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Assessment of the Genotoxic and Antioxidant Activities of Several Vegetables, Spices and Herbs in Combination with Cyclophosphamide and 4-Nitroquinoline n-Oxide in Drosophila Melanogaster

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Abstract

It has been demonstrated that the juices from several vegetables, spices and herbs protect against certain carcinogens. The antioxidant capacity of several vegetables has been documented. The aims of the present study were to evaluate the genotoxicity of celery, coriander, epazote, parsley and watercress using the Somatic Mutation and Recombination Test (SMART) on the wings of Drosophila melanogaster using Standard (ST) and High Bioactivation (HB) crosses with regular and high levels of metabolizing cytochrome P450 enzymes, respectively. The protective effects of the extracts against 4-Nitroquinoline n-Oxide (4NQO) 4NQO, an oxidant compound, and Cyclophosphamide (CP), an alkylating agent, were evaluated. Both promutagens were employed alone as positive controls and in combination with the extracts. The majority of the extracts were non-genotoxic, although several positive results were observed. Parsley induced spots at all concentrations assayed in the ST cross and at the lowest concentration in the HB cross. Watercress, coriander and epazote produced some significant results in both crosses. CP in combination with the highest extract concentration exhibited a potentiation-synergistic effect while an inhibition-antagonic effect with 4NQO. In addition, the radical-scavenging activities of the extracts were investigated using the colorimetrical DPPH oxidative assay. The radical scavenging activity order from the highest to the lower was watercress > parsley > coriander > celery > epazote.

Keywords: Celery (*Apium graveolens*); Coriander (*Coriandrum sativum*); Epazote (*Chenopodium ambrosioides*); Parsley (*Petroselinum crispum*); Watercress (*Nasturtium officinale*); Genotoxicity; Wing spot assay; *Drosophila melanogaster*

Abbreviations: CP: Cyclophosphamide; 4NQO: 4-Nitroquinoline n-oxide; RSA: Radical-scavenging activity; ROS: Reactive Oxygen Species

Introduction

The evolution of aerobic metabolic processes, such as respiration and photosynthesis, has resulted in the production of Reactive Oxygen Species (ROS) in mitochondria, chloroplasts and peroxisomes. A common feature among different ROS types is oxidative damage to proteins, DNA and lipids [1]. Oxidative stress has been implicated in numerous chronic diseases, such as neurodegenerative, cardiovascular and diabetes ROS have been implicated in ageing and cancer [2-5]. ROS is a collective term describing oxygen-centred radicals, such as superoxide, hydroxyl and non-radical oxygen derivatives, namely hydrogen peroxide and singlet oxygen [6,7]. This oxidative stress could be decreased through the increased dietary intake of antioxidants. Antioxidants are defined as substances that delay or prevent the oxidation of lipids or other biomolecules or substrates. Antioxidant activity has been attributed to redox properties that function as reducing agents and act as hydrogen donors, singlet oxygen quencher and metal chelators [8]. A recent study of natural antioxidants showed that these molecules are ubiquitous in fruits and medicinal plants and can protect biomolecules from damage. The culinary properties of vegetables, herbs and spices have been attributed to several components and secondary metabolites known as phytochemicals. Among these, polyphenolics and phenolics, responsible for colour, flavour and taste, and flavonoids have been studied due to the effect of these molecules in reducing oxidative stress and several chronic and degenerative diseases [9–20]. The antioxidant properties of these secondary metabolites contribute to their anticancer, antimicrobial and anti-inflammatory properties [21,22]. The reductive abilities of plant extracts could provide antioxidative protection through the donation of electrons to radicals, thus antioxidant properties protect cells from the adverse effects of oxidative stress through ROS [23]. The edible and aromatic culinary plants celery (*Apium graveolens*), coriander (*Coriandrum sativum*), epazote (*Chenopodium ambrosioides*), parsley (*Petroselinum crispum*) and watercress (*Nasturtium officinale*) are included in the human diet, and to the best of our knowledge, these vegetables have not been tested for genotoxicity on the somatic cells of *Drosophila melanogaster*.

The wing-spot assay in Drosophila melanogaster is a sensitive, efficient and reproducible in vivo assay extensively used to investigate the genotoxicity of different chemical and natural compounds alone or in mixtures. Comparisons between the response levels of ST and HB crosses are based on the high expression of cytochrome P450 enzymes , present in the latter and leading to the increased sensitivity of promutagens [24–26].

The carcinogenic drug Cyclophosphamide (CP) is nitrogen mustard alkylating agent that produce alkyl adducts on DNA via alkyl radicals. CP has been widely used as an immunosuppressor and antineoplastic drug. The therapeutic effects of CP have been well documented in a variety of disorders, such as systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis and cancer [27]. The carcinogenic compound and 4-Nitroquinoline n-Oxide (4NQO), a quinoline derivative, induces DNA lesions through ROS production, which are typically corrected through nucleotide excision repair. ROS induction might result from the enzymatic reduction of the nitro group [28].

