25, 2013; **Accepted:** Aug 28, 2013; **Published:** Aug 29, 2013

Abstract: *Aloe vera* L. (AV) plant leaves had been collected from Al-Beida farms. They were cleaned, chopped, homogenized, extracted with 80% ethanol, centrifuged and then condensed the supernatant with vacuum evaporator and freeze-dried with lyophilizer. Two doses *viz.* 40 and 80 mg kg^{-1} body weight of mice (BW) were orally administrated for four weeks to Swiss albino mice (*Mus musculus*). Cytogentic effect of AV was carried out on mice bone marrow cells. Results showed significant effect of AV extract for both doses on mitotic index (MI) and chromosomal aberration when compared with negative control.

Key words: *Aloe vera* (L.) · Cytogentic · Mice Bone Marrow Cells · Chromosomal Analysis

INTRODUCTION

Plants and their products have been an important source of medicine for thousands of years. Even today, it is estimated that about 80% of the developing countries population was still rely mainly on traditional medicines for their primary health care [1]. AV is one of these plants has been used in folk medicine for over 2000 years. It has been used worldwide due to its medicinal properties [2]. Because of its demand, it is cultivated in large quantities in many parts of the world [3]. AV is a rich source of chemical compounds. There are more than 200 different biologically active substances [4] including anthraquinones; aloin, barbalion, anthranol, cinnamic acid, aloetic acid, emodin, chrysophanic acid, resistanol, salicylic acid and enzymes (including cyclooxygenase and bradykininase), lignin, saponins, sterols together with other compounds such as vitamins, saccharides and amino acids, minerals [5-7].

Several studies reported that the AV was cytotoxic for human and animals [7-15]. This cytotoxity is ascribed to some of active substances like anthraquinones; including aloin and aloe-emodin had genotoxic potential for bacterial and mammalian cells [7-9, 11-13,16,17].

Aloe gel, a clear colorless semi-solid gel, was stabilized and marketed. It is used commercially to produce more than one product. This AV gel beginning in the 50's has gained respect as a commodity used as a base for nutritional drinks, as a moisturizer and a healing agent in cosmetics and OTC drugs [18]. More than one study reported that some of AV gel products were cytotoxic to human and animals [10,19-21] such as; acemannan which is constituted a high level of the gel had significant cytotoxicity against human fibroblasts [10]. An earlier study [7] found AV gel was cytotoxic for human normal and tumor cells *in vitro*. Another study when tested four commercial stabilized AV gel samples and yellow AV sap for cytotoxicity. It discovered that AV sap was lethal to human fibroblasts and the two gel products were cytotoxic to human endothelial cells and fibroblasts [19].

A two-year NTP study on oral consumption of non-decolorized whole leaf AV extract taken internally by rats [21] found clear evidence of carcinogenic activity in male and female rats, based on tumors of the large intestine. Recently, Shao *et al.* [15] support the notion that the high levels of anthraquinone present in orally administered, non-purified whole leaf *Aloe vera* extract may be responsible for the adverse effects observed on the colon. However, NTP reported that certain aloe products increased the number of skin cancers (especially in female mice) caused by ultraviolet light' [14].

Some cytogentic studies showed that AV caused chromosomal damage which was expressed as mitotc index and chromosome aberrations [16, 22,23]. Many types of structural rearrangement of chromosomes have been identified in neoplastic cells, such as deletion, duplication, inversion, insertion and translocation. Such rearrangement gives rise to loss, gain and relocation of genetic material. In addition, numerical aberrations, giving rise to loss or gain of entire chromosomes, are common [24].

Corresponding Author: Dr. Idress Hamad Attitalla, Department of Microbiology, Faculty of Science, Omar Al-Mukhtar University, Al-Bayda, Libya.

