Research Article

Journal of Applied Toxicology

Received: 24 November 2011, Revised: 3 February 2012, Accepted: 5 February 2012 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jat.2746

In vivo antigenotoxic activity of watercress juice *(Nasturtium officinale)* against induced DNA damage

Natalia A. Casanova,[a] Julia I. Ariagno,[b] Marcela M. López Nigro,[a] Gabriela R. Mendeluk,[b] María de los A. Gette,[c] Elisa Petenatti,[c] Luis A. Palaoro[b] and Marta A. Carballo[a]*

ABSTRACT: The present study was carried out to investigate the genotoxicity as well as possible protective activity against damage induced by cyclophosphamide (CP) of the aqueous juice of watercress (*Nasturtium officinale*, W.T. Aiton) *in vivo*. Male and female Swiss mice 7–8 weeks old ($N = 48$) were treated by gavage with 1 g kg^{-1} body weight and 0.5 g kg^{-1} body weight of watercress juice during 15 consecutive days. Genotoxicity and its possible protective effect were tested by the comet assay in peripheral blood cells and the micronucleus test in bone marrow. In addition, biopsies of the bladder, epididymis and testicles of mice were performed to extend the experimental design. Watercress juice *per se* did not induce genetic damage according to the comet assay and micronucleus study, exhibiting a protective activity against CP ($P < 0.05$ and $P < 0.001$, respectively). The comparative analysis of bladder histological changes obtained in the watercress plus CP group against those treated with CP alone suggests a probable protective effect. Further studies are needed in order to establish the protective role of watercress juice against DNA damage. Copyright © 2012 John Wiley & Sons, Ltd.

Keywords: watercress; antigenotoxicity; cyclophosphamide; bladder; biomarkers

INTRODUCTION

Watercress (*Nasturtium officinale*, W.T. Aiton; Retrieved [January, 29, 2012], from the Integrated Taxonomic Information System on-line database, http://www.itis.gov.), a member of Brassicaceae family, is a nutritive vegetable rich in vitamin C (43 mg per 100 g), vitamin A (4700 IU per 100 g) and α-tocopherol (34 mg per 100 g; Hadas *et al*., 1994). It contains a high concentration of glucosinolates per gram weight as well as carotenoids, polyphenols and chlorophyll (Gill *et al*., 2007). These compounds might reduce human cancer risk owing to their relationship with enzymes of phase I and II and antioxidant properties (Higdon *et al*., 2007; Steinkeller *et al*., 2001). Previous studies of our laboratory showed that watercress juice does not act as a cytostatic, cytotoxic, aneunogenic or clastogenic agent *in vitro* (Casanova *et al*., 2010). In addition, it seems to have a protective effect in peripheral blood lymphocytes against hydrogen peroxide-induced DNA damage *in vitro* (Casanova and Carballo, 2011).

Taking into account that *Nasturtium officinale* (W.T. Aiton) is a good source of key nutrients associated with health benefits and considering that our previous results *in vitro* do not imply that in an *in vivo* system the juice behaves in the same way, the present study was carried out to evaluate the lack of genotoxicity as well as the possible protective activity of an aqueous juice of this cruciferous vegetable *in vivo*. The antigenotoxic effect of watercress was tested on the genotoxicity induced by cyclophosphamide (CP) in mice using the micronucleus test and the comet assay, in bone marrow and peripheral blood cells, respectively. In addition, its possible protective effect was evaluated by the study of bladder, epididymis and testicles biopsies of mice for CP-induced cellular changes.

Cyclophosphamide, a nitrogen mustard compound, is a member of the group of cytostatic alkylating agents. Its actions lead to splitting of the DNA molecule as well as crossed linking of DNA's double helix, which interferes with DNA replication and transcription (Goodman and Gilman, 2003). Also, it is known that CP produces alterations in the morphology of epithelial cells of some organs. Thus, CP administration to rats induces modifications of the superficial cells of the urothelium; these changes are so marked that they can be confused with neoplastic cells (Romih *et al*., 2001). Incipient pathocytological changes in controlled experimental designs may be a marker of damage. Genotoxicity may also affect fertility, targeting the spermatogenesis process in males; epididymal sperm morphology and DNA packaging are biological probes of this disorder. The alterations

*Correspondence to: M. A. Carballo, Citogenética Humana y Genética Toxicológica, Instituto de Fisiopatología y Bioquímica Clínica, Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 CABA, Buenos Aires, Argentina.
E-mail: macarballo@ffyb.uba.ar

[a]Citogenética Humana y Genética Toxicológica, Instituto de Fisiopatología y Bioquímica Clínica, Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 CABA, Buenos Aires, Argentina

