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Modifying Effect of Propolis on Dimethylhydrazine-Induced DNA Damage But Not Colonic Aberrant Crypt Foci in Rats

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Propolis is a honeybee product with several biological and therapeutic properties, including antimutagenic and anticarcinogenic activities. 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 was administered orally at 0.01%, 0.03%, 0.1%, and 0.3% in the drinking water, which resulted in doses of approximately 12, 34, 108, and 336 mg/kg body weight/day. Animals were also given a single subcutaneous injection of 40 mg/kg DMH and sacrificed 4 hr later for evaluating DNA damage, or 4 doses of 40 mg/kg DMH, administered 2 doses/week for 2 weeks, and sacrificed 12 weeks after the last injection for evaluating ACF development in the distal colon. Administration of AEP either simultaneously with or after the DMH treatment resulted in no statistically significant reduction of ACF. In contrast, 0.01%, 0.03%, and 0.3% AEP, given simultaneously with DMH, reduced DNA damage induction in the mid and distal colon. However, 0.3% AEP alone increased DNA damage in the colon. In conclusion, AEP had no effect on the formation of DMH-induced ACF in rat colon, but it modulated DMH-induced DNA damage in colon cells. Further investigations are recommended in order to establish the conditions under which propolis produces either protective or deleterious effects. Environ. Mol. Mutagen. 45:8–16, 2005.

Key words: aberrant crypt foci; anticarcinogenesis; antimutagenesis; chemoprevention; comet assay; propolis

INTRODUCTION

Dietary components play an important role in the prevention of various human diseases, including cancer [Rogers et al., 1993; Tanaka, 1997]. The use of natural foods or their active components for preventing chronic diseases is based on the traditional medical practices of various ethnic groups and is supported by epidemiological data on dietary habits and disease patterns [Rao et al., 1995]. Propolis is a natural composite balsam that is produced by honeybees from the gum of various plants. Crude extracts of propolis have long been used in folk medicine. Recently, propolis extracts have gained increasing popularity both as a medicine with antibacterial, antiviral, anti-inflammatory, and antioxidant properties, and as a food to improve health and to prevent disease [Scheller et al., 1989; Marcucci, 1995; Burdock, 1998; Banskota et al., 2000]. Chemical analyses have identified at least 200 compounds in propolis, including fatty and phenolic acids and esters, flavonoids, terpenes, aromatic aldehydes,

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alcohols, sesquiterpenes, β-steroids, and naphthalene [Burdock, 1998]. Several studies have suggested that this honeybee product and some of its components have antimutagenic and anticarcinogenic properties [Rao et al., 1992; Frenkel et al., 1993; Kimoto et al., 1998, 2001; Bazo et al., 2002].

Since carcinogenesis is a multistep process, knowledge of the events occurring during each stage can direct approaches for preventing and inhibiting cancer development. Thus, it becomes important to use methods and protocols capable of detecting damage at different stages of carcinogenesis. Aberrant crypt foci (ACF) in the colon were first described by Bird [1987] and defined as morphologic lesions with elevated crypts, thickened epithelia, and altered luminal openings, and that are larger than adjacent normal crypts. The absence of ACF in healthy and untreated animals and their induction by complete colon carcinogens, but not by noncarcinogens, suggest that ACF are early preneoplastic lesions [McLellan and Bird, 1988]. Since these lesions are considered precursors of colon cancer and since various natural compounds that inhibit ACF development also prevent colon cancer in rodents [Tanaka and Mori, 1996], investigators have used this biomarker both to identify colon carcinogens and to identify chemopreventive agents [Kawamori et al., 1995; Tanaka and Mori, 1996; Bazo et al., 2002]. Also, ACF formation is closely related to genotoxicity, and primary DNA damage in colon cells is an important endpoint in colon carcinogenesis. In this context, the Comet assay has advantages for evaluating DNA damage because it is rapid, sensitive, and detects several classes of DNA injury, such as double-strand breaks, single-strand breaks, alkali-labile sites, incomplete repair of abasic sites, and crosslinks [Singh et al., 1988]. Thus, the use of the ACF and Comet assays, along with the proliferating cell nuclear antigen (PCNA) index, may provide information about events in colon carcinogenesis.

In a previous study, we observed that the chemopreventive potential of propolis was confounded by the toxic effects of a hydroalcoholic extract [Bazo et al., 2002]. Therefore, the present study investigated whether an aqueous extract of propolis (AEP) might produce protective effects against colon ACF induced by 1,2-dimethylhydrazine (DMH) in male Wistar rats. Chemoprotective effects were evaluated in the initiation and promotion stages of colon carcinogenesis by using the ACF and Comet assays.