MATERIALS AND METHODS

Preparation of Plant Extract: Fresh clean leaves of *Aloe vera* were collected from some farms of Al-baeda region, northeast Libya. They were washed under running water and then let for 1h to dry. The inner mucilaginous viscous parenchymatous tissue of leaves was separated out with the help of a sterile knife and chopped. A 250 g of chopped tissue was homogenized in a warring blender at maximum speed for 30 min. The homogenized mass was then transferred to sealing1000 ml conical flask and soaked in 500 ml 80% ethanol at room temperature for 48 h. Then the aloe soaked infusion was filtered and squeezed through 8 layers of muslin cloth and centrifuged at 3000 rpm at 5°C for 15 min. The supernatant was collected and condensed by vacuum rotary evaporator system at 45° C and 90 rpm to obtain jelly like extract. Then the jelly mass was freeze-dried in lyophilizer. The resulting powder was stored in an airtight container in refrigerator for further experimental studies.

Animals: Swiss albino mice (*Mus musculus,* Balb/C), 6-7 weeks old with body weight of 25-30 g from an inbred colony were used for the present study. These animals were maintained under controlled conditions at a temperature of 24±4°C and with a 12-h light/dark cycle. Utmost care was taken to maintain cage hygiene and also provide good ventilation and aeration in the room where the animals were housed. Five mice were housed in polypropylene cages. Sterile woodwork sawdust wood shavings were used as bedding material and the animals were fed with standard diet and water ad libitum throughout the experiment.

Three groups were treated orally daily with gavages needle for 4 weeks with the freeze-dried powder of alcoholic *Aloe vera* extract in doses of 40, 80 mg/kg, in DW respectively and one group as control with using DW.

Chromosome Analysis and Mitotic Preparation: Cytogenetic damage in bone marrow cells was studied by chromosomal aberration analysis and IM estimated. At 29th day the animals of the study were injected intraperitoneal with 0.5 ml of 0.05% Colchicine 2h prior to sacrifice of these animals. Then the mice were sacrificed by cervical dislocation and bone marrow cytogenetic assay was employed to determine any clastogenic effects in dividing cells.

The bone marrow was processed and slides were prepared by routine standara air dry technique [25], as described by Guruprasad *et al*. [26]. In brief, the femur bones were dissected and bone marrow was aspirated washed with 0.567% KCl (5 mL) with using 5ml syringe with a 26 gauge needle. The suspension was then incubated at 37°C for 15 to 20 minutes. Then, the tubes were centrifuged at 3000 rpm for 10 minutes at 4°C. The supernatant was discarded. Cold Carnoy's fixative (3:1 v/v of methanol: acetic acid, 5 mL) was added to pellet and mixed intensively to avoid clumping of cells. The tubes were kept at room temperature for 30 minutes and once again centrifuged for another 10 minutes at 4°C (3000 rpm) and the supernatant removed. This process was repeated twice over 20 minute intervals. Finally the pellet was resuspended in Carnoy's fixative (0.5 mL) and dropped from a height on a nongreasy chilled glass slide using a fine tipped pasteur pipette. The slides were air-dried, coded and stained with 4% Giemsa for 20-30 minutes. The stained slides were then rinsed thoroughly with running tap water followed by distilled water and dried before being viewed under the microscope (Olympus X30 with camera). A total of 300 metaphase plates were scored per animals, chromosomal aberrations like chromatid breaks, fragment, chromatid deletions, chromatid gap, chromosome breaks, dicentrics chromosome and rings, stickness and polyploid cell were scored. The Mitotic Index (MI) was calculated by using the formula mentioned below.

$$MI\% = \frac{\text{Number of cells in mitosis}}{\text{Total number of cells secored}} \times 100$$

Statistics: The statistical software package, SPSS for windows version 11.0 was used for all statistical analysis. The study of MI and chromosomal aberrations were analyzed and expressed as mean and the standard deviation (Mean ± SD). Each treatment group was compared with the control group. The one-way Analysis of Variance (ANOVA) was done to observe the significant differences between the individual groups. The Tukey's multiple range test was carried to compare the means of all doses and negative control @ 5% level of significance.