[b]Laboratorio de Fertilidad Masculina, Instituto de Fisiopatología y Bioquímica Clínica, Departamento de Bioquímica Clínica, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, 1113 CABA, Buenos Aires, Argentina

[c]Universidad Nacional de San Luis, Av. Ejercito de los Andes 950, D5700HHW, San Luis, Argentina

in the cellular apparatus necessary for chromosome segregation could produce aneuploidy or poliploidy cells. These cells, which show nuclear enlargement, hyperchromatism and increase in the number of nucleoli, can be observed in biopsies of different organs (Mohanty and Dey, 2003; Mosieniak and Sikora, 2010). These data could contribute to the knowledge of the chemoprevention effects of a diet supplemented with watercress.

MATERIALS AND METHODS

Chemical agents

Methanol (purity >99%), Giemsa solution, May–Grünwald's eosine-methylene blue solution modified, NaCl (purity >99%), DMSO (purity >99%), NaOH (purity >99%), Mc Ilvane buffer, pH 7.0, containing 10 mM $MgCl_2$, and hematoxylin and eosine solutions were from Merck (Argentina). Ethidium bromide (purity >95%), acridine orange (purity >95%), Na_2EDTA (purity >99%), trizma (purity >99%), triton X-100, cyclophosphamide, chromomicyn A3 and the standard of total intact glucosinolates were provided by Sigma-Aldrich, Argentina. RPMI 1640 medium, fetal bovine serum, phosphate buffered saline, low-melting-point agarose and normal melting point agarose were purchased from Gibco BRL (Argentina). Paraformaldehyde (purity>95%), was obtained from UBS Corporation. Heparin was from Abbot.

Watercress Juice

The cress plant was purchased from an organic market garden and then was chopped and put into a commercial juice processor on ice. The juice was centrifuged (14 000 rpm, 20 min, 4 °C), then the supernatants were sterilized (0.22 μm), aliquoted and frozen until use. All procedures were performed in darkness. The doses were chosen following the recommendations described by Hartmann *et al*. (2003) for the comet assay and by MacGregor *et al*. (1987) for micronucleus test.

Determination of Total Intact Glucosinolates

Total intact glucosinolates (TIG) was quantified by reverse-phase HPLC (Gilson 170 chromatograph equipped with a diode array detector, wavelength 254 nm). The analysis was carried out on a Gemini S4 C18 (250–4 mm) column, using as mobile phase a gradient of 30% sodium monobasic phosphate 0.01 M–70% methanol, at a flow rate of 1.5 ml min^{-1}.

Ten milligrams of freeze-dried aqueous juice were dissolved in methanol and an aliquot of 20 μl was injected into HPLC. The quantification of TIG was performed by comparison of the peak area of the sample with the addition of an internal standard of total glucosinolates from rapeseeds. It is certified by the Institute for Reference Materials and Measurements with a certified value of 99 mol kg^{-1} (European Reference Materials, ERM-BC367, Geel, Belgium).

EVALUATION OF PROTECTION CAPACITY

Animals and Treatments

Male and female Swiss mice 7–8 weeks old with body weight 25–30 g, were supplied by the Animal House of the School of Pharmacy and Biochemistry, University of Buenos Aires, Argentina. Animals were housed in plastic cages at 20–25 °C, 60 ± 10% humidity with a 12/12 h light–dark cycle and received food and water *ad libitum* through the entire experimental period. Animal use and care in all experiments were in accordance with ethical laws on animal use.

To investigate the protective effect of *Nasturtium officinale* (W. T. Aiton) against the clastogenicity induced by CP, the animals were divided in six groups with eight individuals per group. In group 1 mice received drinking water (120 μl per day by gavage) for 15 consecutive days and were treated intraperitoneally (i.p.) on day 15 with 0.9% NaCl. Group 2 received drinking water (120 μl per day by gavage) for 15 days, and mice were treated with CP (20 mg kg^{-1} body weight) on day 15. Groups 4 and 6 received solutions of watercress (group 4, 1 g kg^{-1} body weight; group 6, 0.5 g kg^{-1} body weight) by gavage for 15 days before treatment with CP on day 15. Groups 3 and 5 received only treatments with watercress solutions during 15 consecutive days and were i.p.-treated on day 15 with 0.9% NaCl, to investigate a possible effect on spontaneous genotoxic damage.

Three to six hours after the CP injection (24–30 h before sacrifice), blood samples were collected from the cheek to perform comet assay. The mice were killed by cervical dislocation on day 16 to evaluate micronucleus frequency in bone marrow and cytological analysis.