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Antioxidation through plant extracts has been measured worldwide using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) oxidative assay, which quantifies the radical-scavenging activity, hence the capacity of biological reagents to scavenge the DPPH radicals [29]. Radical-scavenging activities of edible plants and culinary herbs towards hydrophobic DPPH radicals are scarce.

Different studies have demonstrated that edible herbs and vegetable plants contain a variety of antioxidant compounds, and studies concerning the interaction of these compounds in the in vivo *Drosophila melanogaster* wing assay, which possesses the enzymatic machinery to activate environmental mutagens/carcinogens, are scarce. Therefore, the aims of the present study were to assess for the genotoxicity and antioxidant activities of several edible and aromatic culinary plants alone or in combination with the carcinogenic compounds CP and 4NQO using the wing spot assay in Drosophila melanogaster. In addition, the radical-scavenging activity of these extracts was evaluated using the DPPH oxidative assay.

Materials and Methods

Chemical compounds

Cyclophosphamide Monohydrate (CP, CAS number 6055-19-2), 4-Nitroquinoline n-Oxide (4NQO, CAS number 56-57-5) and 1, 1-Diphenyl-2-Picryl-Hydrazyl (DPPH) were purchased from Sigma Aldrich Chemical (St. Louis, MO, USA). Fresh herb and edible plants were purchased from the local market. Plants were determined by Rosa Maria Fonseca Juárez and vouchers were deposited at the Herbarium of the Sciences Faculty- UNAM (Universidad Nacional Autónoma de México)

Sample preparation of extracts from the herbs and edible plants

In order to eliminate possible contamination with pesticides the herbs and edible plants were thoroughly washed with tap water and later with distilled water. Senescent leaves were removed. Dried plants were stored at room temperature prior to experimentation. Fresh juices were obtained from a household juice Turmix extractor. The juice from each plant was centrifuged twice at 9000 g at 4°C for 20 minutes. The supernatants were clarified and sterilized using filtration through Whatman number 1 filter paper and maintained on ice for immediate use or stored at -80°C prior to testing. From these fresh extracts all concentrations assayed were prepared. To analyse the toxicity of the herb and plant extracts, a pilot study in which batches of 10 adult flare, Oregon-flare and mwh males per vial were treated with different percentage concentrations of each extract. Three replicas (independent repetitions) were performed. The number of surviving flies was counted at 24, 48 and 72 h after treatment. Only flies treated with epazote die at 50 and 100 percentage concentrations.

Strains and crosses

Three *Drosophila melanogaster* strains were used:

a) Flare-3 (flr3/In(3LR)TM3, ri pp sep l(3)89Aa bx34e and BdS),

b) Oregon-flare-3 (ORR) flr3/In(3LR)TM3, ri pp sep l(3)89Aa bx34e and BdS) and

c) Multiple wing hairs (mwh/mwh). For explanation of genetic symbols see Lindsley and Zimm [30].

Virgin females from flare and Oregon-flare strains were mated to mwh males, and Standard (ST) and High Bioactivation (HB) crosses were respectively performed [24–26]. The eggs from both crosses were independently collected in a thick layer of fermenting live Baker's yeast, supplemented with sucrose and tap water, during 8 h in complete darkness at 25 °C and 60–80% relative humidity. After three days, the third instar larvae (72 ± 4 h) were washed out of the bottles using tap water (25 °C) through a fine meshed stainless steel strainer.

Wing spot assay and statistical analysis

Third instant larvae were placed in vials containing 5 g Drosophila instant medium rehydrated with 15 ml of the respective aqueous herb solution. Negative controls were prepared using water. Positive controls and co-treatments were performed using 4NQO (2 mM) dissolved in a solution of 1Tween 80: 3 absolute EtOH, CP (5 mM) dissolved in distilled water. Vials with larvae samples of negative and positive controls and treatments and co-treatments were cultivated at 25 °C and 65% humidity under dark conditions until imago emergence. The flies were collected and stored in 70% EtOH. The wings of wild-type flies (trans-heterozygous, mwh/flr3) of both sexes from the two crosses were mounted on microscope slides using Faure's solution (30 g arabic gum, 20 ml glycerol, 50 g chloral hydrate and 50 ml water) and analysed microscopically at 40x [24]. For each treatment, 60 flies were analysed. The frequency of each type of spot (small and large single spots or twin spots) and the total frequency of spots per wing for each treatment were compared pair-wise with negative or positive mutagen control frequencies, or inversely, using the SMART computer programme based on the Kastenbaum–Bowman test ($P < 0.05$) [31]. The non-parametric Mann-Whitney and Wilcoxon U-tests ($\alpha = \beta = 0.05$, one sided) were used to exclude false positive or negative diagnoses. The U-test considers the rank values in controls and treatments and considers over-dispersion in a non-normal distribution. Non-significant results suggest a lack of sensitivity or an absence of genotoxicity at the concentration tested in the wing spot test and significant results represent genotoxicity when the treatment frequencies are higher than the control [32].