RESULTS

In current study, the potential cytotoxic and genotoxic effects of ethanol mucilaginous tissue of leaves of AV extract were evaluated by observing cytological parameters such as mitotic index and chromosome aberration, including chromatid gap, chromatid break, chromosome fusion, ring chromosome, dicentric chromosomes, chromatid deletion, stickiness chromosomes in bone marrow of Swiss albino mice (Fig. 1).

Table 1: The genotoxic effect of *Aloe vera* leaves gel extract administration for 4 weeks on mice mitotic index and chromosome aberrations.

Dose mg/kg	Mitotic Index	Chromatid Gap	Chromatid Break	Chromosome Fusion	Ring chromosome	Dicentric Chromosome	Chromatid Deletion	Stickiness	Total
Control									
Mean ±SD	5.501 ±1.049	0.470±0.596	0.48±0.360	0.15±0.725	0.1±0.376	0.07±.252	0.120±0.324	0.1±0.303	
40 Mean ±SD	3.443 ±0.285	4.21±3.119	4.97±1.00	0.96±0.725	1.26±1.167	0.6±.612	5.540±3.452	1.46±1.477	
80Mean ±SD	2.015 ±0.589	4.76±3.060	5.55±	1.12±0.725	1.64±1.232	0.58±.651	5.770±3.683	2.50±2.832	

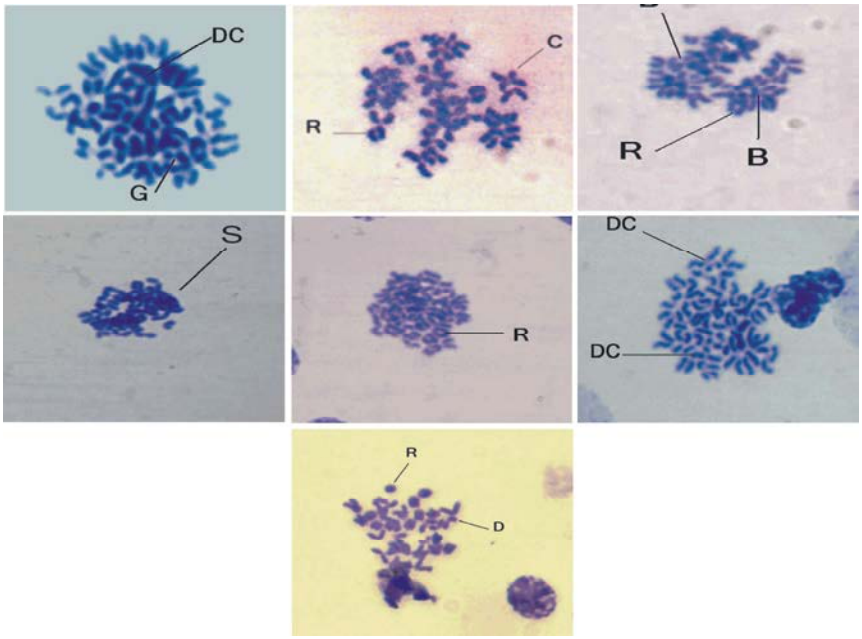

Fig. 1: Chromosome aberrations of mice bone marrow after treatment with *Aloe vera* leaves gel extract; G: chromatid gap; B: chromatid break; C: chromosome fusion; R: ring chromosome; DC: dicentric chromosome; D: deletion and S: stickiness chromosome.

Results of this study have been summarized in Table 1. Two doses of AV extract (Table 1) induced a significant decrease ($p<0.001$) in the mitotic index in treated groups with 40 and 80 mg/kg bw (Mean±SD =3.44±0.285 and 2.0153±0.589 and 2.21±0.02%, respectively) in comparison with the control (Mean±SD =5.501±1.049).

The statistical analysis of results (ANOVA) showed that the increased in all chromosome aberrations (chromatid gap, chromatid break, chromosome fusion, ring chromosome, dicentric chromosomes, chromatid deletion, stickiness chromosomes) in all treated animals groups were highly significant ($p<0.001$) when compared to the negative control.

