Food and Chemical Toxicology xxx (2012) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

Toxicological and mutagenic analysis of *Artemisia dracunculus* (tarragon) extract

Heibatullah Kalantari [a], Hamid Galehdari [a], Zahra Zaree [a], Rudolf Gesztelyi [b], Balazs Varga [b], David Haines [b], Mariann Bombicz [b], Arpad Tosaki [b], Bela Juhasz [b,*]

[a] School of Pharmacy, Ahwaz Jundishapur University of Medical Sciences, Iran
[b] School of Medicine, Faculty of Pharmacy, Department of Pharmacology, University of Debrecen, Debrecen, Hungary

ARTICLE INFO

Article history:
Received 24 February 2012
Accepted 19 July 2012
Available online xxxx

Keywords:
In vitro
Hepatic toxicology
Artemisia dracunculus
Tarragon
Comet assay

ABSTRACT

Mutagenicity and liver toxicity of the herb tarragon (*Artemisia dracunculus*) were evaluated using single cell gel (comet) electrophoresis. Ten microlitres aliquots of peripheral venous human blood were incubated with tarragon extract, saline, or the mutagen sodium dichromate. Cell suspensions dispersed in low-melting agarose were electrophoresed in ethidium bromide. The resulting DNA migration trails were obtained using fluorescent microscopy at 400× magnification, and graded according to the mutagenicity index (MI) for each cell incubation condition. The *in vivo* liver toxicity of *Artemisia dracunculus* was assessed in the blood of mice treated orally with the extract of the herb, using alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as liver function indicators. Liver morphology was assessed using hematoxylin and eosin (HE) staining of liver tissue. The present study demonstrated a direct correlation between tarragon extract dosage and three major outcome variables: MI; serum liver enzyme activity; and liver histopathology. These outcomes are possibly due to the presence in tarragon of methylchavicol and other genotoxic compounds. These findings provide a preliminary guide for risk assessment of tarragon in diet and in possible therapeutic applications.

1. Introduction

The popularity of herbal preparations for prevention and mitigation of disease has increased dramatically in recent years. Their availability outside most government regulatory statutes and without prescription as over-the-counter (OTC) products has made many of them highly cost-effective alternatives to pharmaceutical drugs (Chan, 2003). Moreover, physicians generally find that patient compliance in following a particular treatment regimen is substantially better when medicinal plant extracts are recommended, versus that found with "mainstream" remedies. Nevertheless, herbal medicines will have optimal benefit to the public only if stringent toxicological analysis are conducted incidental to their clinical use. The present evaluation of tarragon (*Artemisia dracunculus* L., or dragon's wort) is an example of this trend.

Tarragon is a perennial member of the Asteraceae family, related to the herb wormwood. The herb is widely used as food seasoning and as a primary flavor for some brands of carbonated beverages. The plant contains compounds that help alleviate pain associated with dental conditions, and that promote bile production and detoxification by the liver, thus aiding digestion, and they may act as a mild sedative (McGuffin et al., 1997; Nestler, 2002). Tarragon may be useful in management of dysregulalated glucose metabolism, including hyperglycaemia, diabetes, and related metabolic syndromes (Cefalu et al., 2008; Ribnicky et al., 2009; Ribnicky et al., 2006; Wang et al., 2008). The herb may also prove useful in the treatment of microbial infections (Benli et al., 2007; Lopes-Lutz et al., 2008), and has potential as an anticoagulant (Shahriyary and Yazdanparast, 2007).

The results of the present study will provide insight into the toxicological profile of this plant and aid physicians and other caregivers in tailoring its use to be an optimally appropriate element of medical treatments. The present report evaluates tarragon's mutagenic (DNA damaging) capacity. This is important information since DNA damage reduces cell viability contributing to tissue damage and is additionally carcinogenic. Several reliable and widely used mutagenicity tests were considered for use in this investigation, notably the micronucleus test and the single cell gel electrophoresis (comet) assay (Collins et al., 1997).

The comet assay was ultimately selected based on its wide use in research, simplicity and reliability. This assay, also known as the Single Cell Gel Electrophoresis (SCGE)-based assay, is applicable for mutagenicity in determining differential migration patterns by DNA fragments of varying length, in order to assess the level of DNA damage to which a cell has been subjected. Typically, blood incubated with a potential mutagen (in the present case, tarragon extract) is suspended in agarose and layered onto a microscope

* Corresponding author. Address: Faculty of Pharmacy, Department of Pharmacology, Health and Science Center, University of Debrecen, Nagyerdei krt. 98, 4032 Debrecen, Hungary. Tel./fax: +36 52 453586.
E-mail address: juhasz.bela@pharm.unideb.hu (B. Juhasz).

0278-6915/$ - see front matter © 2012 Published by Elsevier Ltd.
http://dx.doi.org/10.1016/j.fct.2012.07.052

slide, then treated with non-ionic detergents that lyse all proteins and other components except for DNA, which remains trapped in the cavity once occupied by a DNA-containing leukocyte. An electric field is then applied to the slide causing the negatively-charged DNA to migrate in the direction of the anode (positive electrode). Undamaged DNA, consisting of long strands, remains mostly trapped in the cavities, whereas damaged DNA, comprised of much shorter fragments, migrates out of the cavities in patterns which appear as extended "trails upon staining of the slide with ethidium bromide (EtBr)". These migration trails create comet-like patterns with lengths directly proportional to the degree of DNA fragmentation induced by a particular mutagen. Use of the comet assay in the present investigation yielded insight into tarragon's capacity to induce DNA damage (Cook and Brazell, 1977; Cook et al., 1976; Ostling and Johanson, 1984; Ostling et al., 1987; Singh et al., 1988; Tice et al., 1990, 1991).

