Effects of artichoke (Cynara scolymus) leaf and bloom head extracts on chemically induced DNA lesions in Drosophila melanogaster

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Abstract

The genotoxicity of bloom head (BHE) and leaf (LE) extracts from artichoke (Cynara scolymus L.), and their ability to modulate the mutagenicity and recombinogenicity of two alkylating agents (ethyl methanesulfonate – EMS and mitomycin C – MMC) and the intercalating agent bleomycin (BLM), were examined using the somatic mutation and recombination test (SMART) in Drosophila melanogaster. Neither the mutagenicity nor the recombinogenicity of BLM or MMC was modified by co- or post-treatment with BHE or LE. In contrast, co-treatment with BHE significantly enhanced the EMS-induced genotoxicity involving mutagenic and/or recombinant events. Co-treatment with LE did not alter the genotoxicity of EMS whereas post-treatment with the highest dose of LE significantly increased this genotoxicity. This enhancement included a synergistic increase restricted to somatic recombination. These results show that artichoke extracts promote homologous recombination in proliferative cells of D. melanogaster.

Key words: artichoke, Drosophila melanogaster, recombinogenicity, SMART.

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Introduction

Artichokes, especially Cynara scolymus L. (Asteraceae), have long been consumed as food, especially as a staple component in Mediterranean diets. Several clinical investigations have shown that artichoke extracts can prevent the oxidative modification of blood lipoproteins and reduce blood cholesterol levels (Kirchhoff et al., 1994; Gebhardt, 1998, 2002; Pittler et al., 1998; Zapolska-Downar et al., 2002; Shimoda et al., 2003). Studies of the secondary metabolites of Cynara spp. have shown that polyphenolic compounds, mainly caffeic acid derivatives, as well as triterpenoid saponins and flavonoids, play an important biological role in the action of these extracts (Mucaji et al., 1999, 2001).

Artichoke leaf extracts (LE) have long been used in traditional folk medicine, mainly because of their choleric, diuretic and hypocholesterolemic activities (Speroni et al., 2003). Total LE extracts or their constituents reportedly have a beneficial effect in hepato-biliary diseases and improve liver regeneration after partial hepatectomy (Adzet et al., 1987; Kirchhoff et al., 1994; Kraft, 1997; Speroni et al., 2003). These extracts also have antioxidative and protective properties against hydroperoxide-induced oxidative stress in cultured rat hepatocytes (Gebhardt and Fausel, 1997; Miccadei et al., 2004). The central part of the artichoke flower bud is the edible portion of the plant and is widely consumed in Spain (2.6 g/day/person) (MAPA, 2003). Extracts of Cynara cardunculus L. (ECC) significantly reduced the frequency of 4-nitroquinoline-N-oxide-induced revertants at the ilvl locus and mitotic gene convertants at the trp5 locus in the diploid Saccharomyces cerevisiae strain D7 (Miadokova et al., 2008). An anticlastogenic effect against N-nitroso-N’-methylurea in Vicia sativa L (Miadokova et al., 2008) and against ethyl...
methanesulfonate (EMS) in Drosophila melanogaster has also observed (Miadokova et al., 2006). In contrast, although ECC is not mutagenic in Salmonella typhimurium TA98, it significantly increased the mutagenic effect of 2-aminofluorene and enhanced the cytotoxic/cytostatic effect of cis-Pt (Miadokova et al., 2006). We have also recently demonstrated the genotoxic and antigenotoxic potential of C. scolymus L. leaf extract in vitro (Jaciociunas et al., 2012, 2013).

The aim of this study was therefore: (1) to characterize the mutagenic and recombinogenic activity of artichoke bloom head (BHE) and leaf (LE) extracts, and (2) to explore the antigenotoxic potential of a combination of both extracts against chemical agents capable of inducing distinct types of DNA lesions in eukaryotes. The bioassay used was the Somatic Mutation and Recombination Test (SMART) in D. melanogaster. This test allows assessment of the potential of complex mixtures to cause a loss of heterozygosity in marker genes of somatic cells, expressed in D. melanogaster crosses (ST) (Graf and van Schaik, 1992). Eggs from the two crosses were collected for 8 h on standard medium enriched with baker’s yeast and supplemented with sucrose. After three days, the third instar larvae were washed out of the vials and used for the treatments.

Material and Methods

Plant extracts

The C. scolymus L. specimens used in this work were collected in Gramado (Rio Grande do Sul, Brazil), on a small farm in Apiquárius (latitude 29º27.851’ and longitude 50º49.501’) where the plants were organically cultivated. The artichoke leaves and flowers were collected in the winter of 2007 and a voucher specimen was deposited in the Herbarium of the Department of Botany of the Lutheran University of Brazil (HERULBRA 4288).

Preparation of extracts

Crude aqueous extracts of leaves (120 g) and flowers (160 g) were prepared by infusion with distilled water (plant:solvent ratio of 1:10) at 80 °C for 30 min. The infusion was cooled at room temperature, filtered, frozen and concentrated by lyophilization. The resulting yields were 13.7 g (11.4%) for leaf extracts and 14.8 g (9.3%) for flowers.

The phytochemical profiles of the extracts were determined as described by Harbone (1998) and Simões et al. (2007). These methods involve colorimetric reactions that qualitatively detect flavonoids, tannins, saponins, alkaloids, arthaquinones, coumarins and cardiac glycosides. The presence of saponins, flavonoids and coumarins was also analyzed by thin-layer chromatography (TLC) in silica gel GF254 using eluents and developers indicated by Wagner and Bladt (1996). The phytochemical screening of LE and BHE identified the presence of flavonoids, phenolic compounds and saponins.

Chemicals

The chemical compounds ethylmethanesulphonate (EMS, 62-50-0), liquid form M0880, was obtained from Sigma Chemical Co. (Saint Louis, MO, USA). The bifunctional agent mitomycin C (MMC, 50-07-5) was obtained from Bristol-Myers Squibb (São Paulo, SP, Brazil). Bleomycin sulfate (BLM – 9041-93-4) was obtained from Biossintética (São Paulo, SP, Brazil). These agents and the extracts were dissolved in distilled water immediately before use.

Somatic mutation and recombination test (SMART) in D. melanogaster crosses

Two versions of the SMART were used: (1) standard (ST) cross: flr3/ TM3, Bds females to mwh/mwh males and (2) high bioactivation (HB) cross: ORR/ORR; flr3/ TM3, Bds females to mwh/mwh males (Graf and van Schaik, 1992). Eggs from the two crosses were collected for 8 h on standard medium enriched with baker’s yeast and supplemented with sucrose. After three days, the third instar larvae were washed out of the vials and used for the treatments.