No statistically significant differences had been observed between the effects of 40 and 80 mg/kg bw doses on chromatid gap, chromatid break, chromosome fusion, dicentric chromosomes and chromatid deletion. But there are significantly differences between two doses in their effect on ring chromosome and stickiness chromosomes (Fig. 1).

DISCUSSION

The AV plant had been used worldwide due to its medicinal properties [2]. Several studies reported that the AV was cytotoxic for human and animals [7-11, 13,14,17].

In this study, the effect of the AV leaves gel was evaluated by genotoxic properties like mitotic index and chromosome aberration in albino mice bone marrow cells. It is found that the AV gel had been significant effect on decreasing of mitotic index. This finding agrees with observations of Kayraldiz *et al*. [23] who reported that AV induced the decreasing of the replication mitotic index (MI) in the bone marrow cells of rats. The decrease in the MI may be due to effect of one or more compounds such as anthraquinones; aloin and aloe-emodin which found in AV [27]. This act may be due to the effect of AV on suppression of DNA synthesis or may be caused DNA double-strand breaks through stabilization of topoisomerase (Topo) II-DNA cleavage complexes and inhibition of ATP [28]. However, Madle *et al.* [22]

reported that the mitotic selection of the cells having chromosome abnormalities is capable of decreasing the MI.

In the present study, many types of structural rearrangement of chromosomes had been identified in mouse bone marrow cells after 4 weeks of AV administration. High frequency was showed in some chromosome aberrations like chromatid gap, chromatid break and chromatid, deletion, at 40 and 80 mg/kg bw. And low frequency in chromosome fusion, ring chromosome, dicentric chromosome and stickiness chromomsomes. There was significantly increased in the frequency of all chromosome aberrations such as; chromatid gap, chromatid break, chromosome fusion, ring chromosome, dicentric chromosomes, chromatid deletion and stickiness at all treatment groups when compared to negative control. These results are coinciding with Kayraldiz *et al*. [23] in rat and with Heidemann *et al.* [16] in Chinese hamster ovary cells or in Wistar rats bone marrow cells.

This damage of chromosomes may be induced directly by cytotoxic activities of chemical compounds of AV. These imperfection of chromosomes are justified for more than one reason which associated with physical and chemical agents depend on the lesions induced in the DNA and therefore, upon chemical structure of the genotoxic substance. These can even lead to DNA damage [29].

Several studies reported that substances are capable of causing cytotoxicity by inducing chromosomal abnormalities and DNA double-strand breaks [30,31]. The structural rearrangements have been associated with the chromosomal breakage that can be initiated by telomeric dysfunction, giving rise to unstable dicentric or ring chromosomes [32]. The chromosome aberrations, as a clastogen can trigger the formation of chromosome aberrations by breaking the phosphodiester backbone of DNA Kayraldiz *et al*. [23].

This defect can produce single-strand breaks in the plasmid DNA [33]. More aberrations like chromatid break, ring chromosome, dicentric chromosomes, chromatid and deletion could be resulted from illegitimate reunion (mis rejoining) of free ends from different DNA double-strand breaks [34]. These may be induced by the activities of chemical compounds to prevent the re-establishment of the chromosome under normal repairing of some damage [35]. It may also be induced by disturbing the process of DNA and protein synthesis or during the RNA translocation [35]. It was reported that anthraquinones bound noncovalently to DNA and inhibited topoisomerase II activity [17,20]. This data may be coinciding with idea of Burden and Osheroff [36]; Lynch *et al.* [37] and Kayraldiz *et al.* [23], that all of these compounds induced increases in the formation of topoisomerase II-stabilized cleavage complexes, providing evidence to support a threshold concept for clastogenicity with topoisomerase II poisons. In same field. Epel [38] and Jain *et al.* [38] reported that cytotoxicity of AV might be caused by the decreasing ATP level and the pressure from the functioning of the energy production center.

We can said, there are multiple mechanisms that responsible for genotoxicity likely because of DNA damage induced by the chemicals and herbal medicines and these include reactive oxygen species induction [40,41] DNA polymerase arrest [42], metabolic activation [43] and inhibition of topoisomerase (Topo) [44].