The interaction between positive controls and the highest concentration of the plant extracts was calculated using the Interaction Factor (IF) following the criterion of Schlesinger et al. (1992) with modifications according to Katsifis et al. (1996) and Danesi et al. (2012) [33-35].

$$IF = G1G2G3 - G1 - G2 - G3 + C$$

Where IF= Interaction Factor; G1G2G3 = Frequency of mutant clones in the treatment with combination; G1- G2 = frequency of mutant clones obtained through each mutagen; G3 = frequency of each extract alone; and C = frequency of mutant clones obtained in the control. Interactions might be present in two forms: synergism, when the mixture produced an effect greater than the sum of the effects of the separate exposures (greater than additive); and antagonism, when the mixture yielded an effect less than the sum of the separate effects (less than additive), considering zero as additively [33].

Antioxidant assay

To detect the antioxidant and/or radical scavenging activity of the herb and plant extracts the method of Yen and Chen (1996) was used [36]. The capacity of the extracts to scavenge the lipid soluble 2, 2-Diphenyl-1-Picrylhydrazyl (DPPH) radical, resulting in the bleaching of the purple colour exhibited through the stable DPPH radical was monitored at an absorbance of 517 nm: 1 mL of a 0.1 mM DPPH ethanol solution plus 1 mL of ethanol and 50 µL of the sample solutions at different concentrations and reacted 30 minutes at room temperature under dark conditions [37-39]. After 30 minutes,

the sample was centrifuged at 1000 rpm for 2 minutes, and the first absorbance was measured. Three other lectures at 60, 90 and 120 min were measured the absorbance values were measured and converted into the percentage of Antioxidant Activity (AA) using the following the radical-scavenging activity on an equivalent phenolic content basis was calculated as follows:

Radical scavenging activity (%) = Abs 0 – Abs Sp/Abs 0 X 100%

Where Abs 0 = control, is the optical density of the blank and Abs Sp = sample, the optical density of the sample. Monitoring continued for 90 min. The assays were performed in triplicate, and the results were averaged. The values are expressed as the means ± standard deviation of the three measurements. Ascorbic acid was used as a standard positive control compound.

Results

The objectives of the present study were to

a) Assess for the genotoxicity of several vegetables, spices and herb plants in ST and HB crosses

b) Evaluate whether edible plants have a protective effect against the genotoxic damage induced through CP and 4NQO promutagens assayed in both crosses, and

c) Determine the antioxidant activity of the plant water extracts.

The first group of experiments were conducted to detect whether the plant and herb extracts induced genotoxic damage in the wing spot assay of Drosophila melanogaster. The assay was performed in two independent experiments. The data from each experiment were compared and statistically analysed, followed by pooling for statistical testing. The non-parametric Mann-Whitney and Wilson U-test were used to exclude false positive or negative diagnoses. The data presented in table 1 were obtained after the chronic treatment of flies with the five plant extracts assayed from the ST and HB crosses in the trans-heterozygous mwh/flr3 wings from the Drosophila wing spot assay. No significant differences were observed between the frequencies of mutant spots in flies treated with the four concentrations assayed compared with the negative control in both ST and HB crosses for celery and in the HB cross for coriander, epazote and watercress. Several positive results were obtained: coriander was positive for total spots at the higher concentration in the ST cross and for large spots at the lowest concentration in HB. Epazote showed a slightly positive result for large spots at the lower concentration assayed and for total spots at the higher concentration for the ST cross and was also positive for large spots at two concentrations in the HB cross. Parsley was clearly positive for small and total spots at all concentrations assayed in the ST cross and at the lowest concentration assayed in the HB cross. Watercress was also positive at the lowest concentration in the ST cross and also positive for large spots in the HB cross.

The second group of experiments, summarized in Table 2, was performed to analyse the genotoxicity of each positive control in the wing spot test induced through the alkylating agent CP and the oxidant 4NQO. Both promutagens CP (5 mM) and 4NQO (2 mM) increased the frequencies of single, large and total spots in the ST cross and positive results were obtained for twin spots in the HB cross. The types of spots were completely different between promutagens and crosses. For CP, positive spot induction was observed in the ST cross for small, large and total spots, and the HB cross generated positive results for large spots with all vegetable extracts and twin spots were observed for epazote, parsley and watercress, while total spots for parley and watercress were significantly positive. For 4NQO's cotreatments, significant differences between the ST and HB cross in the induction of all types of spots were observed. Only celery, coriander and epazote generated a positive decrease of small spots in the ST cross, but an increase in large spots was observed for coriander. The effects of 4NQO were all negative for the HB cross.

