Estimation of genotoxic and mutagenic potential of latex and methanolic leaves extract of Euphorbia helioscopia by comet assay and Ames test

Uzma Saleem1*, Saeed Mahmood2, Bashir Ahmad1, Mohammad Saleem1, Aftab Ahmad Anjum3
1University College of Pharmacy, University of the Punjab, Lahore, Pakistan
2Department of Surgery, Lahore General Hospital, Lahore, Pakistan
3Riphah Institute of Pharmaceutical Sciences, Riphah International University, Lahore, Pakistan
4College of Pharmacy, Government College University, Faisalabad, Pakistan
5Department of Microbiology, University of Veterinary and Animal Sciences, Lahore, Pakistan

ARTICLE INFO

Objective: To investigate the genotoxic and mutagenic potentials of Euphorbia helioscopia (Family: Euphobiaceae) latex and methanolic leaves extract.
Methods: Comet assay was adopted to investigate genotoxicity potential and Maron and Ames protocol was followed to evaluate mutagenicity of standardized methanolic leaves extract and latex in concentrations of 10, 5, 2.5, 1.25, 0.625 µg/mL and 1 000, 200, 40, 8, 1.6 µg/mL respectively.
Results: Latex did not damage DNA in lymphocytes and could not produce revertant of Salmonella typhimurium (TA98) at any of the experimental concentrations. While, methanolic leaves extract showed 190.00 ± 0.51 revertants in Salmonella typhimurium (TA98) plate incubated with extract at 10 µg/mL and also damaged lymphocytes DNA at the same concentration. There was statistically significant difference, P < 0.05, at 10 µg/mL/plate concentration with reference to negative control. Pearson correlation was 0.948 showing high consistency between results of comet assay and Ames test.
Conclusion: It was concluded that latex showed no mutagenic and genotoxic potentials at concentrations ranging from 1.6-1 000 µg/mL. Whereas, mutagenic and genotoxic potential threshold level of methanol leaves extract appeared at 10 µg/mL concentration, while remaining used concentrations were devoid of any toxicity.

1. Introduction

Natural products are being consumed from ancient time for the treatment and prevention of various ailments on the basis of knowledge of experienced old persons of the population. Consumption of the herbal medicinal products in the developed countries along with developing countries has been increased from previous few decades[1]. In modern health care system, patient safety is of prime importance. The therapeutic weightage of the drug should be higher than its toxicity in particular disease condition. Genotoxicity and mutagenicity assays were recommended to ensure the quality and safety of natural therapeutic compounds by national and international regulatory agencies[2].

Though medicinal importance of plants has been proved with scientific research but care is needed while selecting the right plant for right indication. Proper authentication and identification of plants is needed to limit their side effects[3-4]. The use of plant based medicines in the treatment of different ailments can be found 5000 years ago[5,6]. More than hundred allopathic medicines are being obtained from plant extracts[7,8]. Digitoxin...
and quinidine are life-saving drugs in cardiac patients, morphine is narcotic analgesic drug used in severely sick patients, colchicine used in gout, phystostigmine and pilocarpine used to treat glaucoma, L-hyoscyamine is antispasmodic and taxol (paclitaxel) is anticancer drug, all these key drugs were obtained from plant source[9,10].

Traditionally, leaves and stems of Euphorbia helioscopia L. (E. helioscopia) are used for vermifuge and febrifuge action, roasted pepper mixed with seed are used in cholera, oil obtained from seeds used in constipation, and roots are used as anthelmintic[11,12]. A number of researchers had explored different pharmacological activities of plant extracts such as insulin secretagogue[13], antibacterial, antifungal, antiviral, phytotoxicity[14-16], vasodepressor[17], anticancer[18], allelopathic[19], antioxidant[14,20,21], anti-allergic and anti-asthmatic[22], breast cancer resistant protein and P-glycoprotein (ABCB1 and ABCG2)[23], cytotoxic[24] and molluscicidal action[25].

