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ScienceDirect
Food and Chemical Toxicology 46 (2008) 1103–1110
www.elsevier.com/locate/foodchemtox

Inhibitory effects of water extract of propolis on doxorubicin-induced somatic mutation and recombination in *Drosophila melanogaster*

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Received 16 May 2007; accepted 7 November 2007

Abstract

Propolis is a substance produced by honeybees (*Apis mellifera* L.). Its components are strong antioxidants and free radical scavengers. The aim of this study was to evaluate the protective effects of a water extract of Brazilian green propolis (WEP) combined with the antitumor agent doxorubicin (DXR) on *Drosophila melanogaster* wing cells through the somatic mutation and recombination test (SMART). Two different crosses were used: The standard (ST) cross and the high bioactivation (HB) cross. The HB cross is characterized by a constitutively enhanced level of cytochrome P450 which leads to an increased sensitivity to a number of promutagens and procarcinogens. Larvae obtained from these two crosses were chronically treated with different concentrations of WEP (12.5, 25.0 and 50.0 mg/mL) alone or combined with DXR (0.125 mg/mL). The results obtained with the two different crosses were rather similar. Neither toxicity nor genotoxicity were observed in WEP treated series. Simultaneous treatment with different concentrations of WEP and DXR led to a reduction in the frequency of recombination compared to the treatment with DXR alone. This anti-recombinogenic effect was proportional to the concentrations applied, indicating a dose–response correlation and can be attributed to the powerful scavenger ability of WEP.

Keywords: Antigenotoxicity; Genotoxicity; SMART; Wing spot test; *Drosophila*

1. Introduction

Propolis (bee glue), is the generic name for the sticky substance produced by honeybees (*Apis mellifera* L.) by mixing their own waxes with resinous substances collected from exudates and leaf buds of various plant sources. Bees use propolis for coating hive parts and the cell interiors of the honeycomb, as sealant of cracks and crevices in the hive, as sterilant in honeybee nests, to exclude draught, protect against external invaders and mummify their carcasses

Abbreviations: 1-NP, 1-nitropyrene; 2AF, 2-aminofluorene; 3,4,5-triCQA, 3,4,5-tri-*O*-caffeoylquinic acid; 3,4-diCQA, 3,4-di-*O*-caffeoylquinic acid; 3,5-diCQA, 3,5-di-*O*-caffeoylquinic acid; 4,5-diCQA, 4,5-di-*O*-caffeoylquinic acid; 4-NO, 4-nitro-*O*-phenylenediamine; 4-NQO, 4-nitroquinoline-*N*-oxide; B[a]P, benzo(*a*)pyrene; CA, caffeic acid; CAS, chemical abstract service; ChA, chlorogenic acid; CHO, Chinese hamster ovary; CP, cyclophosphamide; *D. melanogaster*, *Drosophila melanogaster*; DMC, daunomycin; DNA, deoxyribonucleic acid; DXR, doxorubicin; ECD, 7-ethoxycoumarin-*O*-deethylase; EEP, ethanolic extracts of propolis; EROD, 7-ethoxyresorufin-*O*-deethylase; *flr*, flare; F_M, mutation frequencies; F_R, recombination frequencies; F_T, frequencies of total spots; GSH, glutathione; HB, high bioactivation cross; IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; MMC, mitomycin; MN, micronucleus; *mwh*, multiple wing hairs; NADP, nicotinamide adenine dinucleotide phosphate; NPD, 4-nitro-*O*- phenylenediamine; RNA, ribonucleic acid; *S.cerevisiae*, *Saccharomyces cerevisiae*; SMART, somatic mutation and recombination test; ST, standard cross; WEP, water extract of propolis.

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0278-6915/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.fct.2007.11.005

(Ghisalberti et al., 1978; Ghisalberti, 1979; Pietta et al., 2002; Matsui et al., 2004; Nakajima et al., 2007). Propolis has been used as a folk medicine against inflammation, heart diseases, diabetes and cancer (Matsushige et al., 1996).

Propolis is chemically a complex mixture. Generally it is composed of 50% resin (flavonoids and related phenolic acids), 30% wax, 10% essential oils, 5% pollen and 5% various organic compounds (Pietta et al., 2002). The composition of propolis depends on various factors such as season, vegetation of collection area and kind of extraction used (water or ethanol) (Park and Ikegaki, 1998; Midorikawa et al., 2001; Park et al., 2002, 2004; Volpi and Bergonzini, 2006; Sforcin, 2007).