Co-treatment of the positive controls with the highest concentration of each vegetable extract showed the following results: in combination with CP, small, large and total spots were significantly increased in the ST cross compared with the alkylating agent alone. In the HB cross the results were significantly positive only for large spots in the experiments performed with celery and coriander, while epazote was positive for large and twin spots, watercress was positive for large, twin and total spots and the combined treatment with parsley showed positive increments in the frequencies of all types of spots. The combination of the extracts with 4NQO showed a statistically significant decrease in small and total spots in experiments performed with celery and small and large spots for coriander, while epazote showed a positive decrease in small spots in the ST cross. In experiments with the HB cross the analysis of the data showed that only epazote generated a significant decrease for small spots (Table 2). The wings of 3925 flies were analysed in the present study.

For a quantitative comparison of the effects of vegetable extracts against the genotoxic activity of the promutagens tested, the interaction factor was calculated for total spots after the co-administration of extracts with genotoxins in the two crosses employed, as shown in Table 3. The results were compared, and the significance of any effect was determined in the exposed groups compared with controls to assess the type and extent of any toxicological interaction, which occurred when the effects of combined exposure were either significantly greater or less than additive. The maximum synergistic effect for all types of spots induced through CP was observed for parsley, followed by watercress, coriander, epazote and celery in the ST cross. For the HB cross, parsley produced a higher synergistic effect followed by, watercress, celery, coriander and epazote. The synergistic values obtained with the latter cross were lower than those obtained for the former cross. However, after co-administration of the vegetable extracts with 4NQO, maximum antagonism was observed for celery followed by epazote, parsley, watercress, and coriander for the ST cross. Furthermore, celery was the only edible herb showing an antagonic effect in combination with 4NQO for the HB cross. Surprisingly, the other four herbs did not reduce the effects induced with the oxidant.

A third group of experiments were performed to assess the antioxidant activity of the plant extracts based on the scavenging effect of stable DPPH. Radical scavenging activity of the vegetable extracts studied is shown in Table 4. All the extracts showed a tendency to quench the DPPH free radicals, as indicated through the concentration-dependent increase of activity. The order of radical scavenging activity of the plant extracts from the highest to the lowest was watercress > parsley > coriander > celery > epazote, as shown in Figure 1.

Discussion

The consumption of vegetables, spices and herb plants in the human diet has been associated with healthy nourishment and is almost considered as safe, although, vegetables are complex mixtures that could also contain mutagenic and carcinogenic chemical compounds. Flavonoids are a group of polyphenolic compounds widely distributed in plants that possess different properties, such as antitumor, iron chelating and free radical scavenging and pro-oxidation involved in

