Original Article

Genotoxicity evaluation of hydroalcoholic and aqueous extracts of *Dorema aucheri* by the comet assay

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Abstract

Background: *Dorema aucheri* is a plant of Apiaceae family which is used widely in some states of Iran. Different extracts and essential oil of *Dorema* species contain flavonoids and cumarin compounds which have anti-hypertensive, cholesterol- and triglycerides-lowering properties. This study was undertaken to evaluate the genotoxic properties of hydroalcoholic and aqueous extracts of *D. aucheri* on human hepatoma cells using the comet assay method for safety evaluation.

Materials and Methods: In this method, after incubation of cells with different concentrations of extracts, cell suspensions were added to pre-coated normal agarose slides. After lysis, electrophoresis and neutralization process, staining was done by ethidium bromide and comets were observed using a fluorescence microscope. Tail length, percentage of DNA in tail and tail moment parameters were measured.

Results: Statistical analysis of the results demonstrated that concentrations more than 500 μg/ml of hydroalcoholic and aqueous extract of *D. aucheri* were genotoxic.

Conclusion: It can be concluded from the results that taking the concentrations less than these dosages of extracts are safe but more studies are required to determine genotoxic mechanisms of this plant.

Key Words: Comet assay, *Dorema aucheri*, genotoxicity, $HepG_2$

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INTRODUCTION

In some parts of Iran, various records indicate that the percentage of some kinds of cancer is much higher than the average and many believe that this should be caused by local dietary habits.[1,2] *Dorema aucheri* (called Bilhar in persian) is a member of Apiaceae family grows wild at the end of the spring in Zagros mountains in Iran. The leaf and the stem of this aborigine plant are used widely as a flavor. All parts of this plant contain secretory apparatus producing flavonoids, cumarins and essential oils.[3] Many people believe that all plant-based therapies have less or no side effect in comparison with other therapies and use of *D. aucheri* for treatment of hypertension, high cholesterol and hypertriglyceridemia.[4] *D. aucheri* properties are not well known, but antihyperlipidemic effect and its role on sex hormones and thyroid function have been studied in rat.[5,6] To evaluate the safety of *D. aucheri*, we tested its genotoxicity potential on human hepatoma ($HepG_2$) cells. There

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are several methods for evaluation of DNA damage, and among which the comet assay or single cell gel electrophoresis (SCGE) is the most well known.[7,8] This method is based upon micro electrophoresis and can sensitively detect injurious effects on DNA.[9] In this method, the cells mixed with low melting point (LMP) agarose and then lysed under the alkaline conditions, so they can find unwinding in the DNA helix. Under the effect of the electrical flow in electrophoresis, the DNA molecules move toward anode to form the comet shape. The comet formation pattern is determined by the size of the DNA fragments and the number of broken ends.[10] As the percent of damage increases, the free DNA fragments move more in the tail. 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 of being exposed to any other genotoxic agents.[11] Microscopic observation of DNA migration is possible using ethidium bromide staining and a fluorescent microscope.[12] Numerous advantages offered by the comet assay method including its high sensitivity, inexpensiveness, and applicability for samples of small size have introduced it as one of the simplest and most efficient methods for the biomonitoring of genotoxic properties.[13]

With regard to wide usage of this plant as a flavoring and the presence of active biological compounds, this study was designed to evaluate the genotoxic effects of *D. aucheri* using the comet assay for safety evaluation.

MATERIALS AND METHODS

Materials

Tris, Triton X-100, H_2O_2, NaCl, EDTA, NaOH and NaH_2PO_4 (Merck Co., Germany), low-melting-point agarose (LMA), Na_2HPO_4, KCl and Ethidium bromide (Sigma Co. USA), normal melting point agarose (NMA) (Cinnagen Co., Iran), RPMI-1640, FBS and antibiotic (PAA Co., Australia) were used in this study. The aerial parts of *D. aucheri* were collected from Yasuj Mountains in the western Iran at the end of spring 2012.

Extract preparation

All extracts of aerial parts of *D. aucheri* were prepared in the faculty of pharmacy and pharmaceutical sciences, Isfahan University of Medical Sciences, Isfahan, Iran. Aqueous extract was prepared using 100 g of powdered plant and maceration method, extracted with water at room temperature. Proceeds filtrated and the residual was extracted for two more times. Finally, whole extract was collected, freeze dried and stored in a refrigerator. To prepare hydroalcoholic extract, 100 g of powdered plant enclosed in filter paper and placed in the soxhlet apparatus separately. Ethanol was added and the reflux was performed 15 times. Extract was dried by rotary and stored in the refrigerator.[14,15]

Cell culture

The $HepG_2$ cell line was obtained from Pasture Institute (Iran) and cultured in RPMI medium (contain 10% fetal bovine serum and 250 µl of penicillin/streptomycin to avoid the growth of undesirable and pathogenic bacteria) and incubated under 5% CO_2 at 37°C in micro filter plates.