Propolis and its constituents, have been reported to exhibit a wide range of biological activities, including antioxidant (Nakajima et al., 2007), anti-inflammatory (Paulino et al., 2006), antiproliferative (Bestwick and Milne, 2006), antitumoural (Benkovic et al., 2007; Chen et al., 2007; Sforcin, 2007), antimicrobial (Scazzocchio et al., 2006), antihyperglycemic (Matsui et al., 2004), neuroprotective (Nakajima et al., 2007), antimutagenic activities (Varanda et al., 1999; Jeng et al., 2000; Fu et al., 2004; Moreno et al., 2005; Yang et al., 2006) and immunomodulatory (Sforcin, 2007).

The propolis samples are extracted with water to isolate the charged and relatively polar constituents as the phenolic acids and esters such as cinnamic acid and derivatives (*p*-coumaric acid, artepillin C, drupanin, baccharin); caffeoylquinic acid derivatives (dicaffeoylquinic acid; 3-mono-*O*-caffeoylquinic acid (chlorogenic acid, ChA); 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA); 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA); 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) and 3,4,5-tri-*O*-caffeoylquinic acid (3,4,5-triCQA); caffeic acid (CA) and others (Hilhorst et al., 1998; Midorikawa et al., 2001; Yoshimoto et al., 2002; Matsui et al., 2004; Alves de Lima et al., 2005; Salatino et al., 2005; Teixeira et al., 2005; Nakajima et al., 2007; Sforcin, 2007).

Based on physicochemical characteristics, Brazilian propolis had been classified into 12 groups. One of them was identified in southeastern Brazil, at State of São Paulo and Minas Gerais cerrado (savanna) area, and the botanical origin of the propolis was *Baccharis dracunculifolia* resinaceous exudates. This propolis has been extensively used in foods and beverages to improve health and prevent diseases (Park et al., 2004; Salatino et al., 2005).

Doxorubicin (DXR) is a well known antineoplastic agent used in the treatment of acute leukemia, lymphomas and some solid tumors, such as breast, ovarian and endometrial cancers (Minotti et al., 2004). DXR inhibits the activity of the enzyme topoisomerase II, inducing DNA strand breaks and, as consequence, mutations and chromosomal aberrations in tumor and non-tumor cells (Islaih et al., 2005; Resende et al., 2006). Cellular enzymes are capable of converting DXR into free radical metabolites (Benchekroun et al., 1993; Menegola et al., 2001). The decrease of its genotoxicity in non-tumor cells is the aim that has been achieved experimentally by combined treatments of DXR with free radical scavengers, such as antioxidants (Amara-Mokrane et al., 1996; Antunes and Takahashi, 1998; Gentile et al., 1998; Costa and Nepomuceno, 2006; Tavares et al., 2006; Antunes et al., 2007; Fragiorge et al., 2007).

The *Drosophila melanogaster* wing Somatic Mutation And Recombination Test (SMART) is an one-generation test based on the principle that the loss of heterozygosity of suitable recessive markers (*mwh* and *flr*), due to different genotoxic events (i.e., mitotic recombination, mutation and chromosomal aberration), can lead to the formation of mutant clones of cells in the proliferating imaginal discs of larvae that are then expressed as spots on the wings of the adult flies (Graf et al., 1998). This assay has been successfully used to demonstrate the protective effects of different chemical agents on the genotoxicity of DXR (Costa and Nepomuceno, 2006; Fragiorge et al., 2007). The wide use of propolis in folk medicine prompted us to study the genotoxicity of a WEP alone and the antigenotoxic effects of a WEP on DXR-induced somatic mutation, chromosomal damage and recombination by the wing spot test of *D. melanogaster*.

2. Materials and methods

2.1. Chemical Compounds

Doxorubicin (DXR) (Doxina® – Eurofarma Laboratórios Ltda., São Paulo, Brazil – CAS No. 23214-92-8) was obtained from Hospital de Clínicas da Universidade Federal de Uberlândia, Uberlândia (MG) Brazil. Propolis *in natura* (CAS No. 9009-62-5), also referred as "bee glue", produced by honeybees (*Apis mellifera* L.) in the cerrado (savanna) area near Uberlândia (MG) was supplied by Apiário Girassol, Uberlândia (MG). Ultrapure water (18.2 MΩ) was obtained from a MilliQ system (Millipore, Vimodrone, Milan, Italy).