In contrast, several studies which applied *in vitro* (gene mutation and chromosome aberration tests) and *in vivo* (micro-nucleus test in murine bone marrow) genotoxicity studies, as well as human, animal and bacteria pharmacokinetic data, have reported that Aloe plant constituents were nonmutagenic and non genotoxic [8,14,16,45-47], But recently, a two-year National Toxicology Program (NTP) study on oral consumption of non-decolorized whole leaf extract of *Aloe vera* found clear evidence of carcinogenic activity in male and female rats, based on tumors of the large intestine [27].

REFERENCES

1. Matu, E.N. and J. Van Staden, 2003. Antibacterial and anti-inflammatory activities of some plants used for medical purposes in Kenya. J. Enthopharmacol., 87: 35-41.
2. Joseph, B. and S.J. Raj, 2010. Pharmacognostic and phytochemical properties of aloe veralinn-an overview; Int. J.Pharmace Sc. Rev. and Res., 4:106-110.
3. Newall, C.A., L.A. Anderson and J.D. Phillipason, 1996. Herbal Medicines: A Guide for Health-Care Professionals. Pharmaceutical press, London.
4. Chauhan, L.K., M. Kumar, B.N. Paul, S.K. Goel and S.K. Gupta, 2007. Cytogenetic effects of commercial formulations of deltamethrin and/or isoproturon on human peripheral lymphocytes and mouse bone marrow cells. Environ. Mol. Mutagen, 48: 636-643.
5. Capasso, F., F. Borrelli and R. Capasso, 1998. Aloe and its therapeutic use Phytother Res., 12: 124-127.