MATERIALS AND METHODS

Animals

Nine-week-old male Wistar rats weighing approximately 300 g were obtained from the State University of Maringá (Paraná, Brazil) and acclimated for a period of 2 weeks before beginning experimentation. The animals were maintained in a room under controlled conditions of temperature (22 ± 2°C), humidity (50% ± 10%), and a 12-hr light/dark cycle, with ad libitum access to a commercial diet (NUVILAB, CR1 from Nuvital, Curitiba, Brazil) and water. The University Ethical Committee approved the protocols used in this study.

Carcinogen Treatments

DMH, a colon carcinogen, was obtained from Tokyo Kasei Kogyo (Tokyo, Japan; DO 741-Lot: FCX01), and dissolved in an EDTA solution (37 mg/100 ml distilled water) just before use. For the ACF assay, a total dose of 160 mg/kg body weight (bw) was divided into four subcutaneous injections of 40 mg/kg bw given 2 doses/week for 2 weeks, as described by Takahashi et al. [1992]. For the Comet assay, a single dose of 40 mg/kg bw was injected.

AEP Preparation

Propolis produced by *Apis mellifera* L. was collected on the Chaves Farm (Itapecerica, State of Minas Gerais, Brazil), where the principal plant source is *Baccharis dracunculifolia* in a typical native forest. A single batch containing 6 kg of crude propolis was stored at −20°C and protected from light. Samples of 100 g crude propolis were ground in a blender, added to 900 ml of water, placed in an amber bottle, and kept at 50°C in a water bath for about 8 hr. Then, the suspension was put in a freezer (−20°C) for 1 hr to reach 20°C, filtered, and left at room temperature. On the next day, the filter residues were added to 600 ml of water and processed as described above. On the third day, the same procedure was carried out using 400 ml of water. The three resulting filtrates were mixed and stored at 4°C, protected from light. These procedures were repeated weekly in order to avoid AEP oxidation. The dry weight of propolis in the final solution (AEP) was 0.012 g/ml. Based on this concentration, propolis was administered daily by drinking water (bottles protected from light) at concentrations of 0.01%, 0.03%, 0.1%, and 0.3% in order to achieve doses of 15, 50, 150, and 450 mg/kg bw, respectively. As previously described [Bankova et al., 1998a], gas chromatography-mass spectrometry (GC-MS) was used to identify the compounds present in the AEP.

Animal Treatments

ACF Assay

Each experimental group consisted of 10 animals fed with basal commercial diet during the entire experiment (15 weeks). The negative control (group 1) and positive control (group 2) were treated with EDTA (0.05 ml/10 g bw) or DMH (40 mg/kg bw), respectively, twice a week for 2 weeks. Groups 3a, 3b, 3c, and 3d received AEP at concentrations of 0.01%, 0.03%, 0.1%, and 0.3%, respectively, simultaneously with the DMH treatment. Groups 4a, 4b, 4c, and 4d received AEP at the same concentrations during the 12 weeks after the last DMH injection. Group 5 received only the highest concentration of propolis (0.3%) for the entire 15 weeks of the assay. Body weight and AEP consumption were measured twice a week during the entire experimental period. All the animals were sacrificed 12 weeks after the last DMH dose by exsanguination after anesthesia with sodium pentobarbital (45 mg/kg i.p.; Fig. 1).

Comet Assay

Groups of 9–25 animals were distributed as follows: negative control (group 6; EDTA, 0.05 ml/10 g bw) and positive control (group 7; 40 mg/kg bw DMH), with groups 8a, 8b, 8c, and 8d receiving 0.01%, 0.03%, 0.1%, or 0.3% AEP, respectively, and groups 9a, 9b, 9c, and 9d treated with the different concentrations of AEP for 7 consecutive days and injected with 40 mg/kg DMH on the last day. The animals were sacrificed on day 7, 4 hr after the treatment with DMH or EDTA (Fig. 2). In preliminary experiments, assays conducted 4, 6, and 8 hr after the

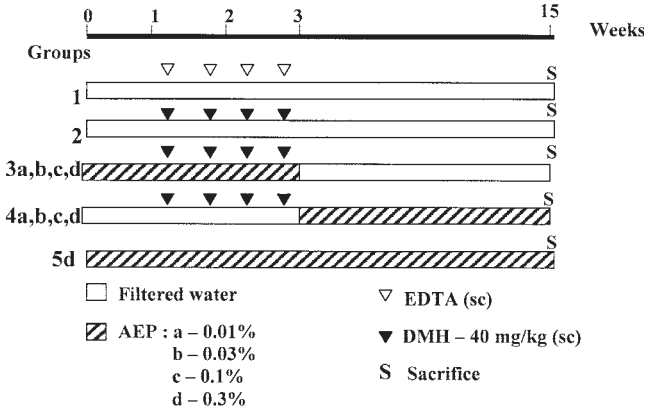

Fig. 1. Experimental protocol for evaluating the effect of propolis on DMH-induced aberrant crypt foci in the rat colon.