2.2. Water extract of propolis (WEP) preparation

Water extract of Brazilian green propolis (WEP) was prepared according to Matsushige et al. (1996). Crude propolis was treated with ultrapure water and kept at 80 °C for 2 h. The insoluble portion was separated by filtration to obtain the water extract, which was evaporated partially and lyophilized. Different concentrations of WEP (12.5, 25.0 and 50.0 mg/mL) were obtained diluting this lyophilized powder with ultrapure water.

2.3. Markers, strains and crosses

The *D. melanogaster* wing assay was performed employing two genetic markers located on the left arm of chromosome 3: multiple wing hairs (*mwh*, 3–0.3), a homozygously viable recessive mutation that produces multiple trichomes per cell instead of one trichome; and flare^3 (*flr*^{3}, 3–38.8), a recessive mutation that produces malformed wing hairs with the shape of a flare. The mutant alleles of *flr* are recessive zygotic lethals. Nevertheless, homozygous cells in the wing imaginal discs are viable and lead to mutant wing cells. The *flr*^{3} allele is kept over a balancer chromosome carrying multiple inversions and a dominant *S* marker that is a homozygous lethal (*flr*^{3}/*TM3*,*Bd*^{S}: Third Multiple 3, Beaded-Serrate) (Graf et al., 1998).

Three *D. melanogaster* strains were used: (i) the *multiple wing hairs*: *y*; *mwh j*; (ii) the *flare-3*: *flr*^{3}/*In(3LR)TM3, ri p*^{p}*sep l(3)89Aa bx*^{34e}*e Bd*^{S}; and (iii) the *ORR; flare-3: ORR; flr*^{3}/*In(3LR)TM3, ri p*^{p}*sep l(3)89Aa bx*^{34e}*e Bd*^{S}. The *ORR; flare-3* strain carries chromosomes 1 and 2 from a DDT-resistant Oregon R(R) line, characterized by an increased level of cyto-

chromes P450, conferring high sensitivity to promutagens and procarcinogens (Dapkus and Merrel, 1977; Hällström and Blanck, 1985).

Two crosses were carried out: Standard (ST) cross and high bioactivation (HB) cross. For the ST cross, virgin females *flr*^{3}/*In(3LR)TM3, ri p*^{p}*sep l(3)89Aa bx*^{34e}*e Bd*^{S} were mated with *y; mwh j* males (Graf et al., 1989). For the HB cross, virgin females *ORR; flr*^{3}/*In(3LR)TM3, ri p*^{p}*sep l(3)89Aa bx*^{34e}*e Bd*^{S} were mated with *y*; *mwh j* males (Graf and van Schaik, 1992). From both crosses, the following progeny are produced: marker-heterozygous (MH) flies (*mwh*+/+*flr*^{3}) with phenotypically wild-type wings; and balancer-heterozygous (BH) flies (*mwh*+/+*TM3, Bd*^{S}) with phenotypically serrate wings.

2.4. Larval feeding

Eggs were collected from females of the ST and HB crosses over an 8 h breeding period in culture flasks containing a solid agar base (3% w/v) and fermenting yeast supplemented with sucrose. Third instar larvae were collected and transferred to glass vials containing 1.5 g instant mashed potato flakes (Yoki Alimentos S. A., São Bernardo do Campo, SP, Brazil) rehydrated with 5 mL of different concentrations of WEP (12.5; 25.0 and 50.0 mg/mL) alone or combined with DXR (0.125 mg/mL). Negative (ultrapure water) and positive (0.125 mg DXR) controls were included in both experiments. Larvae were allowed to feed on the medium until completion of their larval life (~48 h). The experiments were carried out at 25 ± 1°C and 60–70% relative humidity.

2.5. Analysis of adult flies

The wings of MH flies (stored in 70% ethanol) were mounted on slides in Faure's solution and examined for spots under a compound microscope at 400× magnification. Single spots (*mwh* or *flr*^{3}) can result from different genotoxic events: mitotic recombination, mutation and chromosomal aberration. Twin spots (*mwh* and *flr*^{3}) are produced by mitotic recombination between the proximal marker *flr*^{3} and the centromere of chromosome 3. The wings of BH flies were mounted and analyzed only when a positive response was obtained in the MH progeny. In the wings of BH flies, only *mwh* single spots can be recovered. These spots are due to mutational events because recombination is suppressed in inversion-heterozygous cells with the multiply-inverted *TM3* balancer chromosome (Graf et al., 1984; Guzmán-Rincón and Graf, 1995).