Plant extract and concentration (%)	Cross [a]	Number of flies	Spots per fly (Number of spots) Statistical diagnoses [b]				Spots with *mwh* clone	Mean number of cell division cycles
			Small single spots (1-2 cells) m = 2	Large single spots (> 2 cells) m = 5	Twin spots m = 5	Total spots m = 2		
Solvent (water)								
Control	ST	56	0.24 (13)	0.00 (0)	0.00 (0)	0.24 (13)	13	1.08
Control	HB	59	0.48 (28)	0.00 (0)	0.04 (2)	0.50 (30)	30	1.43
CELERY (CEL)	ST							
CELERY 12.5%		56	0.14 (8) -	0.04 (2) -	0.00 (0) -	0.18 (10) -	10	1.6
CELERY 25%		59	0.06 (4) -	0.00 (0) -	0.00 (0) -	0.06 (4) -	4	1
CELERY 50%		59	0.12 (7) -	0.02 (1) -	0.00 (0) -	0.14 (8) -	8	2
CELERY 100%		59	0.22 (13) -	0.06 (3) -	0.00 (0) -	0.28 (16) -	16	1.62
CELERY (CEL)	HB							
CELERY 12.5%		60	0.16 (10) -	0.06 (4) -	0 (0.00) -	0.24 (14) -	14	2.07
CELERY 25%		60	0.18 (11) -	0.06 (3) -	0.04 (2) -	0.26 (16) -	16	2.12
CELERY 50%		60	0.18 (11) -	0.06 (4) -	0 (0.00) -	0.24 (15) -	15	2.07
CELERY 100%		60	0.24 (14) -	0.06 (4) -	0 (0.00) -	0.30 (18) -	17	1.88
CORIANDER (COR)	ST							
COR 12.5%		60	0.22 (13) -	0.04 (2) -	0.00 (0) -	0.24 (15) -	15	1.53
COR 25%		58	0.32 (18) -	0.02 (1) -	0.00 (0) -	0.32 (19)-	19	1.26
COR 50%		57	0.28 (16) -	0.02 (1) -	0.00 (0) -	0.30 (17) -	17	1.35
COR 100%		59	0.40 (24) -	0.06 (4) -	0.00 (0) -	0.48 (28) +	28	1.61
CORIANDER (COR)	HB							
COR 12.5%		60	0.20 (12) -	0.12 (7) +	0.00 (0) -	0.32 (19) -	19	2.68
COR 25%		60	0.24 (15) -	0.06 (3) -	0.04 (2) -	0.34 (20) -	19	2.32
COR 50%		60	0.24 (14) -	0.08 (5) -	0.06 (3) -	0.36 (22) -	21	2.14
COR 100%		60	0.22 (13) -	0.08 (5) -	0.02 (1) -	0.32 (19) -	19	2.79
EPAZOTE (EPA)	ST							
EPA 3.125%		58	0.28 (16) -	0.02 (1) +	0.00 (0) -	0.30 (17) -	17	1,24
EPA 6.25%		58	0.18 (10) -	0.04 (2) -	0.00 (0) -	0.20 (12) -	12	2.17
EPA 12.5%		58	0.18 (11) -	0.04 (2) -	0.00 (0) -	0.22 (13)-	13	1.85
EPA 25%		59	0.42 (25) -	0.06 (4) -	0.00 (0) -	0.50 (29) +	29	1.52
EPAZOTE (EPA)	HB							
EPA 3.125%		60	0.48 (29) .	0.06 (3) -	0.04 (2) -	0.56 (34) -	32	1.62
EPA 6.25%		60	0.34 (20) -	0.08 (5) +	0.02 (1) -	0.44 (26) -	26	2.31
EPA 12.5%		60	0.36 (22) -	0.06 (3) -	0.04 (2) -	0.44 (27) -	27	1.7
EPA 25%		60	0.58 (35) -	0.08 (5) +	0.08 (5) +	0.74 (45) -	44	2.05
PARSLEY (PAR)	ST							
PAR 12.5%		59	0.66 (39) +	0.06 (3) -	0.00 (0) -	0.72 (42) +	42	1.33
PAR 25%		59	0.54 (32) +	0.06 (3) -	0.00 (0) -	0.60 (35) +	35	1.34
PAR 50%		59	0.50 (29) +	0.06 (3) -	0.00 (0) -	0.54 (32) +	32	1.53
PAR 100%		59	0.52 (31) +	0.06 (3) -	0.00 (0) -	0.58 (34) +	34	1.35
PARSLEY (PAR)	HB							
PAR 12.5%		60	0.74 (44) +	0.06 (3) -	0.04 (2) -	0.82 (49) +	48	1.46
PAR 25%		60	0.30 (18) -	0.04 (2) -	0.00 (0) -	0.34 (20) -	20	1.65
PAR 50%		60	0.32 (19) -	0.04 (2) -	0.04 (2) -	0.38 (23) -	23	1.78
PAR 100%		60	0.24 (15) -	0.08 (5) +	0.04 (2) -	0.36 (22) -	22	2.55
WATER CREES (WA)	ST							
WA 12.5%		60	0.64 (38) +	0.06 (3) -	0.00 (0) -	0.68 (41) +	41	1.39
WA 25%		57	0.34 (19) -	0.08 (4) -	0.00 (0) -	0.40 (23) -	23	1.61
WA 50%		59	0.24 (14) -	0.04 (2) -	0.00 (0) -	0.28 (16) -	16	1.5

WA 100 %		58	0.28 (16) -	0.00 (0) -	0.00 (0) -	0.28 (16) -	16	1.19
WATER CREES (WA)	HB							
WA 12.5%		60	0.48 (29)-	0.08 (5) +	0.02 (1) -	0.58 (35) -	35	1.57
WA 25%		60	0.44 (26) -	0.04 (2) -	0.00 (0) -	0.46 (28) -	28	1.36
WA 50%		60	0.36 (22) -	0.04 (2) -	0.02 (1) -	0.42 (25) -	25	1.72
WA 100 %		60	0.48 (29) -	0.08 (5) +	0.00 (0) -	0.56 (34) -	34	1.76

[a]ST standard cross; HB high bioactivation cross

[b] Statistical diagnoses according to Frei and Würgler (1988; 1995), m: minimal riskmultiplication factor for the assesment of negative results. For the final statistical diagnoses of all outcomes: + = positive; - = negativewith the standard SMART software based in the conditional binomial test according toFrei and Würgler (1988, 1995).

Table 1: Summary of results obtained in trans-heterozygous progeny of ST and HB crosses after chronic treatment of larvae with the plant extracts.