Additional toxicological evaluations of the extract were conducted by measuring the activities of toxicant-sensitive liver enzymes (AST/OT and ALT/PT), along with a histological examination of hepatic tissue from mice treated with tarragon.

2. Materials and methods

2.1. Tarragon extract and control reagents

Tarragon extract was purchased from an Iranian manufacturer (Barich Herbal Pharmaceuticals, Kashan, Iran). The sample used in the present study was drawn from Health Ministry lot #5569, MOH serial number 10061, barcode 6260084700072. The component profile for this sample may be obtained at request from the manufacturer (Barich, Kashan).

The preparation of the extract for use as a comet assay reagent was conducted at the Department of Pharmacognosy of the School of Pharmacy, Ahwaz Jundishapur Medical Science University. Briefly, the preparation protocol utilized an ethanol:water maceration method (ratio 7:3). Filtration was performed, and the resulting filtrates were vacuum-evaporated, followed by a measurement of density (940 mg/ml) using a pycnometer. The resulting suspension was suitable both for use as a comet assay reagent, and for oral administration when testing animals in liver toxicity evaluations. For comet assays, a saline buffer was included as a negative control; sodium dichromate, a known mutagen, was used as a positive control to induce DNA fragmentation, an outcome expected to be manifested as long-tailed comet patterns (Bucher, 2007; Buehrlen et al., 2007; Stout et al., 2009),

2.2. Comet assay

Here, comet assays were performed according to the previously described methodology (Collins, 2002; Kobayashi et al., 1995). Peripheral venous blood was freshly collected from healthy, non-smoking student volunteers at Jundishapur University, and10 µl aliquots of were added to six numbered 2-ml screw-top tubes, each containing 1 ml of Hanks Balanced Salt solution (HBSS) and test reagents (tarragon extracts and controls). Four tubes were prepared, containing 94, 188, 376, and 752 mg tarragon extract at a density of 940 mg/ml. Saline buffer, the negative control, was added to a fifth tube; 262 mg of sodium dichromate, the positive control, was added to the sixth tube. Samples containing reagents and blood were incubated in a water bath for 10 min at 37 °C, followed by centrifugation for five minutes at 3500 rpm. The upper layer was discarded, and the pellet was dispersed in 100 µl of 0.5% (low melting point) agarose in phosphate buffer.

The resulting cell suspensions were poured onto agarose-coated microscope slides with cover slips were placed over them. After solidification, the coverslips were removed and the slides were placed in lysis buffer at 4 °C for one hour to dissolve leukocyte cellular structures, leaving cavities containing cellular DNA. The prepared slides were then electrophoresed in alkaline buffer for one hour at 25 V and 300 mA, followed by three 5-min washes with neutral buffer to remove alkali and detergents (which may interfere with the ethidium bromide dye uptake). Next, slides were stained with ethidium bromide to label DNA and examined with a fluorescent microscope.

Analysis for mutagenicity of the tarragon extracts was conducted using the method of Kobayashi et al. (1995), Lankinen and Vilpo (1997), and Tice and Vasquez (1999). Electrophoresis-induced migration patterns of DNA within the agarose were graded according to length of the comet "tail" (the trail of DNA fragments originating at the position of each lysed cell, visible due to staining with EtBr dye). Graded values for comet tail length were established as follows: *Type 1*: no migration (NM); *Type 2*: short migration (SM); *Type 3*: medium migration (MM); and *Type 4:* long migration (LM). The mutagen indices (MI) of tarragon (test) and control solutions were determined as the relative representation of each of the four grades of comet migration pattern in a sample of cells exposed to a particular solution, according to the following relationship:

Eq. (1). Mutagenicity index (MI)

$$MI = \frac{[0 \times (NM) + 1 \times (SM) + 2 \times (MM) + 3 \times (LM)]}{N}, \quad \text{where } N = (NM + SM + MM + LM)$$

Numerical values for the variables NM, SM, MM and LM were obtained by counting the total number of comets in the coverslip-bounded areas on four slides containing electrophoresed and EtBr-stained agarose suspensions of cells exposed to a particular test solution or control. Here, *N* is the sum of comets counted on all four slides. Tarragon extract density was determined by pycnometer. Stain/lysing solution in neutral buffer was prepared according to the McKelvey-Martin procedure with negligible modification (McKelvey-Martin et al., 1993, 1998; Szeto et al., 2002). Reagents used in the present study included high purity-grade agarose (Fluka, Japan), dimethyl sulfoxide (DMSO), sodium dichromate, ethidium bromide, Triton X-100, disodium EDTA, N-laury/sarcosin sodium salt, and trizma (Sigma, St. Louis, USA). Major equipment included a Shandon electrophoresis apparatus (Vokan SAE, England), a TJ6 centrifuge (Beckman-Coulter, Brea CA USA), and a fluorescent microscope (Helmat Hund, Germany).

2.3. Evaluation of tarragon liver toxicity in mice

2.3.1. Animals

Mice used in the present study experiments were Swiss albino males with an average weight of approximately 25 ± 5 g. The animals were fed regular rodent chow *ad libitum* with free access to water. The animals were allowed 1 week to acclimatize before the experiments and were kept at 25 ± 2 °C, with a relative humidity of 55 ± 5% and a 12-h light–dark cycle. All animals included in the present study were handled and received humane care in compliance with the principles of laboratory animal care formulated by the National Society for Medical Research and Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences, and published by the National Institutes of Health (Publication No. NIH 85-23, revised in 1996). All protocols for their use in this investigation were approved by the institutional review board (IRB) of Ahvaz Jundishapur University of Medical Sciences.