Cells were incubated with different concentrations of aqueous and hydro alcoholic extract of *D. aucheri* for 2 h. As described in our previous studies, the upper medium of wells was thrown away and washed with PBS. After trypsinization, they were neutralized by the medium and were poured in five different falcons and centrifuged. Then 1 ml of medium was added to each falcon and 300 µl of cell suspension was used for the comet assay procedure.[14]

Comet assay

The comet assay procedure has been described in our previous studies.[14,16,17] Briefly, incubated cell suspensions (1×10^6 cells/ml) were mixed with of 1% LMP agarose (37°C) and were placed on the pre-coated slides (1% NMP agarose). The slides were respectively incubated with lysis solution (pH = 10.0) and electrophoresis buffer (pH > 13.0) for 40 min. Electrophoresis was done for 40 min (25 V, 300 mA). After this stage, the slides were rinsed with distilled water and were placed in neutralization solution (pH = 7.5) for 10 min. 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 microscopy with an excitation filter of 510–560 nm and barrier filter of 590 nm. All stages of comet assay were performed at room temperature and in dark conditions and all solutions were prepared freshly and used cool.

Statistical analysis

One-way analysis of variance[18] was used to compare the results of comet assay, followed by Tukey's multiple comparison *post hoc* test. The *P* values 0.05 and less were considered as statistically significant.

RESULTS

To determine the genotoxic concentration of the aqueous and hydro alcoholic extracts of the *D. aucheri*, $HepG_2$ cells were separately incubated with different concentrations of these extracts for 2 h followed by the comet assay. Three factors including tail length, percentage of DNA in tail and tail moment (% DNA in

tail × tail length) were measured and compared with the negative control group.

Comet assay results of the alcoholic extract of *D. aucheri*

Three concentrations of hydro alcoholic extract of *D. aucheri* were selected and comet parameters as index of DNA damage were studied [Figure 1] The result of the one-way analysis[18] for the tail length was significant ($P < 0.0001$). According to the results of Tukey's multiple comparison *post hoc* test, two concentrations of the hydro alcoholic extract (500 and 1000 µg/ml) had genotoxic effects on $HepG_2$ cells and increased the tail length significantly ($P < 0.001$) compared with the control group [Figure 1a].

The one-way analysis of the percentage of DNA in tail was significant ($P < 0.0001$). The results of Tukey›s multiple comparison *post hoc* test showed the concentrations of 500 and 1000 µg/ml had significant differences (respectively $P < 0.01$ and $P < 0.001$) compared with the control group [Figure 1b].

The results of the tail moment were completely similar to the tail length [Figure 1c].

Comet assay results of the aqueous extract of *D. aucheri*

The comet properties of aqueous extract of *D. aucheri* were studied [Figure 2]. The result of the one-way analysis[18] for all three factors including tail length, percentage of DNA in tail and tail moment was significant ($P < 0.0001$). According to the results of Tukey›s multiple comparison *post hoc* test, the concentration of 1000 µg/ml have increased the all factors significantly ($P < 0.001$) in comparison with the control group [Figure 2a-c].

DISCUSSION

Genotoxicity or DNA damage can lead to cancer. Research on the genotoxic properties of foreign compounds including native herbs of different regions has an important role in indicating the reason of prevalent genetic disorders of each region and improvement in safety information. There are several *in vitro* and *in vivo* tests for genotoxicity assessment and detecting DNA damage with the comet assay method is one of common methods of this assessment. For this propose, different concentrations of hydro alcoholic and aqueous extracts of *D. aucheri* were prepared and incubated with $HepG_2$ cells for 2 hours. All three important factors (tail length, % DNA in tail and tail moment) were compared with the control group as genotoxicity parameters. Significant increase of studied factors was seen with both extracts of *D. aucheri*. The component of these extracts has genotoxic potency on $HepG_2$ cells.[19] In this study, we compare genotoxic effects of *D. aucheri* with intact cells as negative control and H_2O_2 (200 µM) was used

