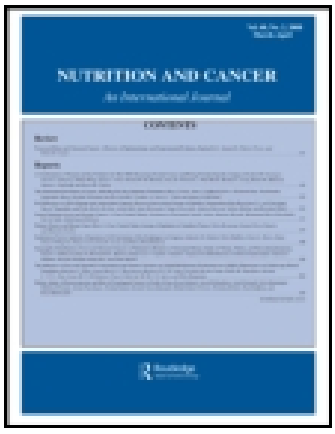

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Reduction in Colon Cancer Risk by Consumption of Kava or Kava Fractions in Carcinogen-Treated Rats

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Reduction in Colon Cancer Risk by Consumption of Kava or Kava Fractions in Carcinogen-Treated Rats

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Epidemiological studies suggest that kava reduces colon cancer risk. However, no experimental studies of the chemopreventive properties of kava toward colon cancer have been reported. Further, there are concerns regarding hepatotoxicity of kava. The goal of this study was to determine whether kava consumption reduces markers of colon cancer in an animal model and to study the safety of kava. An ethanolic extract and polar and nonpolar fractions of the kava extract were fed to rats for 12 days prior to, during, and after administration of dimethylhydrazine, a colon-specific carcinogen. After 14 wk, rats fed the nonpolar extract had a significant reduction in precancerous lesions [aberrant crypt (AC) foci (ACF)] as well as large (≥4 AC/ACF) sialomucin-only expressing foci, an indicator of greater tumorigenic potential, compared to the control group. Groups fed the ethanolic extract and polar kava fraction trended toward reductions in ACF and large sialomucin-only expressing foci. The combined kava groups had significantly fewer total AC, ACF, large ACF, and large sialomucin-only expressing foci compared to the control group. Histological examination found no hepatic lesions in animals consuming the kava diets, suggesting that kava is safe to consume. Our results support that kava may reduce colon cancer risk.

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INTRODUCTION

Colon cancer is the third most common malignancy in the United States and is expected to result in 51,370 deaths in 2010 (1). Numerous epidemiological studies suggest that the cause of colon cancer is primarily environmental, with diet suggested as the primary environmental factor. Consequently, dietary modifications to reduce colon cancer risk are important to identify.

In the search for dietary agents with chemopreventive activity, the very low cancer incidence in certain South Pacific Islands, such as Fiji, Western Samoa, and Vanuatu, is of great interest. For example, the cancer incidence rate in Vanuatu in 1989 was 70.9 per 100,000 in males compared to 307.2 per 100,00 in Los Angeles (2). These differences cannot be accounted for by differences in cancer incidence registration (2), smoking rates (3), dietary components such as dietary fiber, retinol, carotene, or vitamin C, or foods such as carrots, papaya, or tomatoes (4). Kava consumption, however, has been shown to correlate inversely with cancer incidence in these South Pacific Islands, including colon cancer (5). Such a negative correlation suggests that kava may contain chemopreventive constituents.

Mechanistically, kava can inhibit various cytochrome P450 isozymes, including CYP1A2, CYP2C9, and CYP3A4 (6–8). Inhibiting these biotransformation enzymes would prevent the bioactivation of various chemical procarcinogens. Kava is also able to inhibit tumor necrosis factor-α (TNFα)-induced activation of nuclear factor kappaβ (NF-κB) and COX-2 (9–11), which play critical roles in the promotion of carcinogen-induced colorectal tumorigenesis. In fact, inhibiting COX-2 and NF-κB is one mechanism of chemoprevention by various NSAIDs

(12,13). These mechanisms of action suggest that kava contains chemopreventive(s) agent against colon cancer.

Traditional kava is prepared through water or coconut milk extraction of kava root and is a daily beverage in certain South Pacific Islands. Commercial kava is an organic extract of kava root and has been used for anxiety treatment (14,15). It is not currently in use through prescription because of several reported cases of hepatotoxicity, although it is sold as a dietary supplement. This rare hepatotoxicity has been mainly observed among individuals consuming commercial kava, prepared by an ethanol or acetone extraction, as well as 2 individuals consuming traditionally prepared kava (16). Sufficient concern developed regarding the potential for liver injury by kava-containing dietary supplements that the FDA issued a consumer advisory in 2002 (17). However, it has been suggested that this hepatotoxicity is linked to consumption of low-quality kava, not the method of preparation (16). However, given that long-term animal studies of kava have detected either no hepatoxicity (18,19) or hepatoxicity only at high doses of kava (20), that there are very few documented suspected cases of kava hepatotoxicity, and that there is a long history of safe use of traditionally prepared kava, the hepatotoxicity of kava remains an open question.

In this study, we examined whether feeding carcinogen-treated rats a kava extract would reduce morphological markers of colon cancer risk. Furthermore, to understand whether fractions of the kava extract of different polarity differ in their chemopreventive activity, both polar and nonpolar fractions of the kava extract were also fed. Finally, histological examination of the livers was carried out to gauge hepatotoxicity of kava and the kava fractions.

METHODS AND MATERIALS

Chemicals

1,2-dimethylhydrazine was purchased from Aldrich Chemical Co. (Milwaukee, WI). Ethanolic kava extract was purchased from Gaia Herbs (Lot # 083012308; Brevard, NC). Solvents used for column chromatography were used without further purification or distillation, unless otherwise stated.