Genotoxicity test

Chronic treatments (from 48 h until pupation) were done by adding similar-aged larvae (72 ± 4 h) from the ST and HB crosses to vials containing 1.5 g of Drosophila Instant Medium (Carolina Biological Supply Company, Burlington, NC, USA) plus 3 mL of fresh BHE (0.0069, 0.0138, 0.0276 and 0.0552 g/mL) or LE (0.01875, 0.02175, 0.0435 and 0.0875 g/mL), previously diluted in distilled water. The toxicity of these extracts was assessed in a pilot experiment in which batches of 100 flies were treated with different concentrations of each extract. The number of surviving flies was counted and at least 70% of the flies reached the adult stage in all treatments. The extracts were tested in triplicate in two independent experiments. Distilled water was used as a negative control.

Co-treatment

Larvae from the ST cross were transferred to plastic vials containing 1.5 g of Drosophila Instant Medium rehydrated with 3 mL of the test solution containing distilled water alone, mutagenic compound, or extract (LE: 0.0435 and 0.0875 g/mL; BHE: 0.0276 and 0.0552 g/mL) plus the mutagen (12.5 mM EMS; 0.5 mM MMC or 0.01 mM BLM). The larvae were left to feed and then complete development on this medium. MMC, BLM or EMS alone were used as positive controls and distilled water alone was the negative control (Andrade et al., 2004; Sinigaglia et al., 2006).
Post-treatment

Larvae from the ST cross were transferred to Plexiglas vials, the lower end of which was covered with fine nylon gauze. These tubes were then placed in 50 mL beakers containing 0.3 g of powered cellulose (Merck) mixed with 2 mL of distilled water or different mutagen solutions. The larvae were fed on these mutagen-cellulose suspensions through the gauze for 4 h (for EMS and BLM) and 6 h (for MMC). The groups (subjected to acute feeding with water or genotoxin) were then washed and put into plastic vials with 1.5 g of Drosophila Instant Medium containing either distilled water or different concentrations of the extracts (0.0435 g/mL and 0.0875 g/mL). The larvae were allowed to feed on the instant medium until pupation (± 48 h).

Wing scoring

Approximately 10-12 days after the treatments, the emerging adult flies were collected and conserved in 70% ethanol. The mwh x flr3 standard cross produced two types of progeny that were distinguished phenotypically based on the Bdot marker: (1) trans-heterozygous flies for the recessive wing-cell markers multiple wing hair (mwh) and flare (flr3) and (2) heterozygous flies for a balancer chromosome with large inversions on chromosome 3 (TM3). Wings of five females and five males of the two phenotypes were mounted on slides and scored under a microscope at 400X magnification for the occurrence of spots. Induced loss of heterozygosity in the marker-balancer-heterozygous genotype leads to two types of mutant clones: (1) single spots, either mwh or flr3, that result from point mutations, chromosomal aberrations and/or somatic recombination, and (2) twin spots, consisting of both mwh and flr3 sub-clones, that originate exclusively from somatic recombination (Graf et al., 1984). In flies with the balancer-heterozygous genotype, mwh spots reflect predominantly somatic point mutations and chromosomal aberrations since somatic recombination involving the balancer chromosome and its structurally normal homologue is a non-viable event. By comparing the frequencies of these two genotypes it was possible to quantify the modulatory effect of C. scolymus L. on the recombinogenic and mutagenic activities of the genotoxins (Frei et al., 1992).

Statistical analysis

The data were evaluated according to the multiple-decision procedure of Frei and Würgler (1988), which produces four possible diagnoses: positive, weakly positive, negative or inconclusive. The frequencies of each type of mutant clone per fly of a treated series were compared pair-wise, i.e., control vs. modulator; genotoxin alone vs. genotoxin plus modulator, using the conditional binomial test according to Kastenbaum and Bowman (1970). All inconclusive and weak results were analyzed with the non-parametric U-test of Mann, Whitney and Wilcoxon. The U-test takes into account the rank values in controls and treatments and considers over-dispersion in a non-normal distribution (Frei and Würgler, 1995). For both tests p < 0.05 was considered significant. Based on the control-corrected frequencies of clone formation per 105 cells, the percentages of modulator interference were calculated as follows: \[ \text{[(genotoxin alone - genotoxin plus modulator/genotoxin alone) X 100]} \] (Abraham, 1994).

Results

Genotoxicity

The genetic toxicity analyses of both BHE and LE were done in the ST and HB crosses by observing the occurrence of clone spot induction in marker-trans-heterozygous (mwh/flr3) adult flies. For each concentration used, Tables 1 and 2 show the total number of flies analyzed, the frequency of the different mutant clones and the total spots scored, which represent the final genotoxicity of the extracts tested. For all four doses used, neither extract showed a significant difference in relation to the respective negative controls in the ST and HB crosses, which means they were clearly not genotoxic in this test system.

MMC, BLM and EMS were genotoxic and produced somatic recombination in marker-heterozygous (mwh/flr3) flies (Table 3). Likewise, significant mutational responses were observed; each of the compounds increased the frequency of total spots in balancer-heterozygous (mwh/TM3) flies. The frequencies of mutant spots induced by EMS in the later genotype were smaller than those obtained in trans-heterozygous flies (Tables 4 and 5). These findings are consistent with previously reported responses for these compounds in the SMART assay (Sinigaglia et al., 2004, 2006). In addition, the genotoxicity of BLM was preferentially related to the induction of small single and total spots, as previously described by Graf et al. (1984).

Modulator effects

Since MMC, EMS and BLM act as direct genotoxins the modulatory effects of both extracts was analyzed only in the ST cross. In the co-treatment protocol and in the trans-heterozygous genotype, neither BHE nor LE modified the MMC and BLM spot frequencies, which suggested that neither extract interfered with the mechanisms that precede the induction of DNA lesions by these genotoxins. Conversely, there was a significant increase in the frequency of mutant clones in response to EMS for both concentrations of BHE, but not for LE (Tables 3 and 4). In the balancer-heterozygous genotype (TM3), BHE also significantly increased the frequencies of total spots induced by EMS (by ~60 and 130% for 0.0276 and 0.0552 g BHE/mL, respectively), indicating that the extract was both co-mutagenic and co-recombinogenic (Table 4). Figure 1 shows the synergistic effect of co-treatment with BHE on EMS genotoxicity, particularly in relation to mutation and recombination. EMS alone (12.5 mM) induced 41.2 spots through

Statistical analysis

The data were evaluated according to the multiple-decision procedure of Frei and Würgler (1988), which produces four possible diagnoses: positive, weakly positive, negative or inconclusive. The frequencies of each type of mutant clone per fly of a treated series were compared pair-wise, i.e., control vs. modulator; genotoxin alone vs. genotoxin plus modulator, using the conditional binomial test according to Kastenbaum and Bowman (1970). All inconclusive and weak results were analyzed with the non-parametric U-test of Mann, Whitney and Wilcoxon. The U-test takes into account the rank values in controls and treatments and considers over-dispersion in a non-normal distribution (Frei and Würgler, 1995). For both tests p < 0.05 was considered significant. Based on the control-corrected frequencies of clone formation per 105 cells, the percentages of modulator interference were calculated as follows: \[ \text{[(genotoxin alone - genotoxin plus modulator/genotoxin alone) X 100]} \] (Abraham, 1994).
Table 1 - Genotoxicity of leaf extracts (LE) from *Cynara scolymus* L. in the *D. melanogaster* wing spot test using standard (ST) and high bioactivation (HB) crosses.