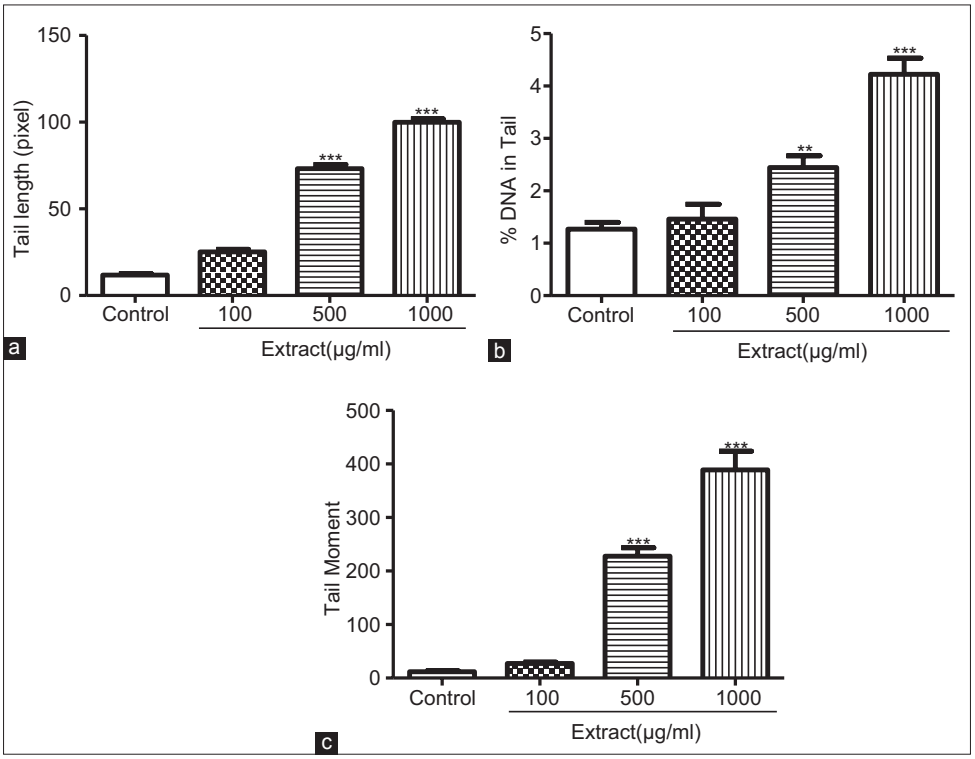

Figure 1: Comparison of (a) Tail length, (b) % DNA in tail and (c) Tail moment of different concentrations of hydroalcoholic extract of *D. aucheri*. Each graph has been represented as Mean ± SEM. ** and ***show respectively significant results of ($P < 0.01$) and ($P < 0.001$) in compare with the control group

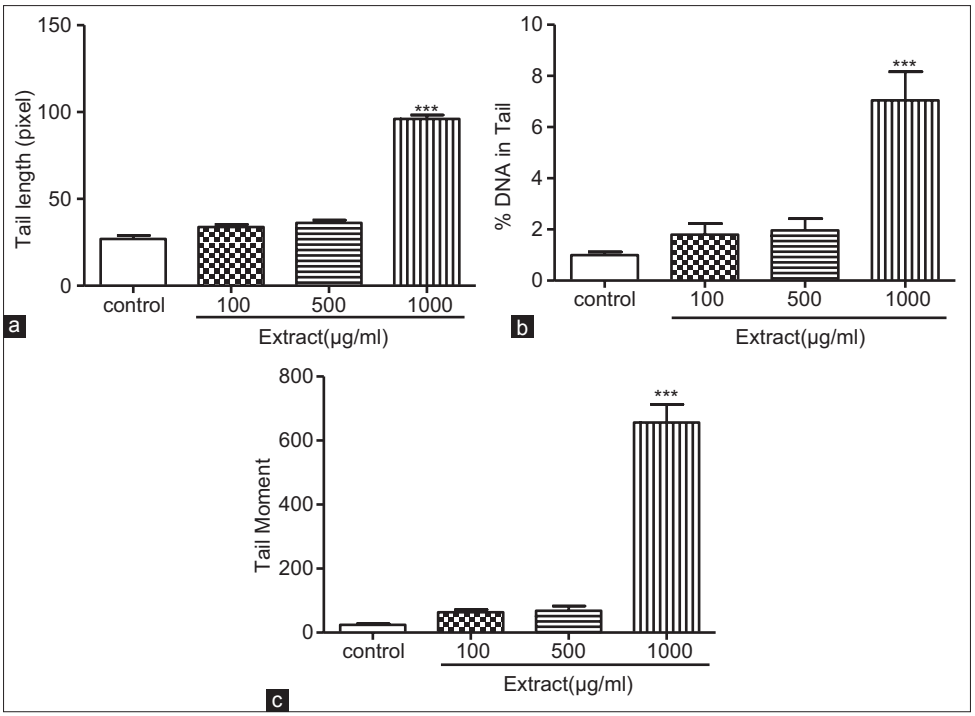

Figure 2: Comparison of (a) Tail length, (b) % DNA in tail and (c) Tail moment of different concentrations of aqueous extract of *D. aucheri*. Each graph has been represented as Mean ± SEM. ***shows significant results of ($P < 0.001$) in compare with the control group