6. Vogler, B.K. and E. Ernst, 1999. Aloe vera: a systematic review of its clinical effectiveness. Br J. Gen Pract, 49: 823-828.
7. Winters, W.D., R. Benavides and W.J. Clouse, 1981. Effect of aloe extracts on human normal andtumor cells *in vitro*. Economic Botany, 35: 89-95.
8. Westendorf, J., H. Marquardt, B. Poginsky, M. Dominiak, J. Schmidt and H. Marquardt, 1990. Genotoxicity of naturally occurring hydroxyl anthraquinones. Mutat Res., 240: 1-12.
9. Krivobok, S., F. Seigle-Murandi, R. Steiman, D.R. Marzin and V. Betina, 1992. Mutagenicity of substituted anthraquinones in the Ames/ Salmonella microsome system. Mutat Res., 279: 1-8.
10. Tello, C.G., P. Ford and A.M. Iacopino, 1998. *In vitro* evaluation of complex carbohydrate denture adhesive formulations. Quintessence Int., 29: 585-593.
11. Mengs, U., W. Grimminger, G. Krumbiegel, D. Schuler, W. Silber and W. Volkner, 1999. No clastogenic activity of a senna extract in the mouse micronucleus assay. Mutat Res., 444: 421-426.
12. Mueller, S.O., I. Eckert, W.K. Lutz and H. Stopper, 1996. Genotoxicity of the laxative drug components emodin, aloe-emodin and danthroninmammalian cells: topoisomerase II mediated. Mutat. Res., 371: 165-173.
13. Mueller, S.O., M. Schmitt, W. Dekant, H. Stopper, J. Schlatter, P. Schreier and W.K. Lutz, 1999. Occurrence of emodin, chrysophanolandphyscion in vegetables, herbs and liquors. Genotoxicity and anti-genotoxicity of the anthraquinones and of the whole plants. Food Chem. Toxicol., 37: 481-491.
14. NTP Technical Report (TR) 553, 2010. NTP technical report on the photococarcinogenesis study of Aloe vera CAS NO. 481-72-1 (Aloe-emodin)] in SKH-1 mice (simulated solar light and topical application studyAvailable at: http:/ /ntp. niehs. nih. gov/ ntp/ htdocs /LT_ rpts/TR553.pd.
15. Shao, A., A. Broadmeadow, G. Goddard, E. Bejar and V. Frankos, 2013. Safety of purified decolorized (low anthraquinone) whole leaf Aloe vera (L) Burm.f. juice in a 3-month drinking water toxicity study in F344 rats. Food Chem Toxicol., 57: 21-31.
16. Heidemann, A., W. Volkner and U. Mengs, 1996. Genotoxicity of aloe emodin *in vitro* and *in vivo*. Mutat Res., 367: 123-133.
17. Mueller, S.O. and H. Stopper, 1999. Characterization of the genotoxicity of anthraquinones in mammalian cells. Biochim Biophys Acta, 1428: 406-414.
18. IASC, Iternational Aloe Science Council, 2002. Aloe book. (http://www. aloelf.com/wp.content / uploads/ aloe-vera/aloebook.pdf).
19. Danhof, I.E. and B.E. McAnally, 1983. Stabilised Aloe Vera: Effect on Human Skin Cells. Drug and Cosmetic Ind., 52: 105-106.
20. Mueller, S.O., H. Stopper and W. Dekant, 1998. Biotransformation of the anthraquinonesemodin and chrysophanol by cytochrome P 450 enzymes. Bioactivation to genotoxic metabolites. Drug. Meta Dispos, 26: 540-546.
21. NTP, National Toxicology Program, 2001. Toxicology and carcinogenesis studies of emodin (CAS NO. 518-82-1) feedstudies in F344/N rats and B6C3F1 mice. Natl Toxicol Program Tech. Rep. Ser., 493: 1-278.
22. Madle, S., B. Beek and C. Nowak, 1993. ZumVerständnis von Chromosomen mutations tests an Somazellen. In: Fahrig R. ed. Mutationsforschung und GenetischeToxicologie.Wissenschaftliche Buchgesellschaft Publishers; pp: 224-242.
23. Kayraldiz, A., A. Yavuzkocaman, E. Rencüzoğullari, E.S. İstifli, H.B. İla, M. Topaktaş and Y.K. Dağlioğlu, 2010. The genotoxic and antigenotoxic effects of *Aloe vera* leaf extract *in vivo* and *in vitro*. Turk J. Biol., 34: 235-246.
24. Anders, E., 1996. Oxidation of hydroxyl benzotriazole by laccase and lignin pexoxidase. Sicience, 274: 2119-2121.
25. Evans, F.P., G. Breckon and C.E. Ford, 1964. Air-drying method for meiotic preparation from mammalian testes. Cytogenetics, 3: 289-294.
26. Guruprasad, K.P., A. Subramanian, V.J. Singh, R. Sudheer, K. Sharma, P.M. Gopinath, V. Sewram, P.M. Varier and K. Satyamoorthy, 2012. Brahmara sayana protects against ethyl methane sulfonate or methyl methane sulfonate induced chromosomal aberrations in mouse bone marrow cells. (http://creativecommons.org).
27. NTP (National Toxicology Program) 2011. Technical report on the toxicology and carcinogenesis studies of 1-bromopropane(CAS NO. 106-94-5) IN F344/N RATS and B6C3F1 MICE(inhalation studies)NTP TR 56,4 NIH Publication, pp: 11-5906.
28. Tripathi, N., S. Bajpai and M. Tripathi, 2009. Genotoxic alterations induced by fluoride in Asian catfish, Clariasbatrachus (L.) Fluoride, 42: 292-296.