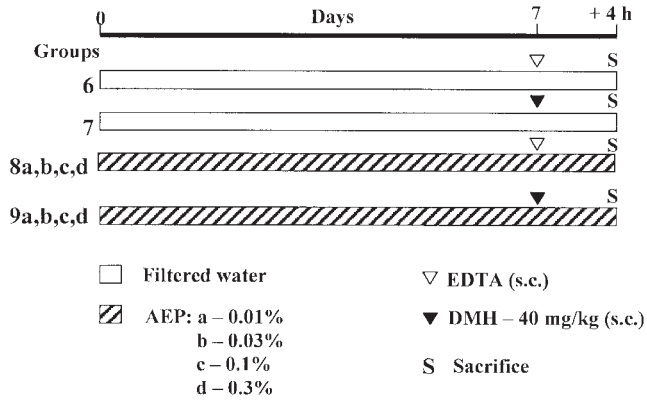

Fig. 2. Experimental protocol for evaluating the effect of an AEP on DMH-induced DNA damage in rat colon cells.

DMH treatment indicated that DNA damage was greatest at the 4-hr time point.

ACF Assay

After laparotomy, the distal colon was excised, flushed with saline, cut open along the longitudinal axis, and fixed in 10% phosphate-buffered formalin (pH 6.9–7.1) for at least 2 days. Just before analysis, the colon was stained with 0.2% methylene blue for 10 min, placed on microscope slides with the mucosal side up, and observed with a light microscope at 200× magnification [Bird, 1987]. Fifty sequential fields of the distal colon were screened for ACF, which were characterized by elongated slit-shaped lumens, surrounded by thickened epithelium that stained more intensely than the surrounding normal crypts (Fig. 3A and B). The number of microscope fields analyzed was established by the progressive mean method as described by Rodrigues et al. [1985]. The number of ACF and the crypt multiplicity (number of crypts in each focus) were recorded. The multiplicity of ACF was expressed as aberrant crypts (AC)/focus.

PCNA Immunostaining and Analysis

The cell proliferation index in colon crypts was scored by using the Proliferating Cell Nuclear Antigen (PCNA) immunostaining technique. Formalin-fixed paraffin-embedded tissue sections (4 μm) were cut and mounted on silanized slides. Paraffin was removed from the embedded sections by three changes of xylene followed by sequential changes of ethanol solutions and water. After removing the paraffin and subsequent hydration, the slides were washed in phosphate-buffered saline (PBS), and the endogenous peroxidase activity was blocked by 3% hydrogen peroxide in PBS for 5 min. Nonspecific protein binding was minimized by using 1% nonfat dried milk in PBS. The slides were incubated for 2 hr at room temperature with the primary monoclonal antibody (PC10, M0879; Dako, Glostrup, Denmark) diluted 1:50 in bovine serum albumin (BSA). The avidin-biotin technique was then performed with matched components (secondary biotinylated antibody and avidin-peroxidase complex) from the Vectasin ABC Kit (Vector Laboratories, Burlingane, CA) for 45 min. Visualization of the labeled cells was achieved by using 3,3′-diaminobenzidine tetrahydrocloride (DAB; Sigma, St. Louis, MO) and 0.025% hydrogen peroxide in 0.1 M Tris-HCl, pH 7.4, for 4 min. All slides were lightly counterstained with hematoxylin, coded, and examined by a single experienced investigator (Fig. 3C). As recommended by Jia and Han [2000], at least 10 vertical U-shaped well-oriented crypts/colon or 750 cells/slide were examined by light microscopy at 400° magnification. PCNA labeling indexes were determined by dividing the number of PCNA-labeled colon cells by the total number of cells scored.

Colon Cell Isolation, Cell Viability, and Comet Assay

After laparotomy, the colon was excised (mid and distal colon), tied at one extremity, and flushed with saline to remove feces. Then, the other extremity was also tied and an enzymatic cocktail (0.3 mg collagenase I + 5 mg trypsin/EDTA) was injected into the colon. Afterward, the colon containing the enzymatic cocktail was placed into Hanks balanced salt solution and kept at 37°C in a water bath for 40 min. One colon extremity was cut off to collect the cell suspension.