Comet Assay

The procedure described by Singh *et al*. (1988) was used with modifications. Briefly, 35 μl of peripheral blood was resuspended in 200 μl of 0.5% low melting point agarose at 37 °C and spread onto slides precoated with 1% normal melting agarose. The gels were stored at 4 °C until solidify and a protective layer of 0.5% low melting point agarose was added. The slides were submerged in cold, freshly prepared lysis solution (2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Trizma, 1%, Triton X-100 and DMSO 10%, pH 10) and left overnight at 4 °C. The slides were placed in cold electrophoresis alkaline buffer (10 M NaOH, 200 mM Na_2EDTA, pH >13) and the embedded cells were exposed for 20 min to allow DNA unwinding to occur, before electrophoresis at 25 V and 300 mA (0.75 V cm^{-1}) for 20 min. The slides were then washed with neutralization buffer (Tris 0.4 M, pH 7.5) and the DNA stained with ethidium bromide (0.02 mg ml^{-1}) and observed using a fluorescent microscope at 40×. All procedures were carried out in darkness to avoid additional DNA damage. Two hundred randomly selected cells (100 per slide) were analyzed visually on a scale of 1–4, depending on the grade of damage, to calculate the damage index $(DI) = (1 \times n_1) + (2 \times n_2) + (3 \times n_3) + (4 \times n_4)$, where n is the number of cells in each category evaluated. The correlation between visual scoring and computer-based image analysis has been established; there is a good correlation between visual score and image analysis parameters (Collins *et al*., 2008). All the slides were coded and scored by the same observer. The percentage of reduction of damage was calculated according Serpeloni *et al*. (2008) by the formula:

$$\text{Reduction } (\%): \text{ mean DI in A} - \text{mean DI in B} / \text{mean DI in A} - \text{mean DI in C}$$

where A is the group treated with CP (positive control), B is the group treated with juice plus CP and C is the negative group.

Cell viability was determined by means of the ethidium bromide/acridine orange assay described by McGahon *et al*. (1995).

Journal of AppliedToxicology

Micronucleus Test

The mouse bone marrow micronucleus test was carried out according to Schmid (1975). The bone marrow cells from both femurs of each animal were flushed in the form of fine suspension into a centrifuge tube containing fetal bovine serum (FBS). This cell suspension was centrifuged at 1000 rpm for 10 min and the supernatant was removed. The pellet was resuspended in FBS before being used for preparing slides. The air-dried slides were stained with May–Grünwald and Giemsa. Two thousand polychromatic erythrocytes (PCEs) were scored per animal to determine the frequency of micronucleated polychromatic erythrocytes (MnPCEs). All the slides were coded and scored by the same observer. The percentage reduction in the frequency of micronuclei (MN) was calculated using the formula mentioned above for the comet assay.

Histology of Bladder, Testicle and Epididymis

The bladders, testicles and epididymis obtained from mice, after being washed with buffer saline solution (PBS), were fixed in 5% formaldehyde, embedded in paraffin, sectioned with microtome in 4–5 μm cuts and stained with hematoxylin–eosin.

Epididymal Sperm Assays and Sperm Morphology

Both epididymis were extracted and reduced with scissors to fine fragments in Petri dishes containing 3 ml of 0.9% NaCl isotonic solution. The sample was homogenized with a Pasteur pipette. Four smears were done with each homogenate; two of them were stained with Papanicolaou method and two were employed for the Chromomicyn A3 test. Sperm were classified according to Wyrobek and Bruce (1975) and WHO as normal and abnormal heads (banana-like form, amorphous, without hook and with two tails).

Chromatin Packaging Quality

The chromatin packaging quality of sperm was assessed using CMA3 staining, which was performed essentially as described before (Bianchi *et al*., 1996). Approximately 15×10^5 of PBS-washed sperm cells were applied to slides and allowed to air dry at room temperature. The cells were then fixed in methanol at −20 °C for 20 min and allowed to air dry again. A 20 μl aliquot of CMA3 solution (0.25 mg ml^{-1} CMA3 in McIlvane buffer, pH 7.0 containing 10 mM $MgCl_2$) was applied and the slide was placed in a dark chamber for 20 min at room temperature. The slide was then air dried and observed with optical microscope with immersion oil (100×). The cells were examined using a fluorescent microscope with oil immersion. For each sample, at least 500 cells were counted: cells positive for CMA3 displayed bright yellow-green fluorescence (presumably defective chromatin packaging), while those negative for CMA3 showed dull yellow staining (normal chromatin packaging).