29. Edwards, J.D. and E.W. Karen, 1994. Chromium (VI) induced cytotoxicity and DNA damage occur via two distinct pathways: An oxidative pathway and direct chromium DNA interaction. Met. Ions. Biol. Med., 3: 175-180.
30. Galloway, S.M., J.E. Miller and M.J. Armstrong, 1998. DNA synthesis inhibition as an indirect mechanism of chromosome aberrations: comparison of DNA-reactive and non-DNA-reactive clastogens. Mutation Res., 400: 169-186.
31. Kirkland, D.J. and L. Muller, 2000. Interpretation of the biological relevance of genotoxicity test results: the importance of thresholds Mutation Res., 464: 137-147.
32. Agapova, L.S., G. Ilyinskaya, N. Turovets, A .Ivanov and B. Kopnin, 1996. Chromosome changes caused by alteration of P53 expression. Mutation Research, 354: 129-138.
33. Paes-Leme, A.A., E.S. Motta and De J.C.P. Mattos, 2005. Assessment of *Aloe vera* (L.) genotoxic potential on Escherichia coli and plasmid DNA. J. Ethnopharmacol., 102: 197-201.
34. Cornforth, M.N. and J.S. Bedford, 1993. Ionizing radiation damage and it's early development in chromosome. Advances in Radiation Biology, 17: 423-496.
35. Chidambaram, A., A. Sundaramoorthy, A. Murugan, K.G. Sankar and L. Baskaran, 2009. Chromium induced cytotoxicity in blackgram (*vigna mungo* L.). Ira J. Environ Health Sci. Eng., 6: 17-22.
36. Burden, D.A. and N. Osheroff, 1998. Mechanism of action of eukaryotictopoisomerase II and drugs targeted to the enzyme. Biochim Biophys Acta, 1400: 139-154.
37. Lynch, A., J. Harvey and M. Aylott, 2003. Investigations into theconcept of a threshold for topoisomerase inhibitor-induced clastogenicity. Mutagenesis, 18: 345-353.
38. Epel, D., 1963. The effects of carbon monoxide inhibition of ATP leveland the date of mitosis in the sea urchin egg. J. Cell. Biol., 17: 315-319.
39. Jain, A.K. and R.K. Sorbhoy, 1988. Cytogenetical studies on the effectsof some chlorinated pesticides. III: Concluding remarks. Cytologia, 53: 427-436.
40. Antomy, S., K.K. Agama, Z.H. Miao, K. Takagi, M. Wright, A.I. Robles, L. Varticovski, M. Nagarajan, A. Morrell and M. Cushman, 2007. Novel indenoisoquinolines NSC 725776 and NSC 724998produce persistent topoisomerase I cleavage complexes and overcome multidrug resistance. Cancer Res., 67: 10397-10400.
41. Zou, J., Q. Che, S. Tang, X. Ji, K. Che, T. Zhang and X. Xiao, 2009. Olaquindox-induced genotoxicity and oxidative DNA damage in human hepatoma G2 (HepG2) cells. Mutat. Res., 676: 27-33.
42. Bridgewater, L.C., F.C. Manning, E.S. Woo and S.R. Patierno, 1994. DNA polymerase arrest by adducted trivalent chromium Mol. Carcinog., 9: 122-133.
43. Zhou, S.F., C.C. Xue, X.Q. Yu and G. Wang, 2007. Metabolic activationof herbal and dietary constituents and its clinical and toxicological implications: an update. Curr. Drug. Metab., 8: 526-553.
44. Smart, D.J., H.D. Halicka, G. Schmuck, F. Traganos, Z. Darzynkiewicz and G.M. Williams, 2008. Assessment of DNA double-strand breaks and gamma H2AX induced by the topoisomerase II poisons etoposide and mitoxantrone. Mutat. Res., 641: 43-47.
45. Liberman, D.F., F.L. Schaefer and R.C. Fink, 1980. Mutagenicity of islandicin and chrysophanol in the Salmonella microsomesystem. Appl. Environ. Microbiol., 40: 476-479.
46. Heidemann, A., H.G. Miltenburger and U. Mengs, 1993. The genotoxicity status of senna. Pharmacology, 47: 178-186.
47. Lang, W., 1993. Pharmacokinetic-metabolic studies with ^{14}C-aloe emodin after oral administration to male and female rats. Pharmacology, 47: 73-77.
48. Mengs, U., D. Schuler and R.R. Marshall, 2001. No induction of chromosomal aberrations in Chinese hamster ovary cells by chrysophanol. Mutation Res., 492: 69-72.