2.6. Data evaluation and statistical analysis

The data were evaluated according to the multiple-decision procedure of Frei and Würgler (1988) to decide whether a result is positive, weakly positive, inconclusive or negative. The frequencies of each type of mutant clone per fly of a treated series were compared pair-wise (i.e., negative control versus WEP; positive control (DXR) alone versus DXR plus WEP) using the conditional binomial test according to Kastenbaum and Bowman (1970), with significance levels set at $\alpha = \beta = 0.05$. Based on clone induction frequencies per 10^5 cells, the recombinogenic activity was calculated as: mutation frequencies (F_M) = frequencies clones BH flies/frequencies clones MH flies; recombination frequencies (F_R) = $1-F_M$. Frequencies of total spots (F_T) = total spots in MH flies (considering *mwh* and *flr*^{3} spots)/No. of flies; mutation = $F_T \times F_M$; recombination = $F_T \times F_R$ (Santos et al., 1999; Sinigaglia et al., 2006). Based on the control-corrected spot frequencies per 10^5 cells, the percentages of WEP inhibition were calculated as: (DXR alone – WEP plus DXR/DXR alone) × 100 (Abraham, 1994).

3. Results

The results of chronic treatment of larvae with different concentrations of WEP alone or combined with one fixed concentration of DXR in the *Drosophila* wing spot assay (SMART) using ST cross flies and HB cross flies are shown, respectively, in Tables 1 and 2. All the samples were tested in two independent experiments. The results were pooled after verifying that there were no significant differences in the responses in the two experiments. Negative (ultrapure water) and positive (DXR) controls were included in each experiment. Wings from the BH progeny were scored whenever positive responses were observed in the MH progeny. For purposes of statistical evaluation, the frequencies of mutant spots per fly of a treated series were compared pair-wise.

No significant differences in the frequency of mutant spots were observed between flies treated with 12.5, 25.0 and 50.0 mg WEP/mL and the negative control in ST cross MH flies (Table 1) and in HB cross MH flies (Table 2).

The positive control, DXR, produced statistically significant induction of all categories of spots in both the ST and HB crosses (Tables 1 and 2).

In the MH flies, simultaneous treatment with different concentrations of WEP and DXR showed a significant inhibitory effect against the frequency of mutant spots compared to the treatment with DXR alone. The inhibition was observed for all DXR-spot categories considered, except for the frequencies of small single spots in the two lowest doses of WEP (12.5 and 25.0 mg/mL) plus DXR in the ST cross MH flies and in the HB cross MH flies. On the whole, these results indicate a dose–response correlation since the lowest WEP concentration was found to be effective and a gradual increase in WEP concentration results in a proportional increase in the inhibition of mutant clone formation (Tables 1 and 2).

Considering the BH flies, in which all recombinogenic events are eliminated, simultaneous treatment with different concentrations of WEP and DXR did not show a significant inhibitory effect against the frequency of mutant spots compared to the treatment with DXR alone, except for the frequencies of all categories of spots in the treated series with 25.0 mg WEP/mL plus DXR and for the frequencies of large single spots in the treated series with 25.0 or 50.0 mg WEP/mL plus DXR in ST cross BH flies. These results indicate the lack of a dose–response correlation since the increase in WEP concentration did not result in a proportional increase in the reduction of mutagenicity (Tables 1 and 2).

Comparisons of the clone frequencies observed in the MH and BH flies of the treated series with DXR alone and DXR plus WEP were done according to Santos et al. (1999) and Sinigaglia et al. (2006) to quantify the mutagenic and recombinogenic potential of the test samples. The results showed that the genotoxicity in MH flies was due to mainly mitotic recombination (Tables 1 and 2). The results obtained show that WEP had anti-recombinogenic rather than antimutagenic activity.