2.3.2. Treatment group design

Mice were randomly assigned to five test groups of eight animals each, based on dosage of tarragon extract. To determine the existence of a dose-responsive relationship between tarragon and hepatotoxicity, members of each group were administered treatments for 7 days in dosages ranging from 0–752 mg/kg, defined as follows: *Group A:* drug-free group (normal saline only); *Group B: 94 mg/kg* tarragon extract; *Group C: 188 mg/kg* tarragon extract; *Group D:* 376 mg/kg tarragon extract; and *Group E:* 752 mg/kg tarragon extract. These dose ranges were selected based on previous evaluation of the capacity of the extract to parameters related to glucose metabolism, obesity, and diabetes in mouse models (Ribnicky et al., 2009; Watcho et al., 2010)

2.3.3. Toxicity assessments

On the 8th day after initiation of treatment with extracts or control (saline), blood was collected from jugular veins of animals and utilized for serum preparation. The blood samples were left to stand at room temperature for 1 h, and then centrifuged at 300*g* for 10 min to obtain the serum used for evaluating liver enzyme activities. The mice were anesthetized with sodium pentobarbital (80 mg/kg, i.p.) and sacrificed under anesthesia; next, their liver and kidneys were removed and fixated in an ice-cold, PBS/10% formalin solution. Tissue specimens were hematoxylin-eosin (H & E)-stained and observed under a light microscope. The following three post-mortem evaluations were then conducted:

(i) *Macroscopic examination:* All mice were given a complete postmortem examination (autopsy). The abdominal, thoracic, and cranial cavities were examined for abnormalities, and the livers were removed, examined, and fixed in 10% formalin.

(ii) *Serum enzyme activities:* Test kits purchased from Asan Pharmaceutical (Seoul, Korea) were used to determine the occurrence toxic effects on liver function by assessment of tarragon-mediated alteration of ALT and AST activities. Changes in the activity of these enzymes are considered valid indicators of the toxicity of a particular agent, and for the purpose of the present study, were assayed by the method of Reitman and Frankel (1957).

(iii) *Histopathologic/microscopic examination:* 6 µm-thick histological sections were prepared from formalin-fixed livers, hematoxylin-eosin (H&E)-stained, and observed under a light microscope. To prepare harvested tissue for examination by light microscopy, livers were removed, preserved in 10% neutral phosphate-buffered formalin, and processed by routine paraffin sectioning and staining with hematoxylin and eosin (H&E). Staining was performed according to the manufacturers' instructions.

Please cite this article in press as: Kalantari, H., et al. Toxicological and mutagenic analysis of *Artemisia dracunculus* (tarragon) extract. Food Chem. Toxicol. (2012), http://dx.doi.org/10.1016/j.fct.2012.07.052

2.4. Statistical methods

The data were expressed as the mean ± SEM. One-way analysis of variance test was first carried out to test for any differences between the mean values of all groups. If differences were established, the values of the treated groups were compared with those of the negative and positive control groups, followed by Tukey's post-testing using GraphPad Prism 5.01. A change of $p < 0.001$ between the negative and positive controls and treated groups was considered to be significant.

3. Results

3.1. Comet assay

Examination of electrophoresed, EtBr-stained, agarose slides using fluorescent microscopy at 400× magnification showed apparently strong correlation between occurrence of characteristic DNA migration (comet) patterns and the contents of the solutions in which cells on a particular slide had been incubated (Fig. 1).

Type 1 comets (no migration – NM) constituted the predominant form on slides containing blood incubated in a saline buffer (Fig. 1a, Table 1) with 94 and 188 mg tarragon extract (Fig. 1c and d, Table 1). *Type 2* comets (short migration – SM) were seen increasingly on slides containing cells incubated with 376 mg of the extract (Fig. 1e). The ratio of the patterns shifts with *Type 3* (medium migration – MM) on slides containing cells treated with 752 mg extract (Fig. 1f); and *Type 4* comet pattern (long migration – LM) was seen in the greatest number on slides with cells incubated in the positive control: 262 mg sodium dichromate (Fig. 1b).

The representation of each grade of DNA migration is shown in Table 1. Distribution of comet pattern forms was, as expected, predominantly skewed in favor of *Type 1*, no migration – NM in slides containing cells incubated with a saline buffer (negative control); and with *Type 4*, long migration – LM on slides with cells stimulated with 262 mg sodium dichromate (positive control). Mutagen indices (MI), calculated according to the relationship given in Section 2 and representing the number of each comet form in a particular set of four slides, relative to the total number of each comet type in slides containing cells exposed to a particular stimulant, exhibited a positive dose-responsive relationship to the dosage of tarragon extract, ranging from $0.40 \pm 0.02^{*\dagger}$ for 94 mg extract to $1.1 \pm 0.04^{*\dagger}$ for cells treated with 752 mg (Fig. 2). MI values for