Statistical Analysis

The results presented are expressed as means ± standard deviation. Differences between controls and treatments means were analyzed by one-way analysis of variance (ANOVA), *post-hoc* Tukey test and Student's t-test. The significance of correlation between comet assay and micronucleus test was estimated by Spearman correlation coefficient. A value of $P < 0.05$ was considered as statistically significant for all the endpoints evaluated (Sigma Stat software).

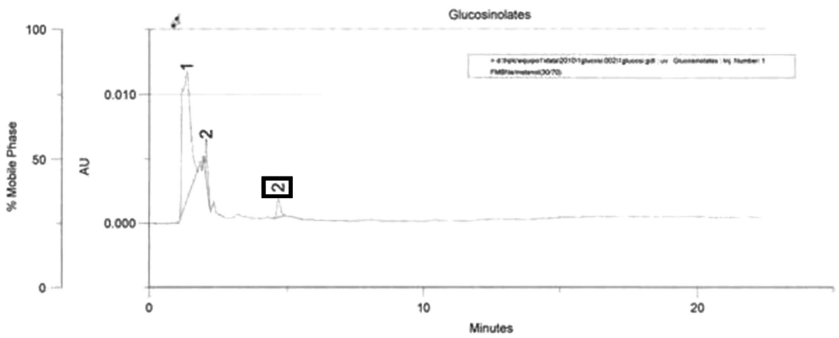
RESULTS

Total Intact Glucosinolates

It is thought that the potential protective effects of cruciferous vegetables against carcinogens could be due to the presence of glucosinolates and their breakdown products. In this work direct analysis of TIG in the juice of watercress was carried out. Figure 1 shows the results of HPLC analysis: peak 2 (retention time 4.5–4.7 min) corresponds to total intact glucosinolates, since the standard used defines them as such to a concentration of 99 mmol kg^{-1}. Its concentration was 295.60 mmol kg^{-1} lyophilizate.

Comet Assay

As no statistical differences were found between males and females, data was pooled. This suggests that the gender does not modify the activity of *Nasturtium officinale* aqueous juice in



	Inj. Number	Peak Name	R. Time	Area	Sample Descrip.	Purity
1	1.00	1	1.34	3501116.50	ilucosinolates	979.02
2	1.00	2	2.07	284575.28	ilucosinolates	982.95
3	1.00	2	4.69	225225.03	ilucosinolates	998.45

Figure 1. HPLC analysis of watercress juice.

these animals. Table 1 presents data from comet assay analysis, i.e. percentage of cells per category, damage index and percentage of reduction of damage in blood peripheral cells of mice (control groups and treated with aqueous juice of watercress or watercress plus Cyclophosphamide). The ANOVA test did not show significant differences between the exposed and control samples regardless of the watercress dose administered ($P > 0.05$). These findings demonstrate the lack of genotoxic effects of watercress juice. However the tail length differed significantly between positive (CP treated) and negative control ($P < 0.001$).

In addition, we observed that the tested doses presented protective effect against CP-induced DNA damage with a reduction in the scores of DI in relation to the positive control group of similar magnitude in analyzed doses ($P < 0.05$).

Micronucleus Test

The potential genotoxic effect of *Nasturtium officinale* (W.T. Aiton) juice *in vivo* and its ability to protect against CP-induced DNA damage are shown in Fig. 2. CP alone induced a clear increase in MN frequency ($P < 0.001$). The results also show that the frequencies of MN in PCE of animals treated only with the juice are not different from those of untreated controls. Since there was no significant difference ($P > 0.05$) between genders regarding the micronucleus frequency in the experimental or the control group, the data for the male and female mice were pooled (Table 2).

When we analyzed the potential protective effect of *Nasturtium officinale* juice we observed that pre-treatment with watercress juice led to a statistically significant reduction ($P < 0.001$) in the frequency of MN in PCEs. The reduction was between 50 and 65%, related to increasing watercress juice content in diets (0.5 and 1.0 g kg^{-1} body weight). These results suggest that *Nasturtium officinale* juice provides protection against the genotoxicity of CP.

Correlation Between MN Frequency and Damage Index

The correlation analysis has revealed a significant positive relationship ($r = 0.986$) between the MN frequency in bone marrow PCEs and damage index in peripheral blood lymphocytes.