Cell viability was determined on portions of the cell suspensions using a dual-dye assay based on a combination of 5–6 carboxyfluorescein diacetate and ethidium bromide [Strauss, 1991]. A 20 μl aliquot of the dye solution was mixed with 20 μl of cell suspension. Under a fluorescent microscope, viable cells that metabolize fluorescein diacetate appear green, whereas the nuclei of dead cells are red. Two hundred cells were counted per animal. Cells were used for the Comet assay only when their viability was $\geq$ 80%.

The alkaline Comet assay was performed according to Singh et al. [1988] and Valverde et al. [1997] under dim indirect light. Briefly, 10 μl of the colon cell suspension containing approximately 3×10^4 cells were mixed with 120 μl of molten 0.5% low-melting-point agarose and layered on a slide precoated with a thin layer of normal-melting-point agarose. The slides were placed into a lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium laurylsarcosine, pH 10; with 1% Triton X-100 and 10% DMSO added just before use) for 24 hr. Then, the slides were washed in PBS and placed into a horizontal electrophoresis unit filled with freshly made alkaline buffer (1 mM EDTA and 300 mM NaOH, pH 13). After a 20-min DNA unwinding period, electrophoresis was carried out in the same buffer at 25 V (0.86 V/cm) and 300 mA for 20 min. Afterward, the slides were neutralized (0.4 M Tris, pH 7.5), fixed with 100% ethanol, and stained with 40 μl of ethidium bromide solution (20 μg/ml H_2O). Images of 50 randomly selected cells from each animal were analyzed at 200× magnification using a fluorescence microscope equipped with an image analysis system (Comet II; Perspective Instruments, Suffolk, U.K.). The parameters scored were tail intensity (% tail DNA) and tail moment (product of the DNA in the tail and the mean distance of migration in the tail).

Statistical Analysis

Mean body weight, diet, and water consumption among the experimental groups were analyzed by one-way ANOVA. The Comet assay