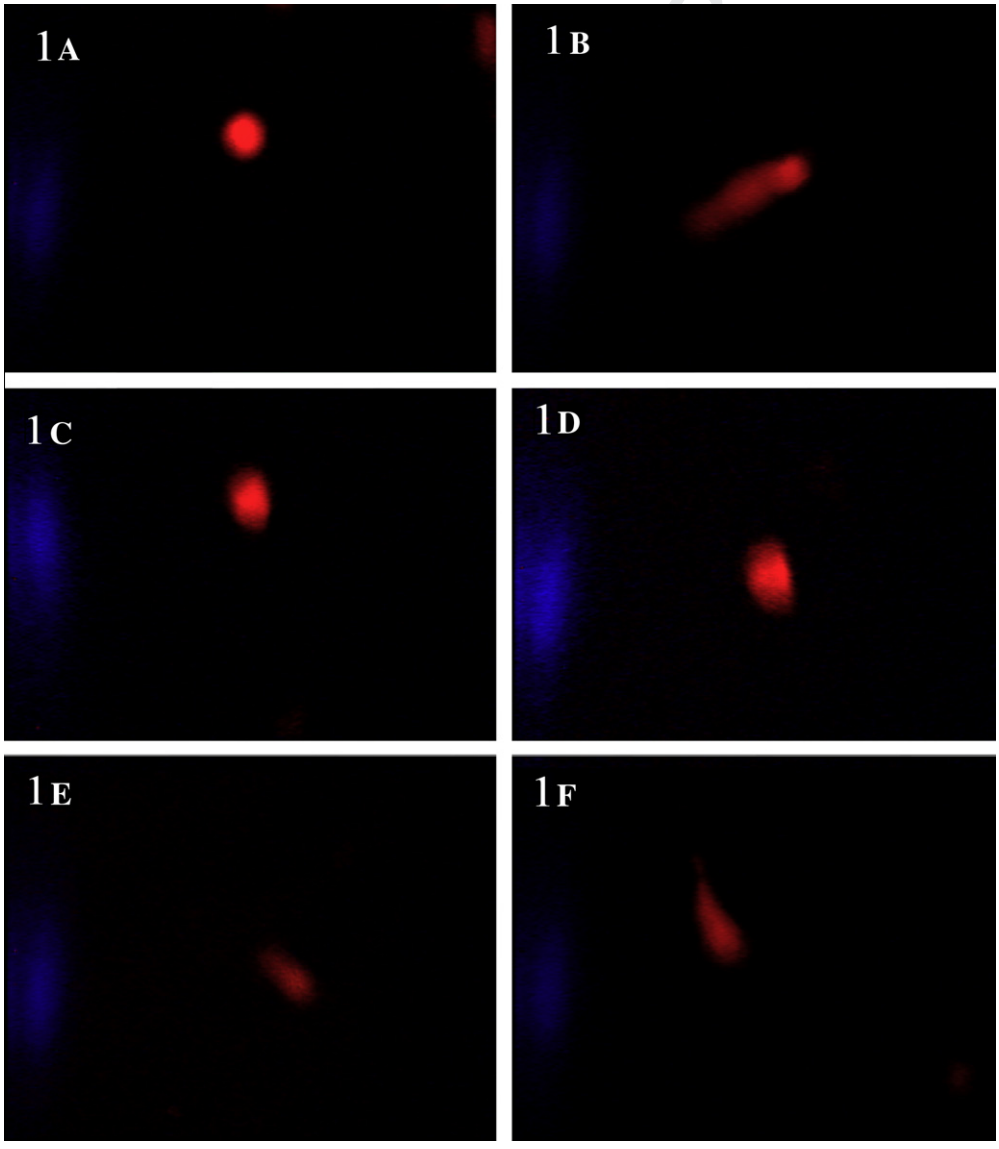

Fig. 1. DNA fragmentation (comet) patterns resulting from treatment of human blood with saline (1A), the mutagen sodium dichromate (1B), or selected doses of Tarragon extract (1C-1F). Examination of electrophoresed, EtBr-stained, agarose slides using fluorescent microscopy at 400X magnification showed apparently strong correlation between occurrence of characteristic DNA migration (comet) patterns and bioactivity of reagents in which cells on a particular slide had been incubated.

Please cite this article in press as: Kalantari, H., et al. Toxicological and mutagenic analysis of *Artemisia dracunculus* (tarragon) extract. Food Chem. Toxicol. (2012), http://dx.doi.org/10.1016/j.fct.2012.07.052

Table 1
Comet assay outcomes. Total number of comets defined by each of the four migration patterns observed by fluorescent microscopy (Fig. 1) in four slides made from blood exposed to extracts or to control reagents. Here, mutagen index (MI), calculated according to Eq. (1) in Section 2, is a measure of the relative representation of each comet pattern all cells exposed to a particular test condition. MI values are elevated in direct proportion to the DNA fragmenting capacity of a particular reagent.

Comet pattern categories	Saline buffer (negative control)	*Artemisia dracunculus* extract				Sodium dichromate (positive control)
		94 mg	188 mg	376 mg	752 mg	
Type 1 (no migration)	181	119	115	113	51	0
Type 2 (short migration)	27	56	68	71	86	0
Type 3 (medium migration)	8	9	18	46	33	175
Type 4 (long migration)	0	0	0	6	18	32
Mutagenicity index (MI)	0.20 ± 0.03†	0.40 ± 0.02*	0.52 ± 0.04*	0.77 ± 0.03*	1.1 ± 0.04*	2.16 ± 0.03*

* $p < 0.05$ compared to corresponding values of saline buffer as negative control.
† $p < 0.05$ compared to corresponding values of sodium dichromate as positive control.