Treatment and concentra-tion (% ormM)	Cross [a]	Number of flies	Spots per fly (Number of spots) Statistical diagnoses [b]				Spots with *mwh* clone	Mean number of celldivision cycles
			Small single spots (1-2 cells) m = 2	Large single spots (> 2 cells) m = 5	Twin spots m = 5	Total spots m = 2		
Control (water)	ST	56	0.24 (13)	0.00 (0)	0.00 (0)	0.24 (13)	13	1.08
CP 5mM		60	2.24 (135)+	0.14 (8)+	0.02 (1)-	2.40 (144)+	144	1.33
CEL 100%+CP		60	3.50 (210) +	0.56 (33) +	0.00 (0) -	4.06 (243) +↑	243	1.56
COR100%+CP		60	4.60 (276) +↑	1.68 (101) +↑	0.00 (0) -	6.28 (377) +↑	376	1.96
EPA 25% + CP		60	4.90 (294) +↑	0.68 (41) +↑	0.00 (0) -	5.58 (335) +↑	335	1.54
PAR 100 % + CP		60	9.64 (578) +↑	1.60 (96) +↑	0.04 (2) -	11.26 (676) +↑	674	1.54
WA 100% + CP		60	4.88 (293) +↑	1.66 (100) +↑	0.00 (0) -	6.56 (393) +↑	393	1.95
Control (water)	HB	59	0.48 (28)	0.00 (0)	0.04 (2)	0.50 (30)	30	1.43
CP 5 mM		60	5.76 (346) +	1.24 (75) +	0.48 (29) +	7.50 (450) +	444	1.83
CEL 100%+CP		60	6.66 (400) -	1.96 (117) +	0.36 (22) -	8.98 (539) -	528	1.89
COR100%+CP		60	5.36 (322) -	2.36 (142) +↑	0.54 (32) -	8.26 (496) -	489	2.15
EPA 25% + CP		60	4.84 (290) -	2.08 (125) +↑	0.84 (50) +↑	7.74 (465) -	454	2.25
PAR 100 % + CP		60	8.62 (517) +↑	3.66 (220) +↑	1.06 (64) +↑	13.36 (801)+↑	775	2.11
WA 100% + CP		60	6.54 (392) -	3.48 (209) +↑	1.04 (62) +↑	11.06 (663) +↑	633	2.21
Control (Tween 80-EtOH)	ST	56	0.24 (13)	0.00 (0)	0.00 (0)	0.24 (13)	13	1.08
4-NQO 2mM		60	0.74 (45) +	0.18 (11) +	0.00 (0) -	0.94 (56) +	56	1.73
CEL 100%+4NQO		60	0.24 (15) +↓	0.20 (12) -	0.00 (0) -	0.44 (27) +↓	27	2.63
COR100%+4NQO		60	0.44 (26) +↓	0.70 (42) +↑	0.00 (0) -	1.14 (68) -	67	3
EPA 25% + 4NQO		60	0.38 (23) +↓	0.32 (19) -	0.00 (0) -	0.70 (42) -	42	2.69
PAR 100 % + 4NQO		60	0.54 (32) -	0.26 (16) -	0.00 (0) -	0.80 (48) -	48	2.44
WA 100% + 4NQO		60	0.46 (28) -	0.38 (23) -	0.00 (0) -	0.86 (51) -	51	2.63
Control (Tween 80-EtOH)	HB	60	0.48 (29)	0.04 (2)	0.04 (2)	0.52 (31)	31	1.52
4-NQO 2mM		60	0.92 (55) +	0.74 (45) +	0.40 (24) +	2.06 (124) +	111	2.55
CEL 100%+4NQO		60	0.72 (43) -	0.58 (35) -	0.26 (16) -	1.56 (94) -	89	2.57
COR100%+4NQO		60	0.94 (57) -	0.84 (50) -	0.36 (22) -	2.16 (129) -	114	2.55
EPA 25% + 4NQO		60	1.12 (67) -	0.82 (49) -	0.44 (27) -	2.38 (143) -	133	2.53
PAR 100 % + 4NQO		60	1.14 (68) -	0.90 (54)	0.40 (24) -	2.44 (146) -	135	2.57
WA 100% + 4NQO		60	1.04 (63) -	0.94 (56)-	0.46 (28) -	2.46 (147) -	137	2.56

[a]ST standard cross; HB high bioactivation cross

[b] Statistical diagnoses according to Frei and Würgler (1988; 1995), m: minimal risk multiplicationfactor for the assessment of negative results. For the final statistical diagnoses of all outcomes: + = positive; - = negative; potentiation (+↑) and inhibition (+↓) with the standard SMART software based in the conditional binomial test according to Frei and Würgler (1988, 1995).