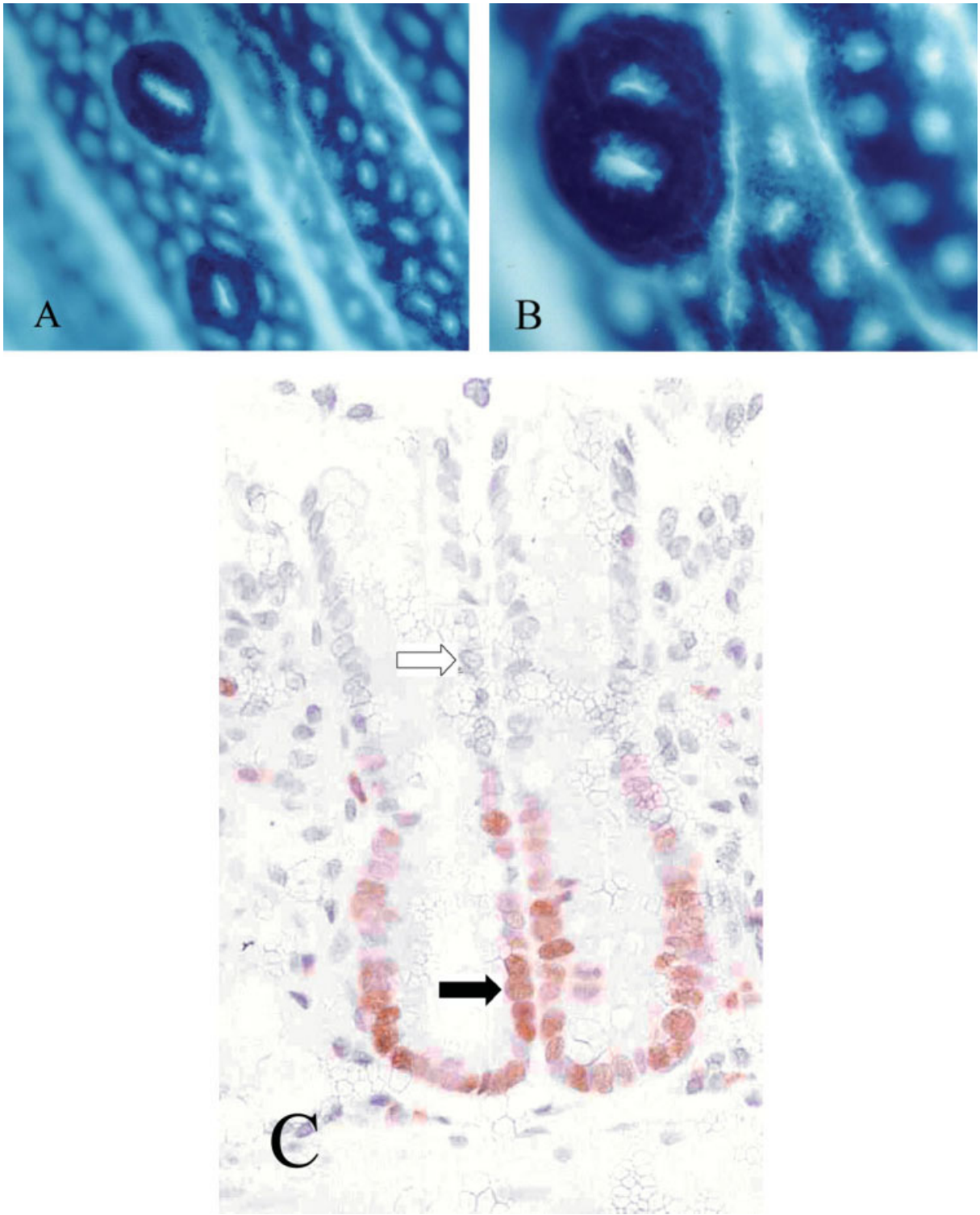

Fig. 3. Photomicrographs (magnification, 400×) of aberrant crypt foci with (**A**) one and (**B**) two aberrant crypts; In (**C**) PCNA-labeled colon cells. Black arrow indicates cryptal proliferating cell ($PCNA^+$); white arrow indicates cryptal nonproliferating cell ($PCNA^-$).

data, ACF, and PCNA labeling index results were analyzed using the Kruskall-Wallis nonparametric test, followed by a post hoc multiple-comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Table I shows the chemical composition of the final AEP (after preparation and storage). The identified substances account for approximately 50% of the components of AEP, the remainder being minor unidentified compounds. The cinnamic acid derivatives, 3,5-diprenyl-*p*-coumaric acid, 3-prenyl-*p*-coumaric acid, and E-*p*-coumaric acid, were found in high concentration.

Table II shows the means of the final body weight, body weight gain, water consumption, and propolis intake during the experimental period. No statistically significant differences were observed among the groups for all these variables. Propolis intake was calculated from its concentration in drinking water, daily water consumption, and the rat body weights. As shown in Table II, groups treated with 0.01%, 0.03%, 0.1%, and 0.3% ingested 12, 34, 108, and 336 mg/kg bw/day of propolis, respectively.

Table III summarizes the data obtained from the ACF assay. No ACF were observed in the negative control and in the group treated with the highest concentration of propolis (data not shown). No statistically significant difference was detected in the numbers of ACF and aberrant crypts (or crypt multiplicity) among the groups receiving AEP simultaneously or as a posttreatment compared to the positive control group that received DMH alone. Nevertheless, a marginally significant trend ($P < 0.08$) for reduction in the number of ACF with propolis dose was observed when propolis was administered during the DMH treatment. No significant difference was observed in the PCNA labeling index between the groups (Fig. 4).

Table IV and Figure 5 show the results obtained in the Comet assay. Significant reductions in the level of DNA damage (tail moment and tail intensity) were detected when 0.01%, 0.03%, and 0.3% AEP were administered simultaneously with DMH (Table IV). However, the highest concentration of propolis (0.3%) by itself induced an increase in DNA damage when compared to the negative control (EDTA; Fig. 5). Cell viability determined prior to the Comet assay was higher than 80% for all the groups, and there were no differences in cell viability between the treatment groups (Table IV).

TABLE I. Chemical Composition of AEP as Determined by GC-MS

Substance	Percentage (%)
Oxopropanoic acid	0.1
Phosphoric acid	0.9
Glyceric acid	0.1
Dihydrocinnamic acid	1.1
Mallic acid	0.1
Hydroxybenzoic acid	0.2
Proline	0.1
Hydroxybutyric acid	0.1
p-hydroxydihydrocinnamic acid	0.3
Z-*p*-coumaric acid	0.2
3,4-dihydroxybenzoic acid	0.1
E-*p*-coumaric acid	3.1
Hexadecanoic acid	0.2
Caffeic acid	1.5
Octadecadienoic acid	0.1
Octadecenoic acid	0.2
Octadecanoic acid	0.1
3-prenyl-*p*-coumaric acid	4.3
9-E-,2-dimethyl-6-carboxyethenyl-8-prenyl-2H-l-benzopyran	0.9
3,5-diprenyl-*p*-coumaric acid	12.4
Hexoses	5.1
Unknown ($M^+ = 562$)	15.6