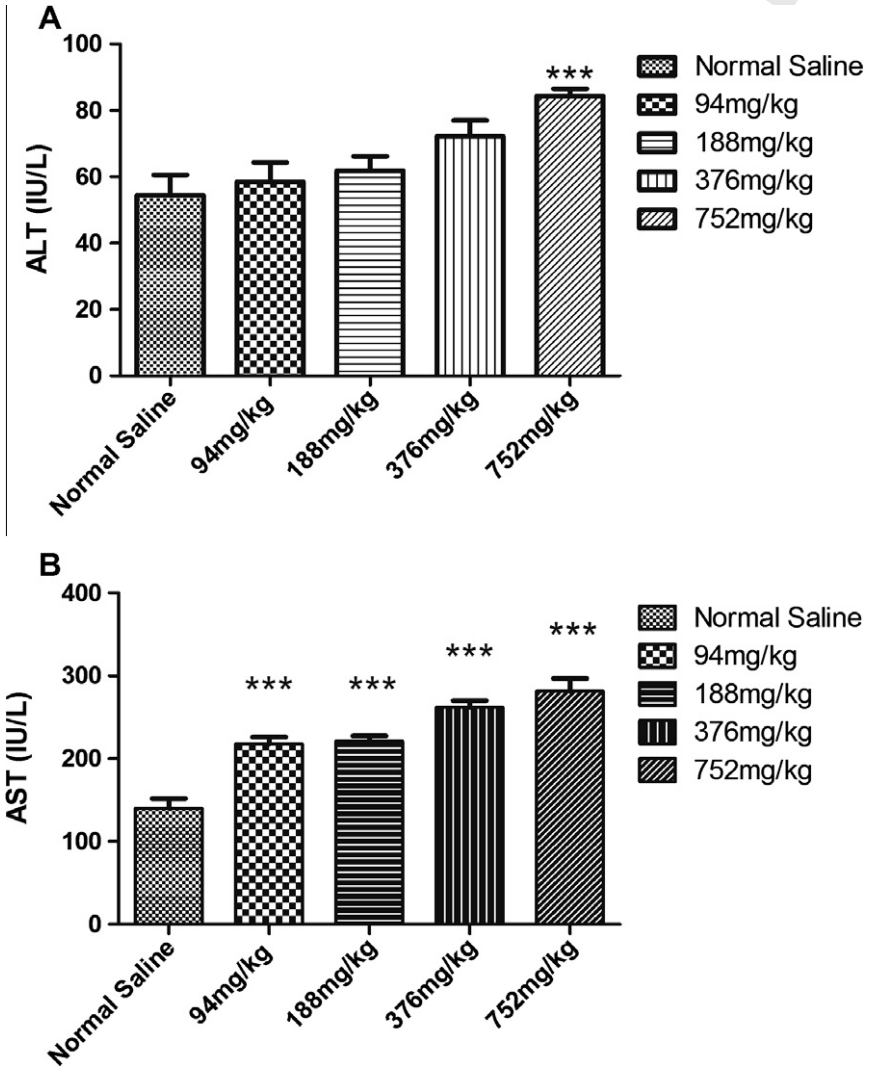

Fig. 2. Serum activity of liver function enzymes. Serum activities of alanine aminotransferase (ALT) (2A) and aspartate aminotransferase (AST) (2B) in mice treated for 7 days with normal saline or Tarragon extract at the indicated combinations. Data shown here reflect the average enzyme activities expressed in International Units (IU) per liter of serum ± SEM in test groups of 8 mice each. ***p < 0.001: Significance of comparison between serum enzyme activity in test groups versus animals treated with normal saline only.

the negative and positive control were 0.20 ± 0.03 and 2.16 ± 0.03, respectively, with a range of intermediate values for slides corresponding to each of the tarragon extracts.

3.2. Liver enzyme activity

Treatment of mice with tarragon resulted in dose-dependent increases in serum ALT (Fig. 2A), and AST (Fig. 2B). As shown in Fig. 2A, ALT levels were increased from 54.44 ± 6.1 IU/l in a serum from negative control animals treated with normal saline, to 58.56 ± 6 IU/l with a tarragon extract dosage of 94 mg/kg; 61.90 ± 4 IU/l at 188 mg/kg; 72.20 ± 5 IU/l at 376 mg/kg, and 84.33 ± 2 IU/l at 752 mg/kg, respectively. Serum AST activities shown in Fig. 2B averaged 140.3 ± 11.35 IU/l in mice treated with normal saline, to 217.8 ± 8.2 IU/l in animals receiving 94 mg/kg tarragon, 221.3 ± 6.3 IU/l in mice receiving188 mg/kg; 262 ± 8.1 IU/l at 376 mg/kg;