as positive control of method. The concentrations of 500 and 1000 µg/ml of hydro alcoholic extract and the concentrations of 500 µg/ml of aqueous extract showed significant results. Separated study of these extracts showed that the potency of hydro alcoholic extract genotoxicity is more than aqueous extract. Then we can assume that these extracts are safe in lower concentrations. Therefore, other genotoxicity tests need to be performed on this plant. *D. aucheri* samples which are sourced from the other parts of the country should also be checked by similar tests.

CONCLUSION

It can be concluded from the discussion that *Dorema aucheri* has genotoxic effect on $HepG_2$ cells. With regard to this finding lower concentration of this plant could be suggested to have no genotoxic effects.

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REFERENCES

1. Pakseresht M, Forman D, Malekzadeh R, Yazdanbod A, West RM, Greenwood DC, *et al*. Dietary habits and gastric cancer risk in north-west Iran. Cancer Causes Control 2011;22:725-36.
2. Semnani S, Sadjadi A, Fahimi S, Nouraie M, Naeimi M, Kabir J, *et al*. Declining incidence of esophageal cancer in the Turkmen Plain, eastern part of the Caspian Littoral of Iran: A retrospective cancer surveillance. Cancer Detect Prev 2006;30:14-9.
3. Wollenweber E, Dörr M, Rustiyan A. *Dorema aucheri*, the first umbelliferous plant found to produce exudate flavonoids. Phytochemistry 1995;38:1417.
4. Narasimhacharya AV, Nitesh K, Desai TN. Prosopis juliflora as an antihypercholesterolemic agent. J Herb Med Toxicol 2010;4:31-4.
5. Azarneushan F, Karami M, Golizadeh L, Davary K. The effect of Dorema aucheri-Hydroalcoholic extracts on thyroids hormones in adult male rats. J Shahrekord Univ Med Sci 2010;12:76-83.
6. Azarnioushan F, Khatamsaz S, Sadeghi HE. The effects of hydroalcoholic extract of Dorema Aucheri on blood concentration of gonadotropin and androgen hormones in adult male rats. Armaghan Danesh 2009;14:63-70.
7. 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. Mol Vis 2008;14:1584-8.
8. Jena GB, Kaul CL, Ramarao P. Genotoxicity testing, a regulatory requirement for drug discovery and development: Impact of ICH guidelines. Indian J Pharmacol 2002;34:86-99.
9. Sardas S. Genotoxicity tests and their use in occupational toxicology as biomarkers. Indoor B E 2005;14:521-5.
10. Rivero MT, Vázquez-Gundín 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.
11. De Méo M, Laget M, Castegnaro M, Duménil G. Genotoxic activity of potassium permanganate in acidic solutions. Mutat Res 1991;260:295-306.
12. Olive PL, Banáth JP, Fjell C. DNA strand breakage and DNA structure influence staining with propidium iodide using the alkaline comet assay. Cytometry 1994;16:305-12.
13. Uhl M, Helma C, Knasmüller S. Evaluation of the single cell gel electrophoresis assay with human hepatoma ($HepG_2$) cells. Mutat Res 2000;468:213-25.
14. 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;17:782-6.
15. Rabbani M, Sajjadi SE, Sadeghi M. Chemical composition of the essential oil from kelussia odoratissima Mozaff. and the evaluation of its sedative and anxiolytic effects in mice. Clinics (Sao Paulo) 2011;66:843-8.
16. Etebari M, Ghannadi A, Jafarian-Dehkordi A, Ahmadi F. Genotoxicity evaluation of aqueous extracts of Co-toneaster discolor and Alhagi pseudalhagi by com-et assay. J Res Med Sci 2012;17:S238-42.
17. 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. Dent Res J (Isfahan) 2012;9:314-20.
18. Stoyanova II, Gulubova MV. Mast cells and inflammatory mediators in chronic ulcerative colitis. Acta Histochem 2002;104:185-92.
19. Mostafavi SH, Fazilati M, Mostafavi SA, Vahhabi MR, Mostafavi F, Omidvarinia S, *et al*. Hepatotoxicity of Dorema aucheri (Bilhar) in albino mice. Arch Iran Med 2013;16:530-2.

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