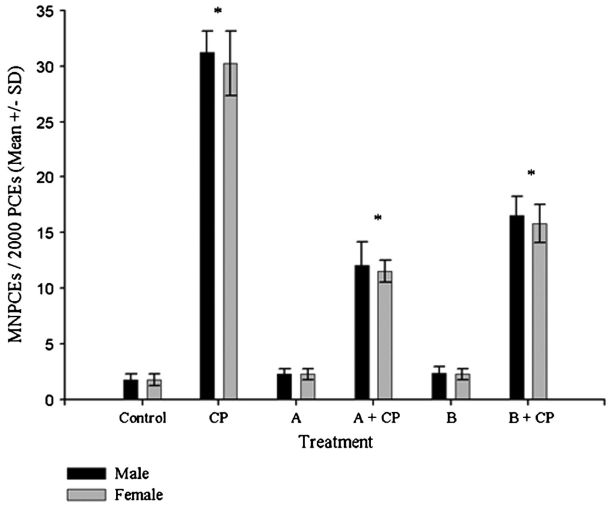


Figure 2. MN frequency (means ± SD) for bone marrow polychromatic erythrocytes (PCEs) from male and female mice treated with *Nasturtium officinale* and co-treated with watercress juice and CP. Control: 0.9% NaCl; CP, cyclophosphamide (20 mg kg^{-1} body weight); (A) 1 g kg^{-1} body weight watercress juice; (B) 0.5 g kg^{-1} body weight watercress juice. * $P < 0.001$, treatment vs control (Student's *t*-test).

Histology of Bladder

Group 2 (positive control) showed some changes, not too pronounced, in superficial cells: a little nuclear enlargement, hyperchromatism and an increase in the number of nucleolus (Figs 3 and 4). These changes resemble the effects that cyclophosphamide produces in the urothelium of patients with malignant lymphoma: hyperplasia with nuclear atypia, large nucleolus and coarse chromatin granules, giving the nucleus a 'salt and pepper' appearance (Forni *et al.*, 1964; Travis *et al.*, 1989). However, groups 3–6 showed no cytophatic effects.

Histology of Testicle, Epididymis and Epididymal Sperm Assays

The mice treated only with CP did not show differences in the histological sections compared with control. Likewise, histological

Table 1. Percentage of cells per category, damage index (DI) and percentage of reduction of damage in blood peripheral cells of mice

Treatment	Categories (%)				Peripheral blood	
	1	2	3	4	DI ± SD	Reduction (%)
0.9% NaCl	49.3	46.4	4.44	0.00	155.4 ± 13.1	—
CP 20 mg kg^{-1}	27.6	48.4	19.7	4.25	200.6 ± 11.1**	—
1.0 g kg^{-1} juice	46.5	48.9	4.53	0.07	158.1 ± 12.5	—
1.0 g kg^{-1} juice + CP 20 mg kg^{-1}	39.5	53.9	6.60	0.07	167.3 ± 5.71*	73.38
0.5 g kg^{-1} juice[a]	40.5	56.2	3.31	0.08	163.0 ± 11.8	—
0.5 g kg^{-1} juice + CP 20 mg kg^{-1}	41.7	49.5	8.69	0.13	167.3 ± 8.02*	73.42

Control: 0.9% NaCl; CP, cyclophosphamide (20 mg kg^{-1} body weight); 1 g kg^{-1} body weight watercress juice; 0.5 g kg^{-1} body weight watercress juice.
Data of each group was pooled ($n = 8$).
Categories: 1 represents no damage or low level of damage; 2–4 represent increasing damage intensities.
[a]One animal died.
* $P < 0.05$, treatment vs positive control;
** $P < 0.001$, positive control vs other groups (Student's *t*-test).

Journal of Applied Toxicology

Table 2. Number of cells analyzed, percentage of reduction and micronucleated polychromatic erythrocytes (MN PCEs) bone marrow from mice treated with N*asturtium officinale* and co-treatment with watercress juice and cyclophosphamide

Treatment	Number of cells analyzed	Number of MN PCEs	Reduction (%)
Control 0.9% NaCl	16 000 ($n = 8$)	14	—
CP 20 mg kg^{-1}	16 000 ($n = 8$)	246*	—
1.0 g kg^{-1} juice	16 000 ($n = 8$)	18	—
1.0 g kg^{-1} juice + CP 20 mg kg^{-1}	16 000 ($n = 8$)	94	65.52
0.5 g kg^{-1} juice	14 000 ($n = 7$)[a]	16	—
0.5 g kg^{-1} juice + CP 20 mg kg^{-1}	16 000 ($n = 8$)	129	50.43

Data of each group was pooled ($n = 8$).
[a]One animal died.
* $P < 0.001$, positive control vs other groups (Student's *t*-test).