4. Discussion

The concentrations of water extract of Brazilian green propolis (WEP) used in the present investigation were chosen based on a previous investigation, where we used the *D.*

Table 1
Summary of results obtained with the *Drosophila* wing spot test (SMART) in the marker-heterozygous (MH) and balancer-heterozygous (BH) progeny of the standard (ST) cross after chronic treatment of larvae with water extract of propolis (WEP) and doxorubicin (DXR)

Genotypes and treatments		Number of flies (N)	Spots per fly (number of spots) statistical diagnosis[a]				Spots with mwh clone[c] (n)	Frequency of clone formation/10^5 cells per cell division[d] (n/NC)[e,f]	Recombination (%)	Inhibition[g] (%)
DXR (mg/mL)	WEP (mg/mL)		Small single spots (1-2 cells)[b] $m = 2$	Large single spots (>2 cells)[b] $m = 5$	Twin spots $m = 5$	Total spots $m = 2$				
mwh/flr^3										
0	0	30	0.57 (17)	0.07 (02)	0.03 (01)	0.67 (20)	18	1.22	–	–
0	12.5	42	0.48 (20) −	0.10 (04) i	0.02 (01) i	0.60 (25) −	25	1.21 [−0.01]	–	–
0	25.0	28	0.46 (13) −	0.00 (00) i	0.07 (02) i	0.54 (15) −	15	1.09 [−0.13]	–	–
0	50.0	40	0.20 (08) −	0.08 (03) i	0.03 (01) i	0.30 (12) −	12	0.61 [−0.61]	–	–
0.125	0	46	2.11 (97) +	4.11 (189) +	3.57 (164) +	9.78 (450) +	414	18.44 [17.22]	89.6	
0.125	12.5	38	2.79 (106) w +	2.21 (84) +	2.66 (101) w +	7.66 (291) w +	280	15.09 [13.87]	88.3	19.4
0.125	25.0	39	2.08 (81) −	1.95 (76) +	1.79 (70) +	5.82 (227) w +	222	11.66 [10.44]	95.0	39.4
0.125	50.0	38	1.47 (56) w +	0.61 (23) +	0.74 (28) +	2.82 (107) +	104	5.61 [4.39]	68.4	74.5
mwh/TM3										
0	0	40	0.10 (04)	0.00 (00)	[h]	0.10 (04)	04	0.20	–	–
0.125	0	29	0.66 (19) +	0.28 (08) +		0.93 (27) +	27	1.91 [1.71]	–	–
0.125	12.5	30	0.77 (23) i	0.10 (03) i		0.87 (26) −	26	1.77 [1.57]	–	–
0.125	25.0	48	0.25 (12) +	0.04 (02) +		0.29 (14) +	14	0.59 [0.39]	–	–
0.125	50.0	30	0.87 (26) i	0.00 (00) +		0.87 (26) −	26	1.77 [1.57]	–	–

Marker-trans-heterozygous flies (*mwh/flr*^{3}) and balancer-heterozygous flies (*mwh/TM3*) were evaluated.

[a] Statistical diagnoses according to Frei and Würgler (1988): +, positive; w+, weakly positive; −, negative; i, inconclusive; $P < 0.05$.

[b] Including rare *flr*^{3} single spots.

[c] Considering *mwh* clones from *mwh* single and twin spots.

[d] Calculated according to Frei et al. (1992).

[e] Number in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls.

[f] $C = 48{,}800$ (approximate number of cells examined per fly).

[g] Calculated according to Abraham (1994).

[h] Balancer chromosome *TM3* does not carry the *flr*^{3} mutation.

Table 2
Summary of results obtained with the *Drosophila* wing spot test (SMART) in the marker-heterozygous (MH) and balancer-heterozygous (BH) progeny of the high bioactivation (HB) cross after chronic treatment of larvae with water extract of propolis (WEP) and doxorubicin (DXR)