Table 2: SMART results after chronic treatment of larvae from ST and HB crosses with CP, 4NQO and in combination with the highest concentration of the plant extracts.

redox cycling processes [21,40-42]. The flavonoid content of the species analysed in the present study has been previously reported. According to Yang et al., parsley has a higher total flavonoid content (73.5 mg/100 g), followed by watercress (37.5) coriander (8) and celery (3.4) [43]. To our knowledge, no data are available for the flavonoid content of epazote. The consensus is that the antioxidant activity of flavonoids

Cross	Genotoxin assayed	Extract	Interaction Factor (IF)[a]
ST	CP 5mM	Celery	1.62
		Coriander	3.64
		Epazote	2.92
		Parsley	8.52
		Watercress	7.12
HB		Celery	1.68
		Coriander	0.78
		Epazote	0.04
		Parsley	6
		Watercress	3.5
ST	4NQO 2mM	Celery	-0.54
		Coriander	-0.04
		Epazote	-0.5
		Parsley	-0.48
		Watercress	-0.12
HB		Celery	-0.3
		Coriander	0.28
		Epazote	0.08
		Parsley	0.52
		Watercress	0.34
[a] According to Schlesinger et al. (1992): IF negative = antagonism; IF positive = sinergism; IF zero = additivity			

Table 3: Interaction factor calculated for total spots after the co-administration of extracts with genotoxins in the two crosses employed.

RAS (Radical Scavenging Activity)[a, b]					
Concentration (%)	Celery	Coriander	Parsley	Watercress	Epazote
3.12%	-	-	-	-	39.09
6.25%	-	-	-	-	42.46
12.50%	0.47	48.22	38.19	68.07	46.71
25%	21.21	54.22	47.73	82.23	55.78
50%	46.49	64.64	55.08	85.62	-
100%	62.02	70.34	74.15	85.48	-
[a]Calculated by the formula RSA = Abs control - Abs extract/ Abs control x100[b] Mean values; standard deviation was calculated and resulted negligible.					

Table 4: Radical scavenging activity of the vegetables, spices and herb plants analyzed.

results from a combination of the iron chelating properties and free radical scavenging, but depending on the experimental conditions, these compounds also behave as pro-oxidant agents involved in redox cycling processes [41]. However, flavonoids exert pro-oxidant activity, including the formation of scavenging of radicals [21,44]. Several flavonoids, such as apigenin and naringenin, have been reported to induce an increase of 30–50 times in the formation of reactive oxidant species when incubated in the presence of GSH and peroxidases from thymus and bone marrow [45].

No significant differences were observed when the frequencies of mutant spots in flies treated with any of the four concentrations assayed for celery were compared with the negative control for both ST and HB crosses. These results could be associated with the phtalides from celery,

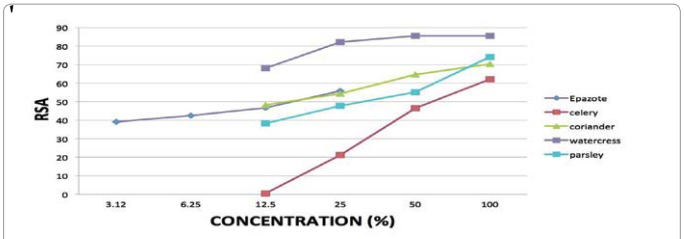

Figure 1: Radical scavenging activity of the five vegetables, spices and herb plants assayed.

which are the most significant bioactive compounds exhibiting many biological activities and health benefits, such as antifungal, antiplatelet, anticonvulsant and antiproliferative activity against two cancer lines, lymphoma and myeloid leukaemia, hence conferring protection against cancer [46]. Sedanolide, another compound isolated from celery oil seeds, induced higher activity of the detoxifying enzyme glutathione-S-transferase. Vitamin C is a known immune system booster that reduces the free radical in the body [47]. The flavonoid apiin, one of the bioactive components of celery was analysed for antioxidant activity, measured through different in vitro and in vivo models showing excellent protection against oxidative stress in mice [48].