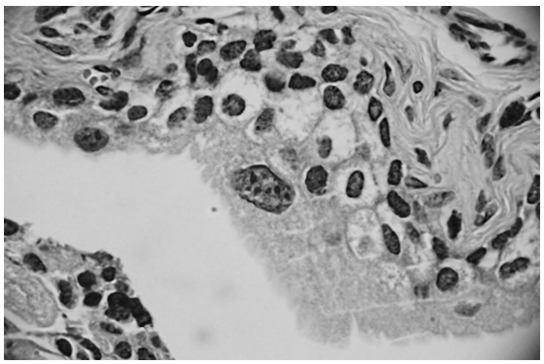


Figure 3. Transitional epithelium of a treated mouse. Umbrella cell showing a big and hyperchromatic nucleus with some nucleolis (hematoxylin–eosin 40×).

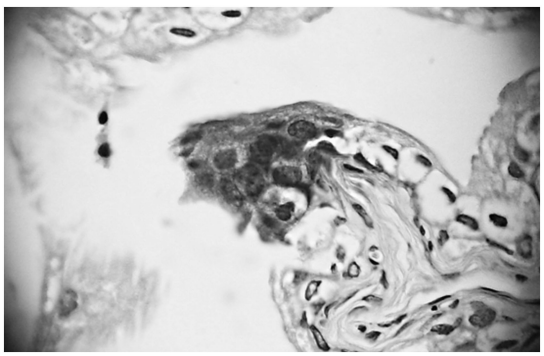


Figure 4. 'Crowded' hyperchromatic nuclei in umbrella cells from urothelium of a treated mouse (hematoxylin–eosin 40×).

changes were not observed in the mice treated with watercress and CP, as well as in those that received only watercress. With respect to sperm morphology and CMA3 data, no differences were observed in any of the treated mice as described above.

DISCUSSION

The effect of natural products on health and development of cancer has been widely studied. To our knowledge, this is the first work that has analyzed the impact of watercress in an experimental design using the micronucleus test and the comet assay in mice.

Since oxidative DNA damage can play a significant role in mutagenesis, cancer, aging and other human pathologies, decreasing oxidative stress seems to be the best strategy possible, achieved by eating food rich in antioxidants and/or by taking supplements containing polyphenols, for example, plant extracts (Kapiszewska *et al*., 2005). The population of the Mediterranean region, according to epidemiological studies, has the lowest prevalence of many degenerative diseases, including cancers, which have been ascribed to reactive oxygen species damage. This phenomenon seems to be associated with the healthy plant-based diet comprising complex polyphenols as well as individual flavonoids.

Biomarkers have considerable potential in aiding the understanding of the relationship between diet and disease or health (Branca *et al*., 2001). Micronuclei and comet assay are widely used methods for the detection of the genetic damage. Micronuclei arise from DNA breaks that lead to acentric chromosome fragments or lagging chromosomes at the interphase. MN assay determines unrepaired DNA strand breaks while the comet assay determines strand breaks (single and/or double) and labile sites (Mughal *et al*., 2010). In the current investigation watercress juice *per se* did not induce genetic damage according to the comet assay, wherever the majority of the cells evaluated were qualified as level 1 DNA damage. Furthermore, no increase in frequency of micronuclei was observed in the groups treated with aqueous juice alone. These results show that *Nasturtium officinale* (W.T. Aiton) would not be genotoxic *in vivo* while the opposite effect was shown by CP, which has been extensively used as a positive control in genetic toxicology schedules (Asita and Molise, 2011).

In addition to these findings, watercress-supplemented diet exhibited protective activity against the *in vivo* DNA damaging effect induced through the indirectly acting alkylating agent CP. Under our experimental conditions, both biomarkers were significantly different when compared with animals treated only with CP ($P < 0.05$ for comet assay and $P < 0.001$ for micronucleus test).

The present study provides a positive correlation in the induction of MN frequency in bone marrow cells with comet assay in peripheral blood leukocytes. Our results clearly demonstrate the correlation between these parameters, showing that the simultaneous application of these effect biomarkers could be useful tools in regulatory genetic damage testing, providing consistent resources for the detection of antigenotoxic chemical's potential.

A wide range of adverse effects, including reproductive toxicity, of CP have been reported in humans and other animals, but

it still has many clinical uses. Adult male patients treated with CP have demonstrated diminished sperm counts and an absence of spermatogenic cycles in their testicular tissue (Howell and Shalet, 1998). Also, CP has been described as a urotoxic agent (Topal *et al*., 2005) and a prototypical bladder toxin in humans and experimental animals, since following bioactivation, acrolein, a potent tissue alkylator, is generated (Frasier and Kehrer, 1993).