DISCUSSION

Propolis and its component chemicals, including flavonoids, aromatic acids, and esters, have shown promising results in preventing carcinogenesis [Rao et al., 1993; Bazo et al., 2002]. In 1997, Matsuno et al. [1997] reported that a compound (PRF-1) isolated from propolis was cytotoxic toward human hepatocellular carcinoma. Later, Varanda et al. [1999] showed that propolis inhibited daunomycin-, benzo(a)pyrene-, and aflotoxin B1-induced mutagenicity in the Salmonella/microsome assay. Recently, Kimoto et al. [2000] reported that propolis protected CD-1 and ddY mice from FeNTA (ferric nitrilotriacetate)-induced renal adenocarcinoma. Although therapeutic doses and formulations have yet to be rigorously established, various studies have been carried out using Brazilian propolis because of its wide-ranging biological activities and good results against some pathologies [Grunberger et al., 1988; Matsuno, 1995; Mitamura et al., 1996; Velikova et al., 2000; Banskota et al., 2001; Kimoto et al., 2001; Akao et al., 2003].

Since colorectal cancer is an important cause of death in many countries, we evaluated the effect of the AEP on this neoplasia. The sequence of events that culminates in colon cancer was fundamental for choosing the bioassays and protocols used in this study. The three endpoints, chemical-induced ACF, DNA damage, and cell proliferation, were employed because they can help determine at which step of colon carcinogenesis propolis acts. Our results indicated that propolis modulated the DNA damage detected by the Comet assay, but it did not affect ACF formation or the PCNA labeling index. Since ACF appear to arise from gene mutations, an increased number of ACF may reflect the initiation step of colorectal carcinogenesis, while the progressive increase in the number of crypts per focus (or AC multiplicity) may correspond to the promotion step of colon tumorigenesis [Zhang et al., 1992; Bird, 1995; Fenoglio-Preiser and Noffsinger, 1999;

TABLE II. Mean Body Weight, Body Weight Gain, Water Consumption, and Propolis Intake (mg/kg/day)*

Treatment (n = 10 rats/group)	Body weight (g)	Body weight gain (g)	Water consumption (ml/kg/day)	Propolis intake (mg/kg/day)
EDTA	401.0 ± 46.7	128.5 ± 23.3	44.1 ± 2.7	
AEP 0.3%	388.0 ± 51.0	118.1 ± 17.2	42.4 ± 3.6	328
DMH	382.4 ± 51.4	120.3 ± 29.4	40.8 ± 2.9	
AEP 0.01% + DMH	384.2 ± 51.6	107.5 ± 19.5	45.3 ± 5.5	12
AEP 0.03% + DMH	376.1 ± 47.1	133.1 ± 23.7	40.6 ± 4.3	32
AEP 0.1% + DMH	394.1 ± 58.6	118.1 ± 16.5	44.5 ± 3.8	113
AEP 0.3% + DMH	383.0 ± 52.5	114.8 ± 18.1	45.6 ± 4.0	357
DMH + AEP 0.01%	379.6 ± 45.3	102.6 ± 22.5	40.7 ± 3.0	11
DMH + AEP 0.03%	371.9 ± 43.7	124.7 ± 19.1	43.9 ± 3.4	35
DMH + AEP 0.1%	382.3 ± 50.1	110.5 ± 08.4	40.0 ± 3.3	104
DMH + AEP 0.3%	380.3 ± 43.8	124.5 ± 28.8	41.1 ± 3.0	324

*EDTA, vehicle control; DMH, 1,2-dimethylhydrazine (160 mg/kg), positive control; AEP + DMH, AEP administered simultaneously with DMH during the 2-week dosing period; DMH + AEP, AEP administered for the 12 weeks after DMH treatment. Values are mean ±SD.