| Crosses / genotypes | LE (g/mL) | No. of flies (N) | Spots per fly (no. of spots)/statistical diagnosis\(^a\) | Total mwh clones\(^c\) (n) | Mean mwh clone size \(^c\) | Total spots\(^b\) (m = 2) | Clone induction frequencies (per 10^5 cells per cell division)\(^d\), \(^e\) (n/NC)\(^f\) |
|---------------------|-----------|------------------|-------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------------------------|
| ST cross            |           |                  |                                                 |                             |                             |                             |                                                 |
| mwh / flr\(^3\)     | NC\(^g\)  | 40               | 0.95 (38)                                       | 0.08 (03)                   | 0.03 (01)                   | 1.05 (42)                   | 42 1.71                                           |
| 0.01875             | 40        | 0.50 (20)        | 0.08 (03)                                       | 0.10 (04)                   | 0.70 (27)                   | 27 2.04                                    | 1.38 [0.77]                                     |
| 0.02175             | 40        | 0.63 (25)        | 0.05 (02)                                       | 0.03 (01)                   | 0.53 (21)                   | 21 1.76                                    | 1.43 [0.72]                                     |
| 0.0435              | 40        | 0.45 (18)        | 0.08 (03)                                       | 0.00 (00)                   | 0.78 (31)                   | 31 1.68                                    | 1.08 [0.08]                                     |
| 0.0875              | 40        | 0.68 (27)        | 0.05 (02)                                       | 0.05 (02)                   | 0.78 (31)                   | 31 1.68                                    | 1.59 [0.56]                                     |
| HB cross            |           |                  |                                                 |                             |                             |                             |                                                 |
| mwh / flr\(^3\)     | NC\(^g\)  | 40               | 0.39 (37)                                       | 0.10 (04)                   | 0.00 (00)                   | 1.03 (41)                   | 41 1.76                                           |
| 0.01875             | 40        | 0.70 (28)        | 0.13 (05)                                       | 0.05 (02)                   | 0.88 (35)                   | 34 1.88                                    | 1.74 [0.36]                                     |
| 0.02175             | 40        | 0.83 (33)        | 0.10 (04)                                       | 0.00 (00)                   | 0.93 (37)                   | 37 1.81                                    | 1.90 [0.20]                                     |
| 0.0435              | 40        | 0.55 (22)        | 0.13 (05)                                       | 0.08 (03)                   | 0.75 (30)                   | 29 2.28                                    | 1.49 [0.61]                                     |
| 0.0875              | 40        | 0.70 (28)        | 0.08 (03)                                       | 0.00 (00)                   | 0.78 (31)                   | 30 1.67                                    | 1.54 [0.56]                                     |

\(^a\)Statistical diagnoses according to Frei and Würgler (1988, 1995). Two-tailed U-test: -, negative; \(m\): minimal risk multiplication factor for the assessment of negative results; significance levels \(\alpha = \beta = 0.05\); \(^b\)Including rare \(flr\) single spots; \(^c\)Considering mwh clones from mwh single and twin spots; \(^d\)Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls; \(^e\)For calculation see Andrade et al. (2004); \(^f\)\(C = 48.800\), i.e., approximate number of cells examined per fly; \(^g\)Negative control (NC).

Table 2 - Genotoxicity of bloom heads extracts (BHE) from *Cynara scolymus* L. in the *D. melanogaster* wing spot test using standard (ST) and high bioactivation (HB) crosses.

| Crosses / genotypes | BHE (g/mL) | No. of flies (N) | Spots per fly (no. of spots)/statistical diagnosis\(^a\) | Total mwh clones\(^c\) (n) | Mean mwh clone size \(^c\) | Total spots\(^b\) (m = 2) | Clone induction frequencies (per 10^5 cells per cell division)\(^d\), \(^e\) (n/NC)\(^f\) |
|---------------------|-----------|------------------|-------------------------------------------------|-----------------------------|-----------------------------|-----------------------------|------------------------------------------------|
| ST cross            |           |                  |                                                 |                             |                             |                             |                                                 |
| mwh / flr\(^3\)     | NC\(^g\)  | 40               | 0.70 (28)                                       | 0.13 (05)                   | 0.03 (01)                   | 0.85 (34)                   | 34 1.65                                           |
| 0.0069              | 40        | 0.65 (26)        | 0.05 (02)                                       | 0.05 (02)                   | 0.75 (30)                   | 30 1.63                                    | 1.54 [0.20]                                     |
| 0.0138              | 40        | 0.55 (22)        | 0.10 (04)                                       | 0.03 (01)                   | 0.68 (27)                   | 26 2.00                                    | 1.33 [0.41]                                     |
| 0.0276              | 40        | 0.50 (20)        | 0.20 (08)                                       | 0.08 (03)                   | 0.78 (31)                   | 31 2.65                                    | 1.59 [0.15]                                     |
| 0.0552              | 40        | 0.53 (21)        | 0.13 (05)                                       | 0.10 (04)                   | 0.75 (30)                   | 30 2.30                                    | 1.54 [0.20]                                     |
| HB cross            |           |                  |                                                 |                             |                             |                             |                                                 |
| mwh / flr\(^3\)     | NC\(^g\)  | 40               | 0.95 (38)                                       | 0.03 (01)                   | 0.08 (03)                   | 1.05 (42)                   | 42 1.57                                           |
| 0.0069              | 40        | 0.88 (35)        | 0.15 (06)                                       | 0.05 (02)                   | 1.08 (43)                   | 43 1.82                                    | 2.25 [0.10]                                     |
| 0.0138              | 40        | 0.80 (32)        | 0.05 (02)                                       | 0.13 (05)                   | 0.98 (39)                   | 39 1.72                                    | 2.00 [0.15]                                     |
| 0.0276              | 40        | 0.95 (38)        | 0.08 (03)                                       | 0.00 (00)                   | 1.03 (41)                   | 41 1.61                                    | 2.10 [0.05]                                     |
| 0.0552              | 40        | 0.73 (29)        | 0.10 (04)                                       | 0.03 (01)                   | 0.85 (34)                   | 34 1.74                                    | 1.74 [0.41]                                     |