Genotypes and treatments		Number of flies (*N*)	Spots per fly (number of spots) statistical diagnosis[a]				Spots with mwh clone[c] (*n*)	Frequency of clone formation/10^5 cells per cell division[d] (n/NC)[e,f]	Recombination (%)	Inhibition[g] (%)
DXR (mg/mL)	WEP (mg/mL)		Small single spots (1–2 cells)[b] $m = 2$	Large single spots (>2 cells)[b] $m = 5$	Twin spots $m = 5$	Total spots $m = 2$				
mwh/flr^3										
0	0	50	0.74 (37)	0.08 (04)	0.08 (04)	0.90 (45)	45	1.84	–	–
0	12.5	40	0.83 (33) −	0.13 (05) i	0.08 (03) i	1.03 (41) −	41	2.10 [0.26]	–	–
0	25.0	41	0.85 (35) −	0.17 (07) i	0.00 (00) −	1.02 (42) −	41	2.04 [0.20]	–	–
0	50.0	55	0.47 (26) −	0.11 (06) i	0.02 (01) −	0.60 (33) −	33	1.22 [−0.62]	–	–
0.125	0	40	2.25 (90) +	4.00 (160) +	6.33 (253) +	12.58 (503) +	479	24.53 [22.69]	93.3	–
0.125	12.5	32	3.69 (118) +	2.91 (93) w+	2.72 (87) +	9.31 (298) w+	289	18.50 [16.66]	91.1	26.6
0.125	25.0	41	3.54 (145) w+	1.95 (80) +	2.51 (103) +	8.00 (328) w+	322	16.09 [14.25]	89.4	37.2
0.125	50.0	36	1.86 (67) −	0.81 (29) +	0.89 (32) +	3.56 (128) +	125	7.11 [5.27]	75.8	76.8
mwh/TM3										
0	0	40	0.13 (05)	0.00 (00)	[h]	0.13 (05)	5	0.25	–	–
0.125	0	30	0.70 (21) +	0.10 (03) i		0.80 (24) +	24	1.63 [1.38]	–	–
0.125	12.5	31	0.61 (19) i	0.19 (06) i		0.81 (25) −	25	1.65 [1.40]	–	–
0.125	25.0	30	0.80 (24) −	0.03 (01) i		0.83 (25) −	25	1.70 [1.45]	–	–
0.125	50.0	32	0.78 (25) −	0.06 (02) i		0.84 (27) −	27	1.72 [1.47]	–	–

Marker-trans-heterozygous flies (*mwh/flr*^{3}) and balancer-heterozygous flies (*mwh/TM3*) were evaluated.

[a] Statistical diagnoses according to Frei and Würgler (1988): +, positive; w+, weakly positive; −, negative; i, inconclusive; $P < 0.05$.

[b] Including rare *flr*^{3} single spots.

[c] Considering *mwh* clones from *mwh* single and twin spots.

[d] Calculated according to Frei et al. (1992).

[e] Number in square brackets are induction frequencies corrected for spontaneous incidence estimated from negative controls.

[f] $C = 48{,}800$ (approximate number of cells examined per fly).

[g] Calculated according to Abraham (1994).

[h] Balancer chromosome *TM3* does not carry the *flr*^{3} mutation.

melanogaster wing SMART to show that within these concentrations the WEP was neither a toxic nor a genotoxic agent. Nevertheless, further experiments were necessary to reinforce the results obtained (Valadares et al., 2004).

In the present study, the genotoxicity of a WEP was investigated again and the results confirmed the previous observations. We used the wing somatic mutation and recombination test in *D. melanogaster* because it represents a rapid and inexpensive way to evaluate the genotoxic/antigenotoxic activity of single compounds as well as of complex mixtures (Graf et al., 1996) and is most suited to the detection of recombinogenic activity of genotoxic chemicals (Spanó et al., 2001).

Doxorubicin (DXR) was selected in this study because it has shown to be a strong direct-acting genotoxic agent, with mutagenic, aneugenic and clastogenic properties (Buschini et al., 2003; Dhawan et al., 2003) that, on the *Drosophila* wing spot test, was capable of inducing all types of spots on the wings (Frei et al., 1985).

In our present study, DXR treatment gave positive results for all types of spots in the MH progeny. Due to these positive responses, the wings of BH flies were also mounted and analyzed. A comparison of the results obtained from MH and BH flies was used to quantify the mutagenic and recombinogenic potential of the test samples. According to previous observations (Lehmann et al., 2003; Costa and Nepomuceno, 2006; Fragiorge et al., 2007), DXR preferentially induced recombination rather than other genotoxic events.

Homologous recombination is one of the mechanisms that can result in a loss of heterozygosity or genetic rearrangements, which may play a primary role in carcinogenesis, or to be involved in secondary and subsequent steps of carcinogenesis by which recessive oncogenic mutations are revealed (Bishop and Schiestl, 2002).