Most of the plant based products possessed genotoxic potential[26,27]. Nonetheless to date, no data are available that can rule out the genotoxic potential of the E. helioscopia. This study was planned to probe into genotoxic and mutagenic potential of latex and methanolic leaves extract of E. helioscopia by comet assay and Ames test respectively to evaluate the safety level.

2. Materials and methods

2.1. Plant collection

E. helioscopia (Family: Euphorbiaceae) was collected from suburbs of Lahore, Pakistan in the months of February and March. After identification and authentication by a taxonomist of Botany Department, Government College University, Lahore, Pakistan, a voucher specimen (1501) was deposited to the herbarium. Leaves and stem were separated and dried under shade then ground to fine powder separately which were later on used in extraction. The latex was collected in dried bottles by cutting the leafy part from the stem.

2.2. Preparation of extract

The pulverized material from both parts of the plant was extracted separately at room temperature by maceration in water and ethanol as solvents. Then both the materials were extracted sequentially using solvents (petroleum ether, chloroform, and methanol) in the order of increasing polarity by Soxhlet apparatus. The solvents were removed from the extracts on rotary evaporator at 40°C.

Latex and methanolic leaves extract were selected in the present research based on their promising in vitro antioxidant activities in our previous study[28].

2.3. Standardization of extract and latex

All the extracts and latex have been standardized by applying following analytical techniques: high performance liquid chromatography, UV and Fourier transform infrared spectroscopy fingerprints[29,30]. Quercetin, myricetin, and kaempferol were used as biomarkers in high performance liquid chromatography.

2.4. Preparation of test samples solutions

Phosphate buffer saline was used as vehicle in the preparation of working solutions of test samples. Latex was used in fivefold serial dilutions ranging from 1 000-1.6 µg/mL and similarly methanolic leaves extract concentrations ranged from 10-0.625 µg/mL. The concentrations were selected on the base of our study on cytotoxicity[31].

2.5. Genotoxicity assay

In genotoxicity assay, comet is formed when nucleated cell got damage as a result of exposure to chemicals/toxins[32]. Genotoxic potential of latex (1 000, 200, 40, 8, 1.6 µg/mL) and methanolic leaves extract (10, 5, 2.5, 1.25, 0.625 µg/mL) was determined by comet assay following the protocol of Singh et al.[33]. Briefly, normal melting point agarose gel (0.75%) was poured (80 µL) on clean glass slides (n = 36) and allowed to solidify for 45 min. Lymphocytes, separated from blood of sheep, were poured (100 µL) into 96-well plate and incubated with selected concentrations for 2 h at 37°C. The suspension pipetted out from each well (100 µL) was poured on previously prepared agarose slides. Later on, low melting agarose gel (0.5%) was poured (80 µL) on each slide and allowed to solidify. All of the slides were dipped in (500 mL) of lyses solution kept under refrigeration for 12 h. On second day, lyses solution was removed and slides placed in (500 mL) alkaline buffer for 25-45 min. All of the slides were placed in electrophoresis chamber having buffer [Tris base, 10.8 g, boric acid (MP Biomedicals, LLC), 5.5g and disodium ethylene diamine tetraacetic acid, 0.93 g were dissolved in 700 mL of double-distilled water and finally, volume was made upto 1 000 mL] and run at 25 v for 45 min. Slides were removed from electrophoresis chamber, rinsed two times with neutralizing buffer (Tris base, 24.25 g was mixed with 400 mL of double-distilled water and final volume was 500 mL) and finally stained with 50-80 µL of ethidium bromide solution. Slides were observed immediately under fluorescent microscope at 100× magnification with 560 nm excitation filter and 590 nm barrier filter. Sodium azide (Sigma-Aldrich) and lymphocytes alone were used as positive and negative control respectively.
Results were presented as olive tail moment (tail DNA% × tail moment) and extent tail moment (tail DNA% × length of tail) in arbitrary units\cite{33,34}. All the values were calculated in pixels using computerized image analysis software (image j).