\(^a\)Statistical diagnoses according to Frei and Würgler (1988, 1995). Two-tailed U-test: -, negative; \(m\): minimal risk multiplication factor for the assessment of negative results; significance levels \(\alpha = \beta = 0.05\); \(^b\)Including rare \(flr\) single spots; \(^c\)Considering mwh clones from mwh single and twin spots; \(^d\)Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls; \(^e\)For calculation see Andrade et al. (2004); \(^f\)\(C = 48.800\), i.e., approximate number of cells examined per fly; \(^g\)Negative control (NC).
| Genotypes | Controls and compounds MUT\textsuperscript{b} + LE (g/mL) | No. of flies (N) | Spots per fly (no. of spots)/statistical diagnosis \textsuperscript{a} | Total \textit{mwh} clones\textsuperscript{d} (n) | Mean \textit{mwh} clone size class\textsuperscript{d} | Clone induction frequencies (per 10\textsuperscript{5} cells per cell division)\textsuperscript{e,f} (n/NC)\textsuperscript{g} |
|------------|------------------|-----------------|-------------------------------------------------|-------------------|-------------------|-----------------------------|
| \textit{mwh} / \textit{flr}\textsuperscript{3} | | | | | | |
| MMC | NC\textsuperscript{h} | 40 | 0.65 (26) | 0.15 (06) | 0.00 (0) | 0.80 (32) | 32 | 2.00 | 1.64 |
| MMC | 40 | 34.30 (1372)* | 31.17 (1247)* | 13.53 (541)* | 79.00 (3160)* | 3003 | 2.90 | 153.84 [152.20] |
| MMC + 0.0435 | 40 | 33.73 (1349) | 29.30 (1172) | 12.75 (510) | 75.78 (3031) | 2588 | 2.94 | 132.58 [130.94] |
| MMC + 0.0875 | 40 | 32.62 (1305) | 29.93 (1197) | 13.20 (528) | 75.76 (3030) | 2480 | 2.57 | 131.26 [129.62] |
| BLM | | | | | | |
| \textit{mwh} / \textit{flr}\textsuperscript{3} | | | | | | |
| NC\textsuperscript{h} | 30 | 0.70 (21) | 0.10 (03) | 0.10 (03) | 0.90 (27) | 27 | 1.81 | 1.84 |
| BLM | 30 | 3.07 (92)* | 0.53 (16)* | 0.03 (01) | 3.63 (109)* | 109 | 1.85 | 7.45 [5.60] |
| BLM + 0.0435 | 30 | 2.50 (75) | 0.27 (08) | 0.10 (03) | 2.87 (86) | 86 | 1.70 | 5.87 [4.03] |
| BLM + 0.0875 | 30 | 2.76 (83) | 0.27 (08) | 0.00 (00) | 3.03 (91) | 91 | 1.66 | 6.22 [4.37] |
| EMS | | | | | | |
| \textit{mwh} / \textit{flr}\textsuperscript{3} | | | | | | |
| NC\textsuperscript{h} | 30 | 0.70 (21) | 0.10 (03) | 0.10 (03) | 0.90 (27) | 27 | 1.81 | 1.84 |
| EMS | 30 | 93.63 (2809)* | 36.60 (1098)* | 21.60 (648)* | 151.83 (4555)* | 4336 | 2.25 | 296.17 [294.33] |
| EMS + 0.0435 | 30 | 100.37 (3011) | 34.07 (1022) | 14.40 (432) | 148.84 (4465) | 4397 | 2.22 | 293.34 [291.35] |
| EMS + 0.0875 | 30 | 105.43 (3163) | 26.93 (808) | 17.27 (518) | 149.63 (4489) | 4378 | 2.22 | 299.04 [294.20] |

\textsuperscript{a}Statistical diagnoses according to Frei and Würgler (1988, 1995). Two-tailed U-test: * positive; p ≤ 0.05 vs. untreated control; +, positive and -, negative, p ≤ 0.05 vs. MMC, BLM or EMS alone; m: minimal risk multiplication factor for the assessment of negative results; \textsuperscript{b}Mutagen: MUT; \textsuperscript{c}Including rare \textit{flr}\textsuperscript{3} single spots; \textsuperscript{d}Considering \textit{mwh} clones from \textit{mwh} single and twin spots; \textsuperscript{e}Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls; \textsuperscript{f}For calculation see Andrade \textit{et al.} (2004); \textsuperscript{g}C = 48.800, \textit{i.e.}, approximate number of cells examined per fly; \textsuperscript{h}NC = negative control.
Table 4 - Summary of results obtained in the \textit{D. melanogaster} wing spot test. Co-treatments with MMC, BLM and EMS in combination with bloom heads extracts (BHE) from \textit{Cynara scolymus} L., 48 h feeding of 3-day-old larvae of the standard (ST) cross: marker-trans-heterozygous flies (\textit{mwh/flr} \textsuperscript{3}) and balancer-heterozygous (\textit{mwh}/\textit{TM3}) flies.

| Genotypes | Controls and compounds MUT\textsuperscript{a} + BHE (g/mL) | No. of flies (N) | Spots per fly (no. of spots)/statistical diagnosis\textsuperscript{a} | Total \textit{mwh} clones\textsuperscript{b} (n) | Mean \textit{mwh} clone size\textsuperscript{b} | Clone induction frequencies (per 10\textsuperscript{5} cells per cell division)\textsuperscript{c} (n/NC)\textsuperscript{d} | Enhancement\textsuperscript{d} (%) |
|------------|--------------------------------------------------|-----------------|--------------------------------------------------------------|--------------|-----------------|---------------------------------------------|------------------|
| \textit{mwh}/\textit{flr}\textsuperscript{3} | NC\textsuperscript{e} | 40 | 0.65 (26) | 0.15 (06) | 0.00 (0) | 0.80 (32) | 32 | 2.00 | 1.64 |
| MMC | | | 34.30 (1372)* | 31.18 (1247)* | 13.53 (541)* | 79.01 (3160)* | 3003 | 2.90 | 153.84 [152.20] |
| MMC + 0.0276 | | | 35.03 (1401) - | 32.55 (1302) - | 14.75 (590) - | 82.33 (3293) - | 3137 | 2.89 | 160.71 [159.07] |
| MMC + 0.0552 | | | 33.33 (1333) - | 32.25 (1290) - | 14.08 (563) - | 79.66 (3186) - | 3053 | 2.94 | 156.40 [154.76] |
| BLM | \textit{mwh}/\textit{flr}\textsuperscript{3} | NC\textsuperscript{e} | 40 | 0.73 (29) | 0.13 (05) | 0.10 (03) | 0.93 (37) | 37 | 1.76 | 1.90 |
| BLM | | | 3.20 (128)* | 0.40 (16)* | 0.03 (01) | 3.95 (145)* | 144 | 1.73 | 7.38 [5.48] |
| BLM + 0.0276 | | | 3.65 (146) - | 0.20 (08) - | 0.10 (04) - | 3.95 (158) - | 158 | 1.66 | 8.09 [6.20] |
| BLM + 0.0552 | | | 2.80 (112) - | 0.20 (08) - | 0.03 (01) - | 3.03 (121) - | 121 | 1.70 | 6.20 [4.30] |
| EMS | \textit{mwh}/\textit{flr}\textsuperscript{3} | NC\textsuperscript{e} | 30 | 0.70 (21) | 0.10 (03) | 0.10 (03) | 0.90 (27) | 27 | 1.81 | 1.84 |
| EMS | | | 93.63 (2809)* | 36.60 (1098)* | 21.60 (648)* | 151.83 (4555)* | 4336 | 2.25 | 296.17 [294.33] |
| EMS + 0.0276 | | | 138.83 (4165) + | 48.53 (1456) + | 28.83 (865) + | 216.19 (6486) + | 6233 | 2.08 | 425.75 [423.91] |
| EMS + 0.0552 | | | 159.30 (4779) + | 47.43 (1423) + | 39.97 (1199) + | 246.70 (7401) + | 6901 | 2.00 | 471.38 [469.54] |
| \textit{mwh}/\textit{TM3} | NC\textsuperscript{e} | 30 | 0.47 (14) | 0.00 (0) | 0.00 (0) | 0.47 (14) | 14 | 1.21 | 0.96 |
| EMS | | | 33.53 (1006)* | 5.70 (171)* | 39.23 (1177)* | 1177 | 1.54 | 80.40 [79.44] |
| EMS + 0.0276 | | | 53.67 (1610) + | 9.00 (270) + | 62.67 (1880) + | 1880 | 1.57 | 128.42 [127.46] |
| EMS + 0.0552 | | | 78.27 (2348) + | 11.37 (341) + | 89.64 (2689) + | 2689 | 1.52 | 183.67 [182.72] |