The comparative analysis of bladder histological changes obtained in watercress plus CP groups against those treated with CP alone indicates that watercress exercises a protective effect, although the induced histological changes are not severe. Consequently, in order to bear out the probable protective effect of watercress against bladder cancer or male infertility, higher doses of CP or longer periods of exposure should be tried.

In the present study, we examined total intact glucosinolates content because of the evidence of their potential health promoting effects (Fig. 1). Several reports in the literature have established that cancer prevention associated with cruciferous consumption is attributed to glucosinolates (Traka and Mithen, 2009), although the mechanistic pattern is not straightforward (Hayes *et al*., 2008). Their hydrolysis products (isothiocyanates) are related to anti-carcinogenic effects, including the inhibition of phase I enzymes and/or activation of phase II enzymes (Canistro *et al*., 2004).

Further watercress juice component analysis and *in vivo* investigations with a chronic schedule are necessary in order to establish if it can be used to develop food products with optimized functional characteristics, as well as to explore the characteristics of *Nasturtium officinale* as a good health promoter.

Acknowledgments

Grants from the University of Buenos Aires (UBACYT 20020100100123 2011–2014 and B031) were essential for this research. The authors would like to acknowledge the staff of the Animal House from the School of Pharmacy and Biochemistry, University of Buenos Aires for providing and keeping the animals used in this experimental design.

References

Asita A, Molise T. 2011. Antimutagenic effects of red apple and watermelon juices on cyclophosphamide-induced genotoxicity in mice. *Afr. J. Biotechnol.* **10**(77): 17763–17768.

Bianchi P, Manicardi G, Urner F, Campana A, Sakkas D. 1996. Chromatin packaging and morphology in ejaculated human spermatozoa: evidence of hidden anomalies in normal spermatozoa. *Mol. Hum. Reprod.* **2**: 139–144.

Branca F, Hanley A, Pool-Zobel B, Verhagen H. 2001. Biomarkers in disease and health. *Br. J. Nutr.* **86**(1): S55–S92.

Canistro D, Della Croce C, Iori R, Barillari J, Bronzetti G, Poib G, Cini M, Caltavuturo L, Perocco P, Paolini M. 2004. Genetic and metabolic effects of gluconasturtiin, a glucosinolate derived from cruciferae. *Mutat. Res.* **545**: 23–35.

Casanova N, Carballo M. 2011. Antigenotoxic activity of Watercress juice in an in vitro mammalian system using comet assay. *Phytother. Res.* **25**: 1743–1746; doi: 10.1002/ptr.3474

Casanova N, López Nigro M, Curieses S, Carballo M. 2010. In vitro evaluation of genotoxicity of watercress juice through effect biomarkers considering human intake. *Lat. Am. J. Pharm.* **29**(7): 1120–1125.

Collins A, Azqueta Oscoz A, Brunborg G, Gaiva I, Giovannelli L, Kruszewski M, Smith C, Stetina R. 2008. Review: The comet assay: topical issues. *Mutagenesis*. **23**(3): 143–151.

Forni A, Koss L, Geller W. 1964. Cytological study of the effect of cyclophosphamide in the epithelium of the urinary bladder in man. *Cancer* **17**: 1348–1355.

Frasier L, Kehrer J. 1993. Effect of indomethacin, aspirin, nordihydroguairetic acid, and piperonyl butoxide on cyclophosphamide-induced bladder damage. *Drug Chem. Toxicol.* **16**(2): 117–133.

Gill C, Haldar S, Boyd L, Bennett R, Whiteford J, Butler M, Pearson J, Bradbury I, Rowland I. 2007. Watercress supplementation in diet reduces lymphocyte DNA damage and alters blood antioxidant status in healthy adults. *Am. J. Clin. Nutr.* **85**: 504–510.

Goodman L, Gilman A. 2003. *As bases farmacológicas de terapêutica* (10th edn). McGraw-Hill: São Paulo.

Hadas S, Meir S, Akiri B, Kanner J. 1994. Oxidative defense systems in leaves of three edible herb species in relation to their senescence rates. *J. Agric. Food Chem.* **42**: 2376–2381.

Hartmann A, Aguerri E, Beepers C, Brender-Schwaab S, Burlinson B, Clay P, Collins A, Smith A, Speit G, Thybaud V, Tice R. 2003. Recommendations for conducting the in vivo alkaline Comet assay. *Mutagenesis* **18**: 45–51.

Hayes J, O'Kelleher M, Eggleston I. 2008. The cancer chemopreventive actions of phytohemicals derived from glucosinolates. *Eur. J. Nutr.* **47**(2): 73–88.