2.6. Mutagenecity assay

Mutagenic potential of latex (1000, 200, 40, 8, 1.6 µg/mL) and methanolic leaves extract (10, 5, 2.5, 1.25, 0.625 µg/mL) was determined by Ames test following the protocol described by Maron and Ames\cite{35}. Histidine dependent Salmonella typhimurium (TA98) (S. typhimurium) without (- S9) metabolic activation mixture was used to check the mutagenicity by plate incubation method. Sodium azide (1%) and normal saline were used as positive and negative control respectively.

Briefly, all the selected concentrations of latex and methanolic leave extract, positive and negative control (200 µL) were incubated with bacterial culture (100 µL) and 0.2 mol/L phosphate buffer (500 µL) at 37 °C in sterile tubes for 20 min. Molted agar (2 mL) containing 9.6 µg/mL histidine (0.05 mmol/L) and 12.4 µg/mL biotin (0.05 mmol/L) was added to each test tube. Then each test tube was poured on separate sterile glucose minimal agar plate. Top agar layer took 2-4 min to solidify, then all the plates were inverted and incubated at 37 °C for 48 h and the His+ revertant colonies were manually counted. Thinning of background lawn (i.e. auxotrophic background) or decreased number of histidine revertant colonies (His+) was used as indicator to test the sample toxicity. Results were described in terms of mean number of revertants per plate ± SD for each concentration.

2.7. Statistical analysis

Results were presented as mean ± SD. Statistically significant difference was measured with ANOVA followed by post hoc Tukey Multiple comparison on SPSS version 12. Treatment groups were significantly different from negative control group at P < 0.05. Pearson correlation was applied to find out relationship between comet (genotoxicity) and Ames (mutagenicity) tests. Correlation was significant at 0.01 level (2-tailed).

3. Results

Latex produced no comet at selected concentration range indicating that latex did not cause DNA damage (Figure 1a). The highest concentration (10 µg/mL) of methanolic extract of leaves caused DNA damage whereas other used concentrations were safe (Figure 1b and 1c). The result was presented as olive tail moment (%tail DNA × tail moment) and extent tail moment (%tail DNA × tail length) in arbitrary units (Figure 2a). Tail and head length of comet was measured to calculate tail moment (Figure 2b). Tail and head length of comet formed at 10 µg/mL were 26.16 ± 0.07, 36.22 ± 0.02 respectively. Tail moment, cell DNA intensity and % tail DNA were 34.81 ± 0.02, 68.86 ± 0.16 and 38.13 ± 0.12 respectively. Olive tail moment was 1327.31 ± 0.05 and extent tail moment was 997.48 ± 0.08.

Prior to perform the mutagenicity test, S. typhimurium strain TA98 was confirmed and standardized on Salmonella Shigella agar medium. Pink colonies were developed within 24 hours which turned yellow with time and after 48-72 hours a black spot appeared in the center of each colony.

Ames test was standardizes on histidine positive and negative media to check natural revertants (Figure 3). No revertants of S. typhimurium were found in latex treated plates.
Mutagenicity assay (Ames test) standardization by growing histidine dependent *S. typhimurium* on histidine positive and negative media plates.

Revertant *S. typhimurium* colonies were revealed on Petri plate on which bacteria treated with the highest concentration (10 µg/mL) of methanolic leaves extract while rest of the concentrations showed no mutagenic potential (Figure 4). There was statistically significant difference, \( P < 0.05 \), at 10 µg/mL/plate concentration with reference to negative control (Table 1). High consistency was found between results of comet and Ames tests by Pearson correlation (\( r = 0.948 \)).

![Figure 3. Mutagenicity assay (Ames test) standardization by growing histidine dependent *S. typhimurium* on histidine positive and negative media plates.](image)

![Figure 4. Mutagenic potential of methanolic leaves extract of *E. helioscopia* by Ames test.](image)

A: 10 µg/mL (plate showing revertants); B: Positive control; C: 5 µg/mL; D: 2.5 µg/mL; E: 1.25 µg/mL; C-E: Plates showing no revertants.