\textsuperscript{a}Statistical diagnoses according to Frei and Würgler (1988, 1995). Two-tailed U-test: *, positive; p ≤ 0.05 vs. untreated control; +, positive and -, negative, p ≤ 0.05 vs. MMC, BLM or EMS alone; m: minimal risk multiplication factor for the assessment of negative results; significance levels α = β = 0.05; \textsuperscript{b}Mutagen: MUT; \textsuperscript{c}Including rare \textit{flr}\textsuperscript{3} single spots; \textsuperscript{d}Considering \textit{mwh} clones from \textit{mwh} single and twin spots; \textsuperscript{e}Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls; \textsuperscript{f}For calculation see Andrade \textit{et al.} (2004); \textsuperscript{g}C = 48.800, i.e., approximate number of cells examined per fly; \textsuperscript{h}Calculated according to Abraham (1994) using the control corrected clone induction frequencies: (MUT alone - MUT plus BHE / MUT alone) x 100; \textsuperscript{i}Negative control: NC; \textsuperscript{j}Only \textit{mwh} single spots were observed in \textit{mwh}/\textit{TM3} heterozygotes as the balancer chromosome TM3 does not carry the \textit{flr}\textsuperscript{3} mutation.
mutational events and 110.6 related to somatic recombination. BHE (0.0276 and 0.0552 g/mL) increased the mutagenic activity of EMS to 65.2 and 96.1 spots, respectively (increments of 1.6 and 2.3 fold). In contrast, BHE (0.0276 and 0.0552 g/mL) had only a minor effect on EMS recombinogenicity (~151 spots, 1.4 fold increase for both concentrations). These results for the co-genotoxicity of BHE with EMS and the lack of effect on the genotoxicity of BLM and MMC probably reflect differences in the mechanisms of action of alkylating agents (MMC and EMS) compared to the intercalating drug BLM.

In the post-treatment protocols, BHE did no significantly affect the genotoxicity of the agents tested. Likewise, LE did not interfere with the mutagenic and recombinogenic action of MMC and BLM. These data indicate that post-treatment with both extracts had no effect on the mechanisms involved in the MMC- and BLM-induced lesions (Tables 5 and 6). The outcome of LE on EMS-induced activity was quite different since this extract significantly increased the frequency of EMS-induced spots by 131% at the highest dose tested. These effects were observed solely in mwh/flr′ flies since in mwh/TM3 flies post-treatment with LE did not alter the frequency of EMS-induced spots (Table 5).

Discussion

The non-mutagenic and recombinogenic effect of artichoke BHE and LE was demonstrated in the wing SMART assay in a standard cross of D. melanogaster (basal metabolism) and in a high bioactivation cross (HB). The metabolic differences between the two crosses reflect variation in their cytochrome P450 (CYP450) levels. The ORR-flare strain has chromosomes 1 and 2 from a DDT-resistant Oregon R(R) line, that contribute to high levels of CYP450. In particular, the CYP6A2 level is increased, primarily as a result of a mutation of the CYP450 regulatory gene Rst(2)DDT. Our data indicate the absence of direct and indirect BHE- and LE-mediated mutagenic and recombinogenic activities. Only one report in the literature has examined the genotoxicity of C. scolymus L. and found that leaf and flower extracts did not induce chromosomal mutation in peripheral blood and bone narrow cells, as assessed by the micronucleus test; these extracts were also not genotoxic in the Comet assay, except at the highest concentration of leaf extract (2000 mg/kg) (Zan MA, 2008, MSc dissertation, Universidade Luterana do Brasil, Porto Alegre, Brazil). Cynara cardunculus is also not mutagenic in the Ames test and Saccharomyces cerevisiae assay, and not clastogenic in Vicia sativa (Miadokova et al., 2008).

The usefulness of SMART for studying antigenotoxic effects is reinforced by the finding that some modulators that decrease the incidence of mutational effects are equally able to increase the occurrence of somatic recombination. This means that modulating agents should be evaluated not only in terms of their action on mutagenic events (point and chromosomal mutations), but also in relation to their effects on somatic recombination. Because trans-heterozygous flies express all of these genetic endpoints, SMART offers an additional advantage over other assays in that it allows one to establish the pharmacological behavior of modulating agents, as described earlier (Santos et al., 1999; Sinigaglia et al., 2004, 2006).

In the co-treatment protocols, neither BHE nor LE modified the frequencies of MMC- or BLM-mutant spots, indicating that neither extract interfered with the steps that precede the DNA-induced lesions, such as antioxidant activity, the suppression of metabolic activation and the stimulation of detoxification via the induction of glutathione S-transferase (Aboobaker et al., 1994; Morse et al., 1995).

Since BLM and MMC can induce oxidative damage (Cederberg and Ramel, 1989; Povirk and Austin, 1991; Tomasz, 1995), we may infer that the mixture represented for LE and BHE had no scavenger activity to prevent drug-induced oxidative damage. Phytochemical analyses
Table 5 - Summary of results obtained in the *D. melanogaster* wing spot test. Acute exposure to MMC (6 h), BLM and EMS (4 h) followed by post-treatment with leaf extracts (LE) from *Cynara scolymus* L., 3-day-old standard (ST) cross larvae: marker-trans-heterozygous (mwh/flr<sup>3</sup>) and balancer-heterozygous (mwh/TM3) flies.