TABLE III. ACF in the Distal Colons of Rats Treated With AEP and DMH*

	Number of ACF with						
Treatment	1 crypt	2 crypts	3 crypts	≥ 4 crypts	Number of ACF	Number of AC	AC/ACF
DMH	19.4 ± 6.5	11.9 ± 4.6	5.6 ± 3.8	4.2 ± 1.7	41.1 ± 14.7	78.2 ± 37.3	1.84 ± 0.3
AEP + DMH							
0.01%	11.1 ± 4.5	16.6 ± 8.2	10.4 ± 4.1	8.7 ± 2.6	46.8 ± 17.7	116.2 ± 53.0	2.42 ± 0.4
0.03%	8.7 ± 4.7	12.0 ± 9.5	7.1 ± 5.2	7.2 ± 2.2	35.0 ± 20.2	87.9 ± 62.0	2.38 ± 0.6
0.1%	10.4 ± 5.2	11.8 ± 3.3	7.6 ± 3.5	4.2 ± 1.2	34.0 ± 10.6	76.6 ± 31.8	2.21 ± 0.4
0.3%	10.7 ± 3.6	9.9 ± 5.00	5.2 ± 2.9	2.0 ± 0.7	27.8 ± 8.3[a]	55.5 ± 19.5	1.99 ± 0.4
DMH + AEP							
0.01%	9.2 ± 4.0	11.8 ± 4.0	7.0 ± 3.5	3.7 ± 1.2	31.7 ± 9.9	70.2 ± 27.1	2.17 ± 0.3
0.03%	10.1 ± 3.5	11.6 ± 5.5	6.5 ± 3.8	6.7 ± 1.8	34.9 ± 12.9	83.1 ± 33.6	2.37 ± 0.5
0.1%	11.7 ± 7.5	11.8 ± 5.3	5.7 ± 4.8	5.6 ± 1.6	34.8 ± 16.7	78.7 ± 42.6	2.25 ± 0.6
0.3%	11.7 ± 7.0	10.8 ± 6.8	7.3 ± 6.2	7.9 ± 2.7	37.7 ± 24.6	93.6 ± 84.9	2.25 ± 0.7

*Mean per animal; n = 10 animals/group. DMH, 160 mg/kg; AEP + DMH, AEP administered for 2 weeks simultaneously with the DMH treatment; DMH + AEP, AEP administered during the 12 weeks following DMH treatment; AC, aberrant crypts. Values are mean ± SD.
[a]$P < 0.08$ for trend.

Rodrigues et al., 2002; Hirose et al., 2003]. Therefore, our results indicate that the AEP neither prevented the initiation nor the promotion of DMH-induced colon carcinogenesis. Nevertheless, the results obtained in the Comet assay demonstrated a protective effect of AEP on DMH-induced genotoxicity. Although the Comet assay detects several classes of DNA alterations [Singh et al., 1988], not all DNA damage detected by this technique is converted into mutations involved in carcinogenesis. It is also the case that DNA damage was measured in the mid and distal colon while ACF were scored only in the distal colon.

In a previous study, we observed an inconsistent reduction in AC multiplicity in the distal colon when a hydroalcoholic extract of propolis was administered after DMH, suggesting that the extract might inhibit the proliferation of initiated cells [Bazo et al., 2002]. Qualitative and quantitative variations in the composition of the aqueous and hydroalcoholic propolis extracts could explain these distinct responses. Bazo et al. [2002] used propolis samples collected in the State of São Paulo (Botucatu),

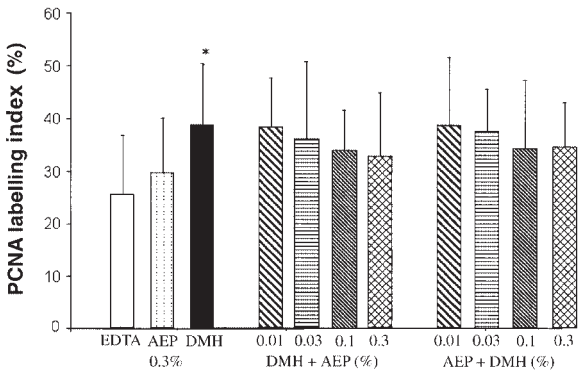

Fig. 4. PCNA labeling index in distal colon of rats treated with AEP and with DMH. Asterisk, $P < 0.05$ (DMH > EDTA).

Brazil, which are rich in phenolic compounds (flavonoids, aromatic acids, benzopyranes), di- and triterpenes, and essential oils, among others [Bankova et al., 1998a, 1998b]. In the present study, we used samples from the State of Minas Gerais (Itapecerica), Brazil, which are rich

TABLE IV. DNA Damage (Tail Moment and Tail Intensity) in Colon Cells of Male Wistar Rats Treated With AEP and DMH*

Treatment	Number of animals[a]	DNA damage		Cell viability (%)
		Tail moment	Tail intensity	
EDTA	25	0.32 ± 0.16	3.59 ± 1.04	87.95 ± 7.36
DMH	25	4.42 ± 1.92[b]	20.49 ± 6.23[b]	86.55 ± 8.23
AEP 0.01% + DMH	16	1.65 ± 1.26[c]	8.85 ± 4.99[c]	86.40 ± 7.23
AEP 0.03% + DMH	9	2.16 ± 1.37[c]	10.78 ± 5.60[c]	86.58 ± 9.33
AEP 0.1% + DMH	10	3.03 ± 1.18	14.02 ± 4.87	86.50 ± 8.36
AEP 0.3% + DMH	15	1.38 ± 0.84[c]	7.89 ± 3.77[c]	89.35 ± 7.94

*EDTA, negative control; DMH, 40 mg/kg, positive control; AEP + DMH, AEP administered simultaneously with DMH over 7 days. Values are mean ± SD.
[a]Some animals were included in a pilot experiment.
[b]$P < 0.01$, statistically different from negative control.
[c]$P < 0.05$, statistically different from negative control.