In addition, phenolic compounds also protect genomic stability, reflecting the biological properties of these compounds, including free radical scavenging [49]. However, some polyphenols might exhibit pro-oxidant effects, which could be associated the mutagenicity of coriander at the highest concentration in the ST cross [50,51]. Recently, it was reported that linalool, an acyclic monoterpene alcohol, is the primary component of coriander, showing a pro-oxidant effect in sarcoma-180 solid tumour tissue and an antioxidant effect in the liver [52]. A positive correlation between the phenolic content of the roots of Coriander sativum was observed with the scavenging activities of DPPH [23]. Coriander was not mutagenic for Salmonella typhimurium strains TA98 and TA100 with and without S9 mix [53]. In the present study, parsley shows a clear positive genotoxic activity at the four doses assayed in the ST cross. Parsley extracts and the anticancer drug vincristine did not induce sex linked recessive lethals in Drosophila melanogaster either in single and in combined treatments but showed significant increase of cholinesterase enzyme activities within single and combination treatment [54]. In another study it was demonstrated pro-oxidant tendencies of parsley extracts in a protein-based model and DNA-based in vitro model, the cytotoxic effect could reflect the generation of hydrogen peroxide from components within the culture media reacting with the phenolic components within the extract [55]. The main components of Chenopodium ambrosioides are monoterpenes with ascaridole, representing more than 70% of compounds as antiprotozoan agents against Trypanosoma cruzi, Plasmodium falciparum and Leishmania amazonensis [56–58]. These components also induced chromosomal aberrations (chromatid type), SCE and a decrease in the mitotic index in cultured human lymphocytes [59]. Epazote in the present in vivo study induced a positive genotoxic effect only at the highest dose assayed with ST.

CP is metabolized through P-450 enzymes in the liver via two pathways. In the first pathway, CP is catalysed through cytochrome P-450 2B and P-450 2C forming the DNA cross-linking agent, phosphoramide mustard, and the toxic metabolite, acrolein, producing superfluous ROS [60,61]. Phosphoramide mustard, forms DNA cross links between and within DNA strands at guanine N-7 positions. The effect is irreversible and leads to cell apoptosis. Alternatively, in trans,

the CYP3A4-mediated N-dechloroethylation of CP to 3-dechloroethyl cyclophosphamide generates the toxic by-product chloroacetaldehyde [62]. CP in mice and rats induced genotoxicity and also produced oxidative stress and inhibited the activities of the anti-oxidant enzymes CAT, SOD, and GSH [63]. The results with CP are not consistent with those of Spano et al. who showed that CP was equally genotoxic in both crosses, and the highest concentration reported in that study (5 mM) was toxic, and only some flies survived in the ST cross, while none survived in the HB cross [64]. In the present study, CP generated positive results alone in both crosses, although in the HB series, the increase was approximately three times higher than that obtained from the ST cross. CP was clearly positive in the ST cross, with all the extracts combined with the alkylating agent, generating a ranking order for the respective genotoxic effectiveness of parsley > watercress > coriander > epazote > celery. Only positive results were obtained in the experiments run with parsley in combination with CP in the HB cross, in addition to the recombinogenic effect induced, evidenced as the frequencies of twin spots. Watercress had a protective effect against DNA damage induced through CP in the bladder cells of mice [65]. CP showed a uniformly toxic effect for solid tumour tissue and for the liver [52]. These results suggest that the higher response of the HB cross could be associated with the constitutively high levels of cytochrome P450, particularly CYP6A2, and the genotoxic metabolites of CP.

Both 4NQO and the metabolite 4 hydroxyaminoquinoline 1-oxide binds covalently to cellular macromolecules, such as nucleic acids and proteins [66]. In addition, 4NQO, a carcinogen that increases ROS, is a UV-mimetic agent that produces purine adducts through CYP450 metabolism [67,68]. In the present study, 4NQO induced small, large and total spots in the ST cross, consistent with the results reported in previous studies [25,69–72]. A significant increase in the frequencies of twin spots indicates that both recombinogenic activity and mutagenic effect were obtained with the HB cross. These results are consistent with those obtained in previous studies using the same promutagen [73,74]. Garcia-Rubio et al. showed that 4NQO causes synergistic increases of homologous recombination in Saccharomyces cerevisiae [75].

In the present study, extracts in combination with CP showed a potentiation effect, while in combination with 4NQO, inhibition activity was observed with the same juices/concentrations in the ST cross. Fernandes et al. showed this behaviour in the ethanol juice of propolis using the Drosophila wing somatic assay [51]. These authors suggested the presence of "Janus-type" substances that induce and prevent genotoxic effects. The interaction factor showed that CP with the extracts in the ST cross showed a clear synergistic effect, while the synergistic effects were lower in the HB series. The combination of the extracts with 4NQO in the ST cross were clearly antagonic, while in the HB cross only celery showed an inhibition effect.

DPPH is a free radical that is stable at room temperature and produces a purple solution in ethanol, with a colour that disappears in the presence of the radical scavenger in the reactive system and when the odd electron of nitrogen in the DPPH is paired. This reduction, in the presence of an antioxidant molecule, has been colorimetrically shown as a yellow coloured diphenyl-picryl hydrazine. The antioxidant strength of plant extracts analysed in the present study was dependent on the concentration. Hence, a direct relationship between the concentration of the vegetable extracts and radical scavenging activity was observed. We did not detect any correlation between the previously reported flavonoid content of the vegetables and plant extracts with the antioxidant activity observed in the present study [43].

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