| Genotypes | Controls and compounds MUT<sup>b</sup> + LE (g/mL) | No. of flies (N) | Spots per fly (no. of spots)/statistical diagnosis<sup>a</sup> | Total mwh clones<sup>e</sup> (n) | Mean mwh clone size class<sup>d</sup> | Total mwh clone size (per 10<sup>5</sup> cells per cell division)<sup>f</sup> (n/NC)<sup>g</sup> | Enhancement<sup>h</sup> (%) |
|-----------|-----------------------------------------------|-----------------|--------------------------------------------------|------------------|------------------|-------------------------------|-----------------|
| MMC       |                                              |                 | Small single spots<sup>c</sup> (1-2 cells) (m = 2) | Large single spots<sup>c</sup> (> 2 cells) (m = 5) | Twin spots<sup>c</sup> (m = 5) | Total spots<sup>c</sup> (m = 2) |                      |                 |
| mwh/flr<sup>3</sup> |                                              |                 | 0.75 (30)                                        | 0.08 (03)        | 0.08 (03)        | 0.91 (36)                     | 36               | 1.69            | 1.84            |
| NC<sup>i</sup> |                                              | 40              | 1.78 (71)*                                       | 7.78 (311)*      | 2.63 (105)*      | 12.18 (487)*                  | 440             | 4.73            | 22.54 [20.70]   |
| MMC       |                                              | 40              | 1.50 (60)                                        | 8.05 (322)*      | 3.05 (122)*      | 12.60 (504)                   | 468             | 4.61            | 23.98 [22.13]   |
| MMC + 0.0435 |                                              | 40              | 1.28 (51)                                        | 8.80 (352)*      | 3.38 (135)*      | 13.45 (538)                   | 504             | 4.69            | 25.82 [23.98]   |
| MMC + 0.0875 |                                              | 40              | 1.87 (56)                                        | 0.17 (05)        | 0.03 (01)        | 2.07 (62)                     | 62              | 1.68            | 4.23 [2.46]     |
| BLM       |                                              |                 |                                                  |                  |                  |                               |                 |                 |                 |
| mwh/flr<sup>3</sup> |                                              |                 | 0.70 (21)                                        | 0.10 (03)        | 0.07 (02)        | 0.87 (26)                     | 26              | 1.96            | 1.78            |
| NC<sup>i</sup> |                                              | 30              | 1.74 (52)*                                       | 0.23 (07)        | 0.03 (01)        | 2.00 (60)*                    | 58              | 1.67            | 3.96 [2.19]     |
| BLM       |                                              | 30              | 1.87 (56)                                        | 0.17 (05)        | 0.03 (01)        | 2.07 (62)                     | 62              | 1.68            | 4.23 [2.46]     |
| BLM + 0.0435 |                                              | 30              | 1.83 (55)                                        | 0.20 (06)        | 0.00 (00)        | 2.03 (61)                     | 61              | 1.80            | 4.17 [2.39]     |
| BLM + 0.0875 |                                              | 30              | 1.90 (57)*                                       | 0.83 (25)*       | 2.73 (82)*       | 5.60 (464)                    | 82              | 2.06            | 5.60 [4.64]     |
| EMS       |                                              |                 |                                                  |                  |                  |                               |                 |                 |                 |
| mwh/flr<sup>3</sup> |                                              |                 | 0.63 (19)                                        | 0.03 (01)        | 0.00 (0)         | 0.66 (20)                     | 20              | 1.90            | 1.37            |
| NC<sup>i</sup> |                                              | 30              | 3.30 (99)*                                       | 3.17 (95)*       | 2.40 (72)*       | 8.87 (266)*                   | 224             | 2.79            | 15.30 [13.93]   |
| EMS       |                                              | 30              | 4.57 (137)                                       | 3.80 (114)       | 2.37 (71)        | 10.74 (322)                   | 277             | 2.62            | 18.92 [17.55]   |
| EMS + 0.0435 |                                              | 30              | 7.73 (232)                                       | 6.67 (200)       | 5.50 (165)       | 19.90 (597)                   | 492             | 2.69            | 33.61 [32.24]   |
| EMS + 0.0875 |                                              | 30              | 4.73 (14)                                        | 0.00 (0)         | 0.47 (14)        | 0.47 (14)                     | 14              | 1.29            | 0.96            |
| mwh/TM3   |                                              |                 |                                                  |                  |                  |                               |                 |                 |                 |
| mwh/flr<sup>3</sup> |                                              |                 | 0.47 (14)                                        | 0.00 (0)         | 0.47 (14)        | 0.47 (14)                     | 14              | 1.29            | 0.96            |
| NC<sup>i</sup> |                                              | 30              | 1.90 (57)*                                       | 0.83 (25)*       | 2.73 (82)*       | 5.60 (464)                    | 82              | 2.06            | 5.60 [4.64]     |
| EMS       |                                              | 30              | 2.40 (72)                                        | 1.00 (30)        | 3.40 (102)       | 102                           | 102             | 2.11            | 6.97 [6.01]     |

<sup>a</sup>Statistical diagnoses according to Frei and Würgler (1988, 1995). Two-tailed U-test: *, positive; p ≤ 0.05 vs. untreated control; +, positive and -, negative, p ≤ 0.05 vs. MMC, BLM or EMS alone; m: minimal risk multiplication factor for the assessment of negative results; significance levels α = β = 0.05; Mutagen: MUT; <sup>b</sup>Including rare flr<sup>3</sup> single spots; <sup>c</sup>Considering mwh clones from mwh single and twin spots; Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls; <sup>d</sup>For calculation see Andrade *et al.* (2004); <sup>e</sup>C = 48,800, i.e., approximate number of cells examined per fly; <sup>f</sup>Calculated according to Abraham (1994) using the control corrected clone induction frequencies: (MUT alone - MUT plus LE / MUT alone) x 100; <sup>g</sup>Negative control: NC; <sup>h</sup>Only mwh single spots were observed in mwh/TM3 heterozygotes as the balancer chromosome TM3 does not carry the flr<sup>3</sup> mutation.
Table 6 - Summary of results obtained in the *D. melanogaster* wing spot test. Acute exposure to MMC (6 h), BLM and EMS (4 h) followed by post-treatment with bloom heads extracts (BHE) from *Cynara scolymus* L., 3-day-old standard (ST) cross larvae: marker-trans-heterozygous (*mwh/flr*).