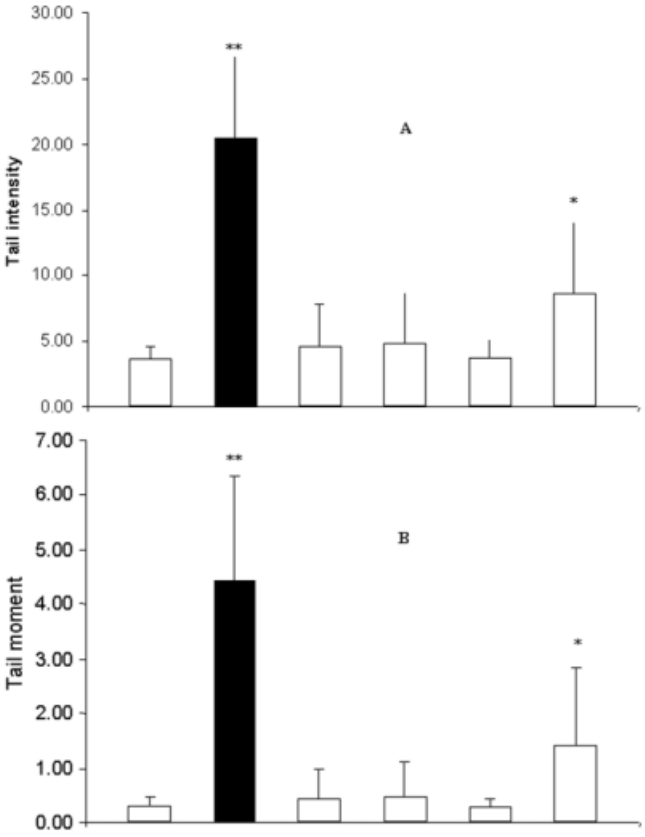

Fig. 5. DNA damage (**A**, tail intensity; **B**, tail moment) evaluated in colonic mucosa cells of rats treated with AEP. DMH, 40 mg/kg bw, positive control; asterisk, $P < 0.05$ (AEP 0.3% > EDTA); double asterisk, $P < 0.01$ (DMH > EDTA).

in cinnamic acid derivatives, more precisely the prenylated p-coumaric acid derivatives, p-coumaric acid and caffeic acid. Distinct antibacterial activities were also observed among Brazilian propolis samples from different geographical regions of the country [Marcucci et al., 2000, 2001; Sawaya et al., 2002; Miorin et al., 2003].

Although the mechanisms involved in chemoprevention by propolis are not understood, interference by one or more propolis components in mutagenic/carcinogenic metabolic pathways, or its putative antioxidant activity [Matsushige et al., 1995; Matsuno et al., 1997], could explain its effects on DMH genotoxicity. Propolis is known to increase the activities of cytochrome P450 and the phase 2 enzymes of rat liver [Siess et al., 1996]. Moreover, chemicals with antioxidant properties have already been found to inhibit DMH- and azoxymethane-induced colon carcinogenesis and DNA damage in animal models [Imaida et al., 1987; Suaeyun et al., 1997; Bazo et al., 2002; Futakuchi et al., 2002]. In the current study, propolis did not inhibit DMH-induced colon genotoxicity in a dose-dependent manner. Concentrations of propolis higher than the low dose of 0.01% did not produce proportionately greater inhibitory effects. Conversely, the highest dose (336 mg/kg) of propolis by itself increased the level of DNA damage in rat colon. According to Ferguson [2001], there is mechanistic evidence indicating that some compounds can both induce and prevent damage. For example, many antioxidants can either accept or donate electrons depending on the redox potential, which may alternatively render them either protective or noxious [Stich and Rosin, 1984; Collins, 2001]. For that reason, prior to establishing a chemoprevention strategy, it is necessary to know under what conditions a compound promotes health and prevents genome damage.

In conclusion, the current study showed that AEP significantly reduced DMH-induced DNA damage in colon cells, but it did not suppress the development of rat ACF in the distal colon. In addition, the results indicate that propolis must be used cautiously, since high doses by themselves can be genotoxic. Further investigations exploiting different target organs and protocols should be conducted in order to establish under what conditions propolis has protective or deleterious activity.

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