### Table 1
*S. typhimurium* revertants appeared at various concentrations of latex and methanolic leaves extract of *E. helioscopia* in Ames test.

| Methanolic leaves extract (µg/mL/plate) | No. of revertants (µg/mL/plate) | Concentration (µg/mL/plate) |
|----------------------------------------|-------------------------------|----------------------------|
| Negative control                       | 0                             | 0                          |
| Positive control                       | 1350 ± 0.21*                  | 0.625                      |
| Positive control                       | 1.6                           | 1.25                       |
| Positive control                       | 8                             | 2.5                        |
| Positive control                       | 40                            | 5                          |
| Positive control                       | 200                           | 10                         |

*Note: P < 0.05 when compared with negative control; Values were expressed as mean ± SD; Positive control: Sodium azide.*

4. Discussion

There are number of biomarkers to detect the DNA damage in cells as a consequence of chemical exposure, but DNA damage measured by single cell gel electrophoresis (comet) is quite rapid and sensitive method. Researchers focused on this technique because of its low cost and sensitivity of method. Any nucleated cell can be employed in this assay[33,34,36-39]. The sensitivity of method allows detecting the DNA damage on individual cell level and it has capability to measure genotoxic potential, quantitatively in prokaryotic and eukaryotic cells[40-47]. Methanol crude extracts of *Euphorbia hirta* (*E. hirta*) showed genotoxicity on *Allium cepa* assay at 1000 µg/mL as reported Bajpayee *et al*., and Lah *et al*., used comet assay to quantify DNA damage[44,48]. Scoring of comet, on the basis of nucleoids with and without tail diameter, method can also be used to quantify the comet[32,47,49]. *E. hirta* extracts showed DNA damage in dose-dependent manner with comet assay[50]. *Calendula officinalis* (Asteraceae) is herbal medicine, traditionally used in the treatment of several disease showed no DNA damage with comet assay[51].

Ames test is more quick and reliable method to evaluate mutagenic potential of plants and chemicals[32,49,52-54]. *E. hirta* extracts (aqueous and methanol) did not show any mutagenic activity on *S. typhimurium* strains TA98 and TA100[55].

Folklore use of herbal products is due to zero people interest in drugs of natural origin and their faith on safety of natural drugs but safety is strictly linked with therapeutic dose for particular indication[56]. Each drug possessed toxic effects but a valuable pharmacologically active compound should have balance between toxic or untoward and therapeutic effects[57]. To ensure the safety and efficacy of natural products, a battery of genotoxic and/or mutagenicity assays are required to be performed to screen the toxicity mechanism[58]. No single test can gather enough data to forecast the chemical hazards to human health.

Mutagenicity and genotoxicity studies were conducted on variety of plants *e.g*. *Acacia nilotica*, *Juglans regia*, *Terminalia chebula*, *Pothomorphe umbellata* and *Physalis angulata*[59-61].

Latex showed no mutagenic and genotoxic potentials and is safe to use for various pharmacological activities. Less than 10 µg/mL concentration of methanolic leaves extract was found safe.

### Conflict of interest statement

We declare that we have no conflict of interest.

### Acknowledgements

For financial and technical support, we are grateful to Higher Education Commission of Pakistan [Grant No. is 117-6490-BM7-
and University of Veterinary and Animal Sciences, Lahore, Pakistan respectively.

References

[1] World Health Organization. General guidelines for methodologies on research and evaluation of traditional medicine. Geneva: World Health Organization; 2000. [Online] Available from: http://whqlibdoc.who.int/hq/2000/WHO_EDM/TRM_2000.1.pdf [Accessed on 10th October, 2014]

[2] Organization for Economic Co-Operation and Development. Hazards of Chemical Substances and Mixtures. 2002. [Online] Available from: http://www.oecd-ilibrary.org/docserver/download/9789264028398.pdf?expires=143314895&id=id&accname=guest&checksum=90E86384198553D84B3EA9826DE8418 [Accessed on 1st October, 2014]

[3] Katiyar C, Gupta A, Kanjilal S, Katiyar S. Drug discovery from plant sources: an integrated approach. Ayu 2012; 33(1): 10-9.

[4] Hussain K, Majeed MT, Ismail Z, Sadikun A, Ibrahim P. Traditional and complementary medicines: quality assessment strategies and safe usage. South Med Rev 2009; 2(1): 19-23.