| Genotypes | Controls and compounds MUT<sup>b</sup> + BHE(g/mL) | No. of flies (N) | Spots per fly (no. of spots)/statistical diagnosis<sup>a</sup> | Total mwh clones<sup>d</sup> (n) | Mean mwh clone size class<sup>d</sup> | Clone induction frequencies (per 10<sup>5</sup> cells per cell division)<sup>e,f</sup> (n/NC)<sup>i</sup> |
|-----------|-------------------------------------------------|-----------------|-------------------------------------------------|-----------------|-----------------|-------------------------------------------------|
| mwh / flr<sup>3</sup> | NC<sup>i</sup> | 50 | 0.72 (36) | 0.10 (05) | 0.10 (05) | 0.92 (46) | 46 | 1.86 | 1.89 |
| MMC | 50 | 2.40 (120)<sup>*</sup> | 8.12 (406)<sup>*</sup> | 2.62 (131)<sup>*</sup> | 13.14 (657)<sup>*</sup> | 604 | 4.52 | 24.75 [22.87] |
| MMC + 0.0276 | 50 | 1.36 (68) | 8.02 (401) | 3.12 (156) | 12.50 (625) | 592 | 4.90 | 24.26 [22.38] |
| MMC + 0.0552 | 50 | 2.08 (104) | 7.92 (396) | 2.74 (137) | 12.74 (637) | 601 | 4.55 | 24.63 [22.75] |
| BLN | 30 | 0.70 (21) | 0.10 (03) | 0.07 (02) | 0.87 (26) | 26 | 1.96 | 1.78 |
| BLM | 30 | 1.72 (52)<sup>*</sup> | 0.23 (07) | 0.03 (01) | 2.00 (60)<sup>*</sup> | 58 | 1.67 | 3.96 [2.19] |
| BLM + 0.0276 | 30 | 1.36 (41) | 0.23 (07) | 0.03 (01) | 1.63 (49) | 49 | 2.33 | 3.35 [1.57] |
| BLM + 0.0552 | 30 | 2.13 (64) | 0.10 (03) | 0.03 (01) | 2.26 (68) | 68 | 1.68 | 4.64 [2.87] |
| EMS | 30 | 0.63 (19) | 0.03 (01) | 0.00 (0) | 0.66 (20) | 20 | 1.90 | 1.37 |
| EMS | 30 | 3.30 (99)<sup>*</sup> | 3.17 (95)<sup>*</sup> | 2.40 (72)<sup>*</sup> | 8.87 (266)<sup>*</sup> | 224 | 2.79 | 15.30 [13.93] |
| EMS + 0.0276 | 30 | 2.87 (86) | 3.30 (99) | 1.60 (48) | 7.77 (233) | 207 | 2.89 | 14.14 [12.77] |
| EMS + 0.0552 | 30 | 4.00 (120) | 4.37 (131) | 2.40 (72) | 10.77 (323) | 268 | 2.75 | 18.31 [16.94] |

<sup>a</sup>Statistical diagnoses according to Frei and Würgler (1988, 1995). Two-tailed U-test: *, positive; p ≤ 0.05 vs. untreated control; +, positive and -, negative, p ≤ 0.05 vs. MMC, BLM or EMS alone; m: minimal risk multiplication factor for the assessment of negative results; significance levels α = β = 0.05; <sup>b</sup>Mutagen: MUT; <sup>c</sup>Including rare *flr* single spots; <sup>d</sup>Considering mwh clones from mwh single and twin spots; Numbers in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls; <sup>e</sup>For calculation see Andrade et al. (2004); <sup>f</sup>C = 48.800, i.e., approximate number of cells examined per fly; <sup>i</sup>Calculated according to Abraham (1994) using the control corrected clone induction frequencies: (MUT alone - MUT plus BHE / MUT alone) x 100; <sup>j</sup>Negative control: NC.
of both extracts have identified flavonoids, such as chlorogenic acid, that act as antioxidants and pro-oxidants (Cao et al., 1997). However, Sotirbán et al. (2011) demonstrated that flavonoids, including chlorogenic acid, are unable to induce oxidative stress in D. melanogaster nor protect DNA against paraquat-induced oxidative stress lesions. A similar behavior was also observed in the post-treatment protocol. Conversely, in the co-treatment experiments, both concentrations of BHE significantly increased the frequency of mutant clones in response to EMS in the trans-heterozygous genotype. In TM3 balancer-heterozygous flies there were significant increases in the total number of spots indicative of co-recombinogenic and/or co-mutagenic activities. In this genotype, spots originate exclusively by mutational events since recombination produced unviable configurations (because of multiple-inversions in the heterozygous state of TM3 balancer chromosomes) (Graf et al., 1984).

The increase in spots seen in balancer-heterozygous individuals indicated that co-treatment with BHE affected both endpoints, which were more related to EMS-mutagenic effects on EMS-mediated genotoxicity was restricted to so-


tion (HR), involved in their correction. It is unclear why LE modulates the genotoxicity of EMS (which is preferen-
tially associated with damage caused by N-alkylation dam-
age) and O6-ethyldoxyguanosine.

Our results indicate that the modulatory action of both extracts was quite different since the synergistic ef-

fect against EMS in trans-heterozygous flies (~ 131% in-
crease), but not in TM3 flies. These findings indicate that the synergistic recombinogenic activity of the LE extract was related to the type of lesions induced and, conse-

quently, to the repair processes, e.g., homologous recombi-
nation (HR), involved in their correction. It is unclear why LE modulates the genotoxicity of EMS (which is preferen-
tially associated with damage caused by N-alkylation dam-
age) and O6-ethyldoxyguanosine.

There are no reports on the modulatory effect of Cynara extracts against MMC and BLM. Extract of C. cardunculus (ECC) showed a specific protective effect on yeast cells undergoing mutagenic and convertogenic changes induced by 4-nitroquinoline-N-oxide, and also re-
duced the anticlastogenic effect of N-nitroso-N-methylurea in Vicia sativa in co-treatment experiments. However, this extract significantly increased the mutagenic effect of 2-aminofluorene in Salmonella typhimurium TA98 (Mia-
dokova et al., 2008). This finding correlates well with the results of Ogawa et al. (1987), who observed a flavo-

loid-mediated increase in the mutagenicity of 2-acetyl-
aminofluorene (2-AAF) in the presence of rat liver micro-
somes. On the other hand, ECC reduced the genotoxicity of EMS in the sex-linked recessive lethal mutation (SLRL) in D. melanogaster via the inactivation of EMS (Miadokova et al., 2006).

LE and BHE contained flavonoids, phenolic compounds and saponins. The major flavonoids present in arti-

choke florescences include narirutin (Wang et al., 2003), apigenin (Zhu et al., 2004) and cyanidin (Schutz et al., 2006), whereas the main constituents of leaves are luteolin and luteolin glycosides (Noldin et al., 2003, Wang et al., 2003). In addition to flavonoids, the phenolic acids de-
scribed as leaf constituents include cafféic acid and ferulic acid (Noldin et al., 2003), cynarin and chlorogenic acid (Speroni et al., 2003), also present in florescences. Al-

though apigenin and luteolin have antimutagenic activity (Birt et al., 1986; Czeczot et al., 1990; Duthie et al., 2000; Romanova et al., 2001) these compounds are also muta-

genic and clastogenic in a variety of eukaryotes and in vivo systems (Ogawa et al., 1987; Snyder and Gillies, 2002).

Based on the findings reported here, we suggest that each extract contains a unique complex mixture that can in-

crease the frequency of genotoxic events induced by EMS. The increase in EMS-mediated recombination must be asso-


ciated with different mechanisms, including interference in the steps that precede EMS-induced genotoxicity and in the mechanisms involved in correcting EMS-specific dam-

age.

Homologous somatic recombination may result in a loss of heterozygosity or genetic rearrangements, and these events are involved in the genesis of numerous diseases, in-

cluding cancer (Bishop and Schiestl, 2003). It would be in-

teresting to determine which components in the extracts are responsible for the synergistic effects on EMS genotoxicity and their interference on other genotoxic agents.

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References

Aboobaker VS, Balgi AD and Bhattacharya RK (1994) In vivo effect of dietary factors on the molecular action of aflatoxin B1: Role of non-nutrient phenolic compounds on the catala-

ylic activity of liver fractions. In Vivo 8:1095-1098.