Higdon J, Delage B, Williams D, Dashwood R. 2007. Cruciferous vegetables and human cancer risk: epidemiologic evidence and mechanistic basis. *Pharmacol. Res.* **55**: 224–236.

Howell S, Shalet S. 1998. Gonadal damage from chemotherapy and radiotherapy. *Endocrinol. Metab. Clin. N. Am.* **27**: 927–943.

Integrated taxonomic information system (ITIS) Website. http://itis.gov/

Kapiszewska M, Sołtys E, Visioli F, Cierniak A, Zajac G. 2005. The protective ability of the mediterranean plant juices against the oxidative DNA damage. The role of the radical oxygen species and the polyphenol content. *J. Physiol. Pharmacol.* **56**(1): 183–197.

MacGregor J, Heddle J, Hite M, Margolin B, Ramel C, Salamone M, Tice R, Wild D. 1987. Guidelines for the conduct of micronucleus assays in mammalian bone marrow erythrocytes. *Mutat. Res.* **189**(2): 103–112.

McGahon A, Martín S, Bissonnette R, Mahboubi A, Shi Y, Mogil R, Nishioka W, Green D. 1995. The end of the (cell) line: methods for the study of apoptosis in vitro. *Methods Cell Biol.* **46**: 153–185.

Mohanty S, Dey P. 2003. Serous effusions: diagnosis of malignancy beyond cytomorphology. An analytic review. *Postgrad. Med. J.* **79**: 569–574.

Mosieniak G, Sikora E. 2010. Poliploidy: the link between senescence and cancer. *Curr. Pharm. Des.* **16**: 734–740.

Mughal A, Vikram A, Ramarao P, Jena G. 2010. Micronucleus and comet assay in the peripheral blood of juvenile rat: establishment of assay feasibility, time of sampling and the induction of DNA damage. *Mutat. Res.* **700**(1–2): 86–94.

Romih R, Koprivec D, Stiblar Martincic D, Jezernik K. 2001. Restoration of the rat urothelium after cyclophosphamide treatment. *Cell Biol. Int.* **25**(6): 531–537.

Schmid W. 1975. The micronucleus test. *Mutat. Res.* **31**: 9–15.

Serpeloni J, Bisarro dos Reis M, Rodrigues J, Campaner dos Santos L, Vilegas W, Varanda E, Dokkedal A, Mara S, Cólus I. 2008. In vivo assessment of DNA damage and protective effects of juices from Miconia species using the comet assay and micronucleus test. *Mutagenesis* **23**(6): 501–507.

Singh N, McCoy M, Schneider E. 1988. A simple technique for quantitation of low levels of DNA damage in individuals cells. *Exp. Cell Res.* **175**(1): 184–191.

Steinkeller H, Rabot S, Freyeald C, Nobis E, Scharf G, Chabicovsky M, Knasmuller S, Kassie F. 2001. Effects of cruciferous vegetables and their constituents on drug metabolizing enzymes involved in the bioactivation of DNA-reactive dietary carcinogens. *Mutat. Res.* **480–481**: 285–297.

Topal T, Oztas Y, Korkmaz A, Sadir S, Oter S, Coskun O, Bilgic H. 2005. Melatonin ameliorates bladder damage induced by cyclophosphamide in rats. *J. Pineal Res.* **38**(4): 272–277.

Traka M, Mithen R. 2009. Glucosinolates, isothiocyanates and human health. *Phytochem. Rev.* **8**: 269–282.

Travis L, Curtis R, Boice J Jr, Fraumeni J Jr. 1989. Bladder cancer after chemotherapy for man Hodgkin's lymphoma. *New Engl. J. Med.* **321**: 544–545.

Wyrobek A, Bruce W. 1975. Chemical induction of sperm abnormalities in mice. *Proc. Natl. Acad. Sci.* **72**(11): 4425–4429.

Research Article

In vivo antigenotoxic activity of watercress juice *(Nasturtium officinale)* against induced DNA damage

Natalia A. Casanova, Julia I. Ariagno, Marcela M. López Nigro, Gabriela R. Mendeluk, María de los A. Gette, Elisa Petenatti, Luis A. Palaoro and Marta A. Carballo

In vivo geno- and antigenotoxicity of watercress juice were evaluated using comet assay (blood cells) and micronucleus test (bone marrow). Biopsies of bladder, epididymis and testicles of mice were performed to extend the experimental design. Watercress did not induce genetic damage *per se* and exhibited protective activity. Analysis of histological changes suggests a probable protective effect. Further studies are needed to establish the protective role of watercress juice against DNA damage.