[5] Goldman P. Herbal medicine today and the roots of modern pharmacology. Ann Intern Med 2001; 135(8): 594-600.

[6] Anushia C, Sampathkumar P, Ramkumar L. Antibacterial and antioxidant activities in Cassia auriculata. Glob J Pharmacol 2009; 3: 127-30.

[7] Li JW, Vederas JC. Drug discovery and natural products: end of an era or an endless frontier? Ann Intern Med 2001; 135(1): 19-23.

[8] Atmakuri LR, Dathi S. Current trends in herbal medicines. J Pharm Res 2010; 3: 109-13.

[9] Nadeem M, Rikhari HC, Kumar A, Palni LMS, Nandi SK. Taxol content in the bark of Himalayan Yew in relation to tree age and sex. Phytochemistry 2002; 60: 627-31.

[10] Special Programme for Research & Training in Tropical Diseases (TDR). Operational guidance: information needed to support clinical trials of herbal products. Geneva: TDR; 2005. [Online]. Available from: http://www.who.int/trd/publications/documents/operational-guidance-eng.pdf?ua=1 [Accessed on 10th October, 2014]

[11] Wu TS, Lin YM, Haruna M, Pan DJ, Shingu M, Pan DJ, Shingu T, Chen YP, et al. Antitumor activities in Euphorbia helioscopia L. extracts against human cancer cells. Anat Rec (Hoboken) 2012; 295: 223-33.

[12] M. Anushia C, Sampathkumar P, Ramkumar L. Antibacterial and antioxidant properties of methanolic and ethanolic extracts of Euphorbia helioscopia (L.). Ethnopharmacol 2009; 126(2): 119-25.

[13] Barla A, Birman H, Kultur S, Oksuz S. Secondary metabolites from Euphorbia helioscopia and their vasodepresser activity. Turk J Chem 2004; 28: 577-85.

[14] Nikolova M, Evstatieva L, Nguyen TD. Screening of plant extracts for antioxidant properties. Bot Serb 2011; 35(1): 43-8.

[15] Ben-Mohamed ML, Jelassi A, Hassen I, Ould MSBA. Antioxidant proprieties of methanolic and ethanolic extracts of Euphorbia helioscopia, (L.) aerial parts. Int Food Res J 2012; 19(5): 1125-30.

[16] Park KH, Koh D, Lee D, Jung I, Kyung HK, Lee CH, et al. Anti-allergic and anti-asthmatic activity of helioscopicin A, a polypenol compound, isolated from Euphorbia helioscopia. J Microbiol Biotechnol 2001; 11(1): 138-42.

[17] Barile E, Borriello M, Di Pietro A, Doreau A, Fattorusso C, Fattorusso E, et al. Discovery of a new series of jatrophone and lathyrene diterpenes as potent and specific P-glycoprotein modulators. Org Biomol Chem 2008; 6: 1756-62.

[18] Zhang W, Guo YW. Chemical studies on the constituents of the Chinese medicinal herb Euphorbia helioscopia L. Chem Pharm Bull 2006; 54: 1037-9.

[19] Al-Zanbagi NA, Banaja AA, Barrett J. Molluscicidal activity of some Saudi Arabian Euphorbiales against the snail Biomphalaria pfeifferi. J Ethnopharmacol 2000; 70(2): 119-25.

[20] Mengs U. Toxic effects of sennosides in laboratory animals and in vitro. Pharmacology 1998; 36(Suppl 1): 180-7.

[21] Ferreira-Machado SC, Rodrigues MP, Nunes AP, Dantas FJ, De Mattos JC, Silva CR, et al. Genotoxic potentiality of aqueous extract prepared from Chrysobalanus icaco L. leaves. Toxicol Lett 2004; 151(3): 481-7.

[22] Saleem U, Ahmad B, Hussain K, Ahmad M, Bukhari NI, Ishitaq S. Estimation of antioxidant power in various extracts of Euphorbia helioscopia L. with five different in vitro antioxidant models. Asian J Chem 2014; 26(4): 1241-5.