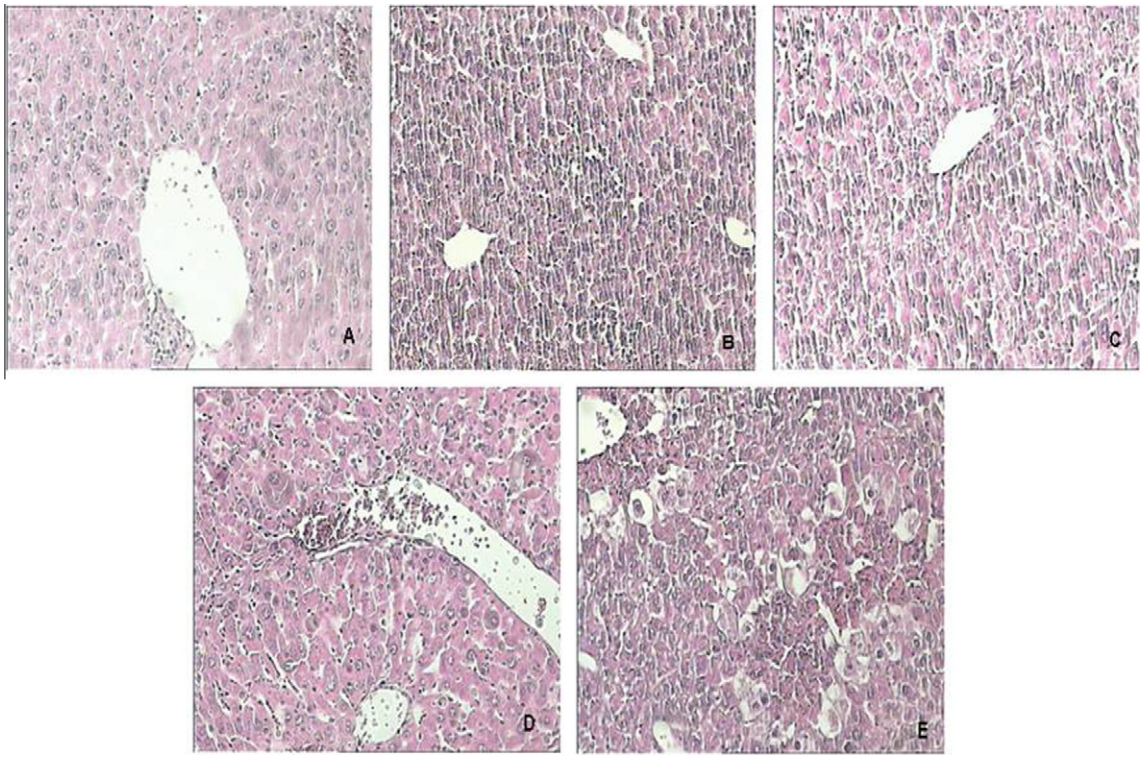

Fig. 3. Histopathological evaluation of liver tissue. 6-micron liver tissue sections harvested from Swiss Albino mice, formalin-fixed and HE-stained, are shown at 400X magnification. Results shown correspond to tissue sections taken from animals treated for 7 days with normal saline (3A), or Tarragon extract at dosages of 94 mg/kg (3B); 188 mg/kg (3C); 376 mg/kg (3D); or 752 mg/kg (3E). Pathological changes in tissue architecture, including inflammation and congestion, centrilobular necrosis, steatosis and swelling of hepatic cytoplasm are observed to occur dose-responsively to Tarragon extract.

and 281.4 ± 15.6 for animals treated with 752 mg/kg of tarragon extract. It is interesting to note that the ALT value was significantly increased only in the case of the highest dose of tarragon extract (752 mg/kg).

3.3. Histological profiles

Histological profiles of liver samples were consistent with the changes of AST and of ALT. The histopathological examination of the liver sections of the control group (normal saline) showed normal cellular architecture with distinct hepatic cells and sinusoidal spaces (Fig. 3A). H&E-stained slides from mice treated with tarragon extract exhibited dose-responsive disruption of tissue microstructure, including increases in inflammatory changes, congestion, centrilobular necrosis, steatosis, and swelling of hepatic cytoplasm (3B–3E).

4. Discussion

As expected, DNA migration (comet) patterns observed in slides containing cells incubated with negative control solution (saline) were mainly *Type 1* (no migration – NM); whereas cells incubated with 262 mg of sodium dichromate (positive control) exhibited predominantly *Type 4* (long migration – LM) (Fig. 1 and Table 1). These outcomes reflected the known behavior of undamaged DNA consisting largely of long strands that mostly remain trapped in cavities within the agarose matrix formed by lysis of cellular material, thereby creating *Type 1* patterns, versus fragmented DNA. That may move out of the cavities in the direction of the electrophoretic electric field gradient, revealed as *Type 4* comets. The dose-responsive increase in mutagen index shown in Table 1 and Fig. 2 indicates that tarragon extract has DNA damaging properties.

Although the data presented here does not allow for the development of a mechanistic explanation of this phenomenon, it is likely that one or more compounds found in the plant is reactive with DNA in ways that cause strand breaks. The plant contains compounds known to affect a diverse range of homeostatic functions, most notably neurologic and gastrointestinal activity. Its essential oil is a complex blend of bioactive phytochemicals that include aromatics, fatty acids, derivatives of isocoumarin (3-(1Z-butenyl)isocoumarin 3-(1E-butenyl)isocoumarin), and polyacetylenes (capillene, 1-and phenyl-2,4-hexadiene) (Aglarova et al., 2008). Compounds present at high percentages in the oil include estragol (methylchavicol), sabinene, and methyleugenol. Tarragon also contains significant quantities of flavonoids such as isorhamnetin, camphorol, quercetin, luteolin, and glycosides of these compounds (Arykova, 1996).

There is wide variability in the content of these and other components, depending on growth conditions for the plant at its place or origin, such as soil composition, salinity, and other environmental factors (Aglarova et al., 2008). Some tarragon components, such as polycyclic aromatic compounds, have potential for mutagenicity based on their structural similarity to DNA bases. Moreover, estragol (methylchavicol), a major component of the plant's essential oil, has known genotoxic properties (Nesslany et al., 2010), a phenomenon that may at least partly account for the results of the present study. Interestingly, another compound in tarragon, capillarisin, has been shown to protect against oxidative damage of the kind that causes DNA fragmentation (Chu et al., 1999).

It is important to emphasize that the data shown here does not permit for the establishment of mechanistic relationships between any particular compound previously demonstrated to be a component of tarragon and the mutagenic capacity of the herb. Nevertheless, future use of tarragon in both diet and healthcare should be undertaken with an awareness of the major components of the

Please cite this article in press as: Kalantari, H., et al. Toxicological and mutagenic analysis of *Artemisia dracunculus* (tarragon) extract. Food Chem. Toxicol. (2012), http://dx.doi.org/10.1016/j.fct.2012.07.052

plant, along with an understanding of its bioactivity and potential toxicity.