One of the proposed mechanisms responsible for the antiproliferative and cytotoxic effects of this anthracycline antibiotic is the formation of free radicals (Bachur et al., 1978; Sinha, 1989). The highly active quinone-containing anticancer drugs, such as DXR, augment the flow of electrons from reduced nicotinamide adenine dinucleotide phosphate (NADP) to molecular oxygen as measured by enhanced reduced NADP oxidation and oxygen consumption. This reaction is catalyzed by microsomal protein and produces a free radical intermediate form of the drug. As free radicals, these drugs have the potential to be "site-specific free radicals" that bind to DNA or RNA and either react directly or generate oxygen dependent free radicals such as superoxide radical or hydroxyl radical to cause chromosomal damage associated with their cytotoxic actions (Bachur et al., 1978). The detection of free radical intermediates from quinone-containing antibiotics in biological systems, however, depends upon cellular bioenvironments, e.g. reducing conditions, and the presence and/or absence of activation and detoxification mechanisms (Sinha, 1989). The importance of oxygen production for DXR toxicity was confirmed by Buschini et al. (2003), who observed that the biological effectiveness of DXR on *S. cerevisiae* was strictly dependent on cell-specific physiological/biochemical conditions, such as a functional respiratory chain and levels of cytochrome P450 and glutathione (GSH).

With the aim of reducing the genotoxicity of DXR in non-tumor cells, many studies have suggested the co-administration of the antineoplastic agent DXR and free radical scavengers such as antioxidants, (Amara-Mokrane et al., 1996; Antunes and Takahashi, 1998; Gentile et al., 1998; Costa and Nepomuceno, 2006; Tavares et al., 2006; Antunes et al., 2007; Fragiorge et al., 2007).

In our study, combined co-treatment with different concentrations of WEP plus DXR led to a statistically significant reduction in the frequencies of spots in MH flies in the ST cross and in the HB cross. WEP revealed an inhibitory effect against the frequency of mutant spots induced by DXR due to an anti-recombinogenic activity. The protective effects were proportional to the concentrations applied, indicating a dose–response correlation which can be attributed to a powerful scavenger ability of WEP.

Recently, research on polyphenols such as flavonoids and related phenolic acids has been prompted by their visible beneficial effects on health (i.e. antimutagenic, anticarcinogenic, antiatherogenic effects) (Volpi and Bergonzini, 2006).

The suppression of tumor growth and antimutagenic activity of WEP, and its constituents, against direct and indirect mutagens have been shown in different organisms.

WEP and caffeic acid reduced the growth of transplantable mammary carcinoma of CBA mouse (Orsolic and Basic, 2007). When the effects of an aqueous extract of propolis (AEP) were evaluated on the formation of 1,2-dimethylhydrazine (DMH)-induced aberrant crypt foci (ACF) and DNA damage in the colon of male Wistar rats by the ACF and Comet assays, respectively, AEP showed no statistically significant reduction of ACF either simultaneously with or after DMH treatment. In contrast, AEP given simultaneously with DMH, reduced DNA damage induction in the mid and distal colon. However, high concentration of AEP alone increased DNA damage in the colon (Alves de Lima et al., 2005).

Some caffeoylquinic acid derivatives isolated from WEP, such as 3-mono-*O*-caffeoylquinic acid (chlorogenic acid, ChA), 3,4-di-*O*-caffeoylquinic acid (3,4-diCQA), 3,5-di-*O*-caffeoylquinic acid (3,5-diCQA), 4,5-di-*O*-caffeoylquinic acid (4,5-diCQA) and 3,4,5-tri-*O*-caffeoylquinic acid (3,4,5-triCQA), and caffeic acid (CA) were also isolated from the sweetpotato (*Ipomoea batatas* L.) leaf and effectively inhibited reverse mutations induced by Trp-P-1 in *S. typhimurium* TA 98. The antimutagenicity of these derivatives was 3,4,5-triCQA>3,4-diCQA = 3,5-diCQA = 4,5-diCQA>ChA in this order (Yoshimoto et al., 2002).

In conclusion, under the present experimental conditions, WEP was not toxic and genotoxic in *Drosophila* somatic cells. Furthermore, simultaneous treatment with different concentrations of WEP and DXR revealed an

inhibitory effect against the frequency of mutant spots induced by DXR due to anti-recombinogenic activity of WEP. The protective effects were proportional to the concentrations applied, indicating a dose–response correlation. Nevertheless, based on previous reports, since propolis composition is completely variable, different solvents solubilize and extract different compounds, and qualitative and quantitative variations in the composition of extracts of propolis can result in distinct responses, further experiments must be carried out for a better understanding of its mechanisms of action and chemoprevention.

Acknowledgements

The authors thank the funding agencies Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES); Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), as well as Universidade Federal de Uberlândia (UFU).

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