[23] Saleem U, Hassan K, Ahmad M, Bukhari NI, Malik A, Ahmad B. Physicochemical and phytochemical analysis of Euphorbia helioscopia (L.). Pak J Pharm Sci 2014; 27(3): 577-85.

[24] Saleem U, Ahmad B, Hussain K, Ahmad M, Bukhari NI, Malik A, Ahmad B. Simultaneous quantification of quercetin, myricetin and kaempferol in extracts and latex of Euphorbia helioscopia using RP-HPLC. Asian J chem 2014; 26(22):
7673-6.

[31] Saleem U, Ahmad B, Ahmad M, Hussain K, Bukhari NI, Anjum AA. Determination of cytotoxicity of latex and methanol extract of Euphorbia helioscopia leaves on vero cell line with MTT assay. Pak J Zool 2014; 46(3): 741-5.

[32] Tsabay MS, Marcarini1 JC, Ferreira DT, Ferraz ERA, Chequer FMD, de Oliveira DP, et al. Evaluation of extracts from Coccoloba mollis using the Salmonella/microsome system and in vivo tests. Genet Mol Biol 2010; 33(3): 542-8.

[33] Singh NP, McCoy MT, Tice RR, Schneider EL. A simple technique for quantification of low levels of DNA damage in individual cells. Exp Cell Res 1988; 175(1): 184-91.

[34] Olive PL, Banáth JP, Durand RE. Heterogeneity in radiation-induced DNA damage and repair in tumor and normal cells measured using the comet assay. Radiat Res 1990; 122(1): 86-94.

[35] Maron DM, Ames BN. Revised methods for the Salmonella mutagenicity assay. Mutat Res 1983; 113(3-4): 173-215.

[36] Ostling O, Johansson KJ. Microelectrophoretic study of radiation-induced DNA damage in individual mammalian cells. Biochem Biophys Res Commun 1984; 123(1): 291-8.

[37] McArt DG, McKerr G, Howard CV, Saetzler K, Wasson GR. Modelling the Comet assay and two recombination assays. Mutat Res 2003; 538: 171-9.

[38] Loh DS, Er HM, Chen YS. Mutagenic and antimutagenic activities of Calendula officinalis extracts in mice treated with methyl methanesulfonate. Adv Life Sci 2012; 2(2): 21-8.

[39] Carillo-Cortés R, Hernández-Ceruelos A, Torres-Valencia JM, González-Avila M, Arriaga-Alba M, Madrigal-Bujaidar E. Antimutagenicity of Stevia pilosa and Stevia eupatoria evaluated with the Ames test. Toxicol In Vitro 2007; 21(4): 691-7.

[40] McArt DG, McKerr G, Howard CV, Saetzler K, Wasson GR. Modelling the comet assay. Biochem Soc Trans 2009; 37(4): 914-7.

[41] Lohr M, Folkmann JK, Sheykzhade M, Jensen LJ, Kermanizadeh A, Loft S, et al. Hepatic oxidative stress, genotoxicity and vascular dysfunction in lean or obese Zucker rats. PLoS One 2015; 10(3): e0118773.

[42] Tice RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, et al. Single cell gel/comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen 2000; 35: 206-21.

[43] Frenzilli G, Negro M, Lyons BP. The Comet assay for the evaluation of genotoxic impact in aquatic environments. Mutat Res 2009; 681(1): 80-92.

[44] Dililitas M, Kocyigit A, Yigit F. A molecular-based fast method to determine the extent of DNA damages in higher plants and fungi. Afri J Biotechnol 2009; 8 (14): 3118-27.

[45] Ghichler T. Differential genotoxicity of ethyl methanesulfonate, N-ethyl-N-nitrosourea and maleic hydrazide in tobacco seedlings based on data of the Comet assay responses in human lymphocytes are not influenced by the menstrual cycle: a case study in healthy Indian females. Mutat Res 2005; 565(2): 163-72.

[46] Ghosh MJM, Sinha S, Chakraborty A, Mallick SK, Bandypadhyay M, Mukherjee A. In vitro and in vivo genotoxicity of silver nanoparticles. Mutat Res 2012; 749(1-2): 60-9.