Changes in liver enzyme activity and histopathology, that were observed to occur dose-responsively in mice administered tarragon extract (Figs. 2 and 3), demonstrated hepatotoxic properties of the herb. Since the liver acts as a primary line of defense against toxic insult, most toxicological analysis evaluate the capacity of a particular agent to adversely affect liver function and tissue integrity. Serum ALT and AST activities are observed to increase significantly in response to a wide range of toxic agents, and are thus considered to be reliable indicators of the toxic risk posed by a particular substance (Taye and Abdel-Raheem, 2012). It is nevertheless important to caveat this observation by emphasizing that the present study was not conducted to determine specific dose ranges of tarragon expected to mediate toxicity in humans or other vertebrates. The data presented here demonstrates only the capacity for mutagenicity and hepatotoxicity of the herb in two well established models. Quantifying the health risk of the herb remains for future investigations.

5. Conclusions

The results of the present study show that tarragon extract exhibits mutagenic properties. However, the data presented here is not comprehensive enough to draw definitive conclusions regarding its potential risk to human health with sustained use, particularly as a therapeutic agent. Validation of its safety as a component of diet and possibly in healthcare is provided by a risk assessment study conducted in 2010 that confirms the mutagenicity of tarragon, but concludes that consumption of the herb poses a minimal risk to human health (Nesslany et al., 2010).

Tarragon has been an element of the human diet for centuries, perhaps millennia; it has also been used extensively in traditional medicine. Hence, occasional consumption is probably not dangerous. Nevertheless, high-dose application of the extract in the treatment of serious disease is not recommended. This was also confirmed by the enzymological assay, and through immunohistochemical examinations. Any agent capable of damaging DNA is potentially carcinogenic. Hence, high-doses of tarragon may increase cancer risk, particularly in persons susceptible to cancer through genetic disposition, disease, or chemical exposure. This report describes a potential adverse effect of tarragon using a Comet mutagen assay, liver function, and liver morphology assay. The main conclusion of the present study is that high-dose usage of tarragon may pose health risks based on mutagenic and hepatotoxic properties observed as a result of the present study.

Conflict of Interest

The authors declare that there are no conflicts of interest.

Acknowledgements

The authors acknowledge the efforts of Stephanie C. Fox, President of *QueenBeeEdit*, Bloomfield, Connecticut, USA, in formatting proofreading and editing this manuscript in preparation for publication. This study was supported by Grants from OTKA 104017, OTKA 78223, and TAMOP-4.2.2-08/1-2008-0007, TAMOP-4.2.1/B-09/1/KONV-2010-0007, TAMOP-4.2.2/B-10/1/-2010-0024, TAMOP-4.2.2.A-111/KONV- and by the cooperation grant of the European Union and Hungarian Government. The work was also supported by the Deputy of Research of Ahwaz Jundishapur University of Medical Sciences, Iran.

References

Aglarova, A.M., Zilfikarov, I.N., Severtseva, O.V., 2008. Biological characteristics and useful properties of tarragon (*Artemisia dracunculus* L.). Pharm. Chem. J. 42, 81–86.

Arykova, T.M., 1996. Proceedings of the Conference. "Theoretical and Applied Aspects of the Study of Medicinal Plants". Theoretical and Applied Aspects of the Study of Medicinal Plants, Tomsk, pp. 19–21.

Benli, M., Kaya, I., Yigit, N., 2007. Screening antimicrobial activity of various extracts of *Artemisia dracunculus* L. Cell Biochem. Funct. 25, 681–686.

Bucher, J., 2007. NTP Toxicity Studies of Sodium Dichromate Dihydrate (CAS No. 7789-12-0) Administered in Drinking Water to Male and Female F344/N Rats and B6C3F1 Mice and Male BALB/c and am3-C57BL/6 Mice. Toxic Rep Ser, 1-G4.

Buehrlen, M., Harreus, U.A., Gamarra, F., Hagen, R., Kleinsasser, N.H., 2007. Cumulative genotoxic and apoptotic effects of xenobiotics in a mini organ culture model of human nasal mucosa as detected by the alkaline single cell microgel electrophoresis assay and the annexin V-affinity assay. Toxicol. Lett. 169, 152–161.

Cefalu, W.T., Ye, J., Zuberi, A., Ribnicky, D.M., Raskin, I., Liu, Z., Wang, Z.Q., Brantley, P.J., Howard, L., Lefevre, M., 2008. Botanicals and the metabolic syndrome. Am. J. Clin. Nutr. 87, 481S–487S.

Chan, K., 2003. Some aspects of toxic contaminants in herbal medicines. Chemosphere 52, 1361–1371.

Chu, C.Y., Tseng, T.H., Hwang, J.M., Chou, F.P., Wang, C.J., 1999. Protective effects of capillarisin on tert-butylhydroperoxide-induced oxidative damage in rat primary hepatocytes. Arch. Toxicol. 73, 263–268.

Collins, A., Dusinska, M., Franklin, M., Somorovska, M., Petrovska, H., Duthie, S., Fillion, L., Panayiotidis, M., Raslova, K., Vaughan, N., 1997. Comet assay in human biomonitoring studies: reliability, validation, and applications. Environ. Mol. Mutagen. 30, 139–146.

Collins, A.R., 2002. The comet assay. Principles, applications, and limitations. Methods Mol. Biol. 203, 163–177.

Cook, P.R., Brazell, I.A., 1977. The superhelical density of nuclear DNA from human cells. Eur. J. Biochem. 74, 527–531.

Cook, P.R., Brazell, I.A., Jost, E., 1976. Characterization of nuclear structures containing superhelical DNA. J. Cell Sci. 22, 303–324.

Kobayashi, H., Sugiyama, C., Morikawa, Y., Hayashi, M., Sofuni, T., 1995. A comparison between manual microscopic analysis and computerized image analysis in the single cell gel electrophoresis assay. MMS Commun. 3, 103–115.