Abraham SK (1994) Antigenotoxicity of coffee in the Drosophila assay for somatic mutation and recombination. Mutagenesis 9:383-438.

Adzet T, Camarasa J and Laguna JC (1987) Hepatoprotective ac-

tivity of polyphenolic compounds from Cynara scolymus L. against CCl4 toxicity in isolated rat hepatocytes. J Nat Prod 50:612-617.

Andrade HHR, Reguly ML and Lehmann M (2004) Wing So-

matic Mutation and Recombination Test (SMART). In: Henderson DS (ed) Drosophila Cytogenetics Protocols. Hu-

mana Press, Totowa, pp 389-412.

Birt DF, Walker B, Tibbel MG and Bresnick E (1986) Anti-

mutagenesis and anti-promotion by apigenin, robenitin and indole-3-carbinol. Carcinogenesis 7:959-963.
Bishop AJ and Schiestl RH (2003) Role of homologous recombination in carcinogenesis. Exp Mol Pathol 74:94-105.

Cao G, Sofic E and Prior RL (1997) Antioxidant and prooxidant behavior of flavonoids: Structure-activity relationship. Free Radic Biol Med 22:749-760.

Cederberg H and Ramel C (1989) Modifications of the effect of bleomycin in the somatic mutation and recombination test in *Drosophila melanogaster*. Mutat Res 214:69-80.

Czeczot H, Tudek B, Kusztelak J, Szymczyk T, Dobrowolska B, Glinkowska G, Malinowski J and Strzelecka H (1990) Isolation and studies of mutagenic activity in the Ames test of flavonoids naturally occurring in medical herbs. Mutat Res 240:209-216.

Duthie GG, Duthie SJ and Kyle JA (2000) Plant polyphenols in cancer and heart disease: Implications as nutritional antioxidants. Nutr Res Rev 13:79-106.

Frei H and Würgler FE (1988) Statistical methods to decide whether mutagenicity test data from *Drosophila* assays indicate a positive, negative, or inconclusive result. Mutat Res 203:297-308.

Frei H and Würgler FE (1995) Optimal experimental design and sample size for the statistical evaluation of data from somatic mutation and recombination tests (SMART) in *Drosophila*. Mutat Res 334:247-258.

Frei H, Clements J, Howe D and Würgler F (1992) The genotoxicity of the anti-cancer drug mitoxantrone in somatic and germ cells of *Drosophila melanogaster*. Mutat Res 279:21-33.

Gebhardt R (1998) Inhibition of cholesterol biosynthesis in primary cultured rat hepatocytes by artichoke (*Cynara scolymus* L.) extracts. J Pharmacoexp Ther 286:1122-1128.

Gebhardt R (2002) Inhibition of cholesterol biosynthesis in HepG2 cells by extract artichoke is reinforced by glucosidase pretreatment. Phytother Res 16:368-372.

Gebhardt R and Fausel M (1997) Antioxidant and hepatoprotective effects of artichoke extracts and constituents in cultured rat hepatocytes. Toxicol In Vitro 11:669-672.

Graf U and van Schaik N (1992) Improved high bioactivation cross for the wing somatic mutation and recombination test in *Drosophila melanogaster*. Mutat Res 271:59-67.

Graf U, Würgler FE, Katz AJ, Frei H, Juon H, Hall CB and Kale PG (1984) Somatic mutation and recombination test in *Drosophila melanogaster*. Environ Mutagen 6:153-188.

Harbome J (1998) Phytochemical Methods. 3rd edition. Chapman & Hall, London, 302 pp.

Jacociunas LV, de Andrade HH, Lehmann M, de Abreu BR, Ferraz Ade B, da Silva J and Dihl RR (2012) Artichoke induces genetic toxicity and decreases ethyl methanesulfonate-related DNA damage in Chinese hamster ovary cells. J Med Food 15:873-878.

Jacociunas LV, de Andrade HH, Lehmann M, de Abreu BR, Ferraz Ade B, da Silva J, Grivichic I and Dihl RR (2013) Artichoke induces genetic toxicity in the cytokinesis-block micronucleus (CBMN) cytome assay. Food Chem Toxicol 55:56-59.

Kastenbaum MA and Bowman KO (1970) Tables for determining the statistical significance of mutation frequencies. Mutat Res 9:527-549.
ment and mode of action. Bioorg Med Chem Lett 13:223-228.

Simões CM, Falkenberg MB and Santos R (2007) Introdução à análise fitoquímica. In: Simões CMO, Schenkel EP, Gossmann G, Mell GCP, Mentz LA and Petrovick PR. Farmacognosia: Da Planta ao Medicamento. UFSC/UFRGS, Florianópolis/Porto Alegre, pp 229-245.

Sinigaglia M, Reguly ML and Andrade HHR (2004) Effect of vanillin on toxicant-induced mutation and somatic recombination in proliferating somatic cells of Drosophila melanogaster. Environ Mol Mutagen 44:394-400.

Sinigaglia M, Lehmann M, Baumgardt P, Amaral VS, Dihl RR, Reguly ML and Andrade HHR (2006) Vanillin as a modulator agent in SMART test: Inhibition in the steps that precede N-methyl-N-nitrosurea-, N-ethyl-N-nitrosurea-, ethylmethanesulphonate- and bleomycin-genotoxicity. Mutat Res 607:225-230.

Snyder RD and Gillies PJ (2002) Evaluation of the clastogenic, DNA intercalative, and topoisomerase II-interactive properties of bioflavonoids in Chinese hamster V79 cells. Environ Mol Mutagen 40:266-276.

Sotibrán ANC, Ordaz-Téllez MG and Rodríguez-Arnaiz R (2011) Flavonoids and oxidative stress in Drosophila melanogaster. Mutat Res 726:60-65.

Speroni E, Cervellati R, Govoni P, Guizzardi S, Renzulli C and Guerra MC (2003) Efficacy of different Cynara scolymus preparations on liver complaints. J Ethnopharm 86:203-211.

Tomasz M (1995) Mitomycin C: Small, fast and deadly (but very selective). Chem Biol 2:575-579.

Wagner H and Bladt S (1996) Plant Drug Analysis: A Thin Layer Chromatography Atlas. 2nd edition. Springer-Verlag, Berlin, 368 pp.

Wang M, Simon J, Aviles I, He K, Zheng Q and Tadmor Y (2003) Analysis of antioxidative phenolic compounds in artichoke (Cynara scolymus L.). J Agric Food Chem 51:601-608.

Zapolska-Downar D, Zapolski-Downar A, Naruszewicz M, Sienicka A, Krasnodebska B and Koldziej B (2002) Protective properties of artichoke (Cynara scolymus) against oxidative stress induced in cultured endothelial cells and monocytes. Life Sci 71:2897-2908.

Zhu X, Zhang H and Lo R (2004) Phenolic compounds from the leaf extract of artichoke (Cynara scolymus L.) and their antimicrobial activities. J Agric Food Chem 24:7272-7278.

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