[47] Valencia-Quintana R, Gómez-Arroyo S, Waliszewski SM, Sánchez-Alarcón J, Gómez-Olivares JL, Flores-Márquez AR, et al. Evaluation of genotoxic potential of dimethyl sulfoxide (DMSO) in meristematic cells of the root of Vicia faba. Toxicol Environ Health Sci 2012; 4(3): 154-60.

[48] Lah B, Zinko B, Narat M, Marinsek Logar R. Monitoring of genotoxicity in drinking water using in vitro comet assay and Ames test. Food Technol Biotechnol 2005; 43(2): 139-46.

[49] Resende FA, Barbosa LC, Tavares DC, de Camargo MS, de Souza Rezende KC, E Silva ML, et al. Mutagenicity and antimutagenicity of (-)-hinokinin in mice treated with methyl methanesulfonate. Adv Life Sci 2012; 2(2): 21-8.

[50] Carillo-Cortés R, Hernández-Ceruelos A, Torres-Valencia JM, González-Avila M, Arriaga-Alba M, Madrigal-Bujaidar E. Antimutagenicity of Stevia pilosa and Stevia eupatoria evaluated with the Ames test. Toxicol In Vitro 2007; 21(4): 691-7.

[51] Issazadeh K, Aliabadi MA. Antimutagenic activity of olive leaf aqueous extract by Ames test. Adv Stud Biol 2012; 4(9): 397-405.

[52] Gomaa IO, Kader MH, Salah TA, Heikal OA. Evaluation of in vitro and in vivo genotoxicity and genotoxicity of magnetite nanoparticles. Drug Discov Ther 2013; 7(3): 116-23.

[53] Loh DS, Er HM, Chen YS. Mutagenic and antimutagenic activities of aqueous and methanol extracts of Euphorbia hirta. J Ethnopharmacol 2009; 126(3): 406-14.

[54] Phillipson JD. Phytochemistry and pharmacognosy. Phytochemistry 2007; 68(22-24): 2960-72.

[55] Munari CC, Alves JM, Bastos JK, Tavares DC. Evaluation of the genotoxic and antigenotoxic potential of Baccharis dracunculifolia extract on V79 cells by the comet assay. J Appl Toxicol 2010; 30(1): 22-8.

[56] Kovacs Z, Pentek I, Prudnikova T, Koller P, Horvath E, Kazmaier Z, et al. Biological activity of leaf extract of Allium cepa against Trypanosoma brucei and Plasmodium falciparum. J Ethnopharmacol 2005; 100: 181-6.

[57] Barragan-Luna S, Castañeda-Vazquez JJ, Pina-Cortes M, Echeverria-Velázquez F, del Rio JP, Pena-Sanchez J, et al. Evaluation of genotoxicity of medicinal plant extracts by the comet and VITOTOX tests. J Environ Pathol Toxicol Oncol 2005; 24(3): 193-200.

[58] Bast A, Chandler RF, Choy PC, Dehnholle LM, Gruenwald J, Halkes SBA, et al. Botanical health products, positioning and requirements for effective and safe use. Environ Toxicol Pharmacol 2002; 12: 195-211.

[59] Arora S, Brits E, Kaur S, Kaur K, Sohi RS, Kumar S, et al. Evaluation of genotoxicity of medicinal plant extracts by the comet and VITOTOX tests. J Environ Pathol Toxicol Oncol 2005; 24(3): 193-200.

[60] de Andrade NS, de Souza MR, Perazzo FF, Bastos JK, Maistro EL. Evaluation of the genotoxic potential of a water-ethanolic extract of Pothomorphe angulata (Piperaceae) aerial parts on wistar rats cells by the comet and micronucleus assay. Cytologia 2005; 70(4): 399-405.

[61] Alves dos Santos R, Cabral TR, Cabral IR, Antunes LM, Pontes Andrade C, Cerqueira dos Santos Cardoso P, et al. Genotoxic effect of Euphorbia hirta L. (Solanaceae) extract on human lymphocytes treated in vitro. Biocell 2008; 32(2): 195-200.