Lankinen, M.H., Vilpo, J.A., 1997. Repair of gamma-irradiation-induced DNA single-strand breaks in human bone marrow cells: analysis of unfractionated and $CD34^+$ cells using single-cell gel electrophoresis. Mutat. Res. 377, 177–185.

Lopes-Lutz, D., Alviano, D.S., Alviano, C.S., Kolodziejczyk, P.P., 2008. Screening of chemical composition, antimicrobial and antioxidant activities of Artemisia essential oils. Phytochemistry 69, 1732–1738.

McGuffin, M., Hobbs, C., Upton, R., Goldberg, A., 1997. American Herbal Products Association's Botanical Safety Handbook. CRC Press, 231p.

McKelvey-Martin, V.J., Green, M.H., Schmezer, P., Pool-Zobel, B.L., De Meo, M.P., Collins, A., 1993. The single cell gel electrophoresis assay (comet assay): a European review. Mutat. Res. 288, 47–63.

McKelvey-Martin, V.J., Ho, E.T., McKeown, S.R., Johnston, S.R., McCarthy, P.J., Rajab, N.F., Downes, C.S., 1998. Emerging applications of the single cell gel electrophoresis (Comet) assay. I. Management of invasive transitional cell human bladder carcinoma. II. Fluorescent in situ hybridization Comets for the identification of damaged and repaired DNA sequences in individual cells. Mutagenesis 13, 1–8.

Nesslany, F., Parent-Massin, D., Marzin, D., 2010. Risk assessment of consumption of methylchavicol and tarragon: the genotoxic potential in vivo and in vitro. Mutat. Res. 696, 1–9.

Nestler, G., 2002. Traditional Chinese medicine. Med. Clin. North Am. 86, 63–73.

Ostling, O., Johanson, K.J., 1984. Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells. Biochem. Biophys. Res. Commun. 123, 291–298.

Ostling, O., Johanson, K.J., Blomquist, E., Hagelqvist, E., 1987. DNA damage in clinical radiation therapy studied by microelectrophoresis in single tumour cells A preliminary report. Acta Oncol. 26, 45–48.

Reitman, S., Frankel, S., 1957. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol. 28, 56–63.

Ribnicky, D.M., Kuhn, P., Poulev, A., Logendra, S., Zuberi, A., Cefalu, W.T., Raskin, I., 2009. Improved absorption and bioactivity of active compounds from an anti-diabetic extract of *Artemisia dracunculus* L. Int. J. Pharm. 370, 87–92.

Ribnicky, D.M., Poulev, A., Watford, M., Cefalu, W.T., Raskin, I., 2006. Antihyperglycemic activity of Tarralin, an ethanolic extract of *Artemisia dracunculus* L. Phytomedicine 13, 550–557.

Shahriyary, L., Yazdanparast, R., 2007. Inhibition of blood platelet adhesion, aggregation and secretion by *Artemisia dracunculus* leaves extracts. J. Ethnopharmacol. 114, 194–198.

Singh, N.P., McCoy, M.T., Tice, R.R., Schneider, E.L., 1988. A simple technique for quantitation of low levels of DNA damage in individual cells. Exp. Cell Res. 175, 184–191.

Stout, M.D., Herbert, R.A., Kissling, G.E., Collins, B.J., Travlos, G.S., Witt, K.L., Melnick, R.L., Abdo, K.M., Malarkey, D.E., Hooth, M.J., 2009. Hexavalent chromium is carcinogenic to F344/N rats and B6C3F1 mice after chronic oral exposure. Environ. Health Perspect. 117, 716–722.

Szeto, Y.T., Collins, A.R., Benzie, I.F., 2002. Effects of dietary antioxidants on DNA damage in lysed cells using a modified comet assay procedure. Mutat. Res. 500, 31–38.

Taye, A., Abdel-Raheem, I.T., 2012. Hepatoprotective effect of the selective mineralocorticoid receptor antagonist, eplerenone against carbon tetrachloride-induced liver injury in rats. Ann. Hepatol. 11, 384–391.

Tice, R., Vasquez, M., 1999. Protocol for the application of the Ph > 13 alkaline single cell gel (SCG) assay to the detection of DNA damage in Mammalian cells. Integrated Laboratory Systems.

Tice, R.R., Andrews, P.W., Hirai, O., Singh, N.P., 1991. The single cell gel (SCG) assay: an electrophoretic technique for the detection of DNA damage in individual cells. Adv. Exp. Med. Biol. 283, 157–164.

Tice, R.R., Andrews, P.W., Singh, N.P., 1990. The single cell gel assay: a sensitive technique for evaluating intercellular differences in DNA damage and repair. Basic Life Sci. 53, 291–301.

Wang, Z.Q., Ribnicky, D., Zhang, X.H., Raskin, I., Yu, Y., Cefalu, W.T., 2008. Bioactives of *Artemisia dracunculus* L. enhance cellular insulin signaling in primary human skeletal muscle culture. Metabolism 57, S58–S64.

Watcho, P., Stavniichuk, R., Ribnicky, D.M., Raskin, I., Obrosova, I.G., 2010. High-fat diet-induced neuropathy of prediabetes and obesity: effect of PMI-5011, an ethanolic extract of *Artemisia dracunculus* L. Mediators Inflamm. 2010, 268547.

Please cite this article in press as: Kalantari, H., et al. Toxicological and mutagenic analysis of *Artemisia dracunculus* (tarragon) extract. Food Chem. Toxicol. (2012), http://dx.doi.org/10.1016/j.fct.2012.07.052