Animals and Diets

Male Wistar rats, with initial an body weight of 55–75 g, were purchased from Harlan Sprague-Dawley (Indianapolis, IN). Animals were housed individually in wire-bottomed cages in rooms maintained at 20°C with a relative humidity of 50%, and a 12-h light/dark cycle. This study was approved by the University of Minnesota Committee on Animal Care and Use. Food and water were available ad libitum throughout the study. Rats were fed the AIN-93G purified diet (21) (control diet) or the control diet containing 6 g/kg diet of 1 of 3 freeze-dried kava preparations (by weight). The 3 kava preparations were the ethanolic extract and polar and nonpolar fractions isolated from the ethanolic extract, prepared as described below. After 12 days on the diets, one group of rats fed the basal diet (DMH-treated control) and all kava-fed groups were injected subcutaneously with 50 mg of the colon-specific carcinogen 1,2-dimethylhydrazine/kg body weight (dissolved in normal saline) twice, 1 wk apart. A second group of rats fed the basal diet received sham injections of saline (saline-treated control). There were 10 rats in the saline-treated control group and 15 rats in each carcinogen-treated group. All animals continued to be fed their respective diets for another 14 wk after the second injection. Body weight and food intake were measured every other week. At the end of the feeding period, rats were anesthetized with isoflurane and the colons and livers excised. Livers were weighed and processed for histological examination. Colons were processed for aberrant crypt (AC) counting as described below. Colon and liver samples were coded after harvest of tissues to allow unbiased enumeration of colonic AC and histological analysis of liver samples.

Traditional Kava Preparation

Traditional kava was prepared by following the protocol of traditional kava beverage preparation (22). In brief, 10 g kava root (a gift from Gaia Herbs, Inc., Brevard, NC) was ground to a fine powder and mixed with double distilled H_2O (100 mL). The mixture was stirred at room temperature for 30 min and centrifuged at 14,000 $\times$ rpm for 10 min. The aqueous supernatant was collected as traditional kava.

Fractionation of Ethanolic Kava Extract

Polar and nonpolar fractions were prepared by addition of 100 g of silica gel (230–400 mesh, Whatman, Piscataway, NJ) to 100 mL of ethanolic kava extract. The solvent was evaporated in vacuo to afford 135 g of kava adsorbed silica gel. Kava adsorbed silica gel (100 g) was subjected to column chromatography on silica gel (230–400 mesh, Whatman, Piscataway, NJ) (750 g). Elution from the column was initiated with hexane, and the polarity of the solvent increased in a stepwise gradient by the addition of 10% portions of ethyl acetate. Fractions were collected based on thin-layer chromatography visualization by UV light and further staining with p-anisaldehyde solutions followed by heating. The nonpolar fraction contained very minor quantities of desmethoxyyangonin, dihydrokavain, and mainly nonpolar constituents present in ethanolic kava extract. The column was further eluted with 10% ethanol in ethyl acetate, to collect the polar fraction. The nonpolar and polar fractions were analyzed by HPLC on a Beckman Coulter System Gold 126 solvent module with a 168 detector. A Clipeus C-18 column (5 μm, 250 $\times$ 4.6 mm) was used for the analyses. The flow rate used was 0.4 ml/min. The mobile phases were water and acetonitrile. The time program used for the analyses was 55% acetonitrile (0–10 min), 55%–99% acetonitrile (10–45 min), and 99% acetonitrile (45–50 min).

Tissue Preparation and AC Enumeration

Colons were flushed with phosphate-buffered saline, cut open along the longitudinal median, fixed flat in 10% buffered formalin, and stored at 4°C. Colons were stained with methylene

blue by a modification of the method described by Bird (23) and AC foci (ACF) counted on 2 × 5 cm sections of the distal colon by stereoscopic microscopy at 40× magnification. The total number of ACF and AC per ACF were enumerated. After ACF determination, colons were kept in 10% formalin solution and later processed in high-iron diamine Alcian blue staining (HID-AB) for visualization of mucin expression (24). Normal colonic crypts predominantly express sulfomucin. However, certain ACF will express sialomucin, which have been shown to exhibit greater dysplasia in the crypt (25,26), a condition believed to represent an ACF with greater likelihood of progressing to a tumor (27). ACF were scored as sulfomucin-only staining, sialomucin-only staining, a mix of sulfomucin and sialomucin, or mucin-depleted. Scoring of colons as mucin-depleted foci (MDF) was done using criteria described by Caderni et al. (24). Briefly, to be considered mucin-depleted, a focus must show absence or minimal expression of mucin in addition to fulfilling 2 of the following: 1) distortion of the opening of the crypt lumen compared to normal surrounding crypts, 2) elevation of the lesion above the surface of the colon, and 3) multiplicity greater than 3.

Six-mm sections were taken from the left lateral, right medial, and caudate lobes of each liver and fixed overnight in 10% buffered formalin at room temperature, and then transferred into 70% ethanol. Appropriately fixed tissues were processed into paraffin blocks using standard histologic techniques, and 5-μm sections were cut and stained with H&E. Histologic slides were examined using light microscopy by an American College of Veterinary Pathologists board-certified pathologist (Michael G. O'Sullivan).

Statistical Analysis

Values shown are means ± SEM. Treatment effects were examined by 1-way ANOVA. Least square means were calculated, and the PDIFF option with SAS used to inspect group differences. $P < 0.05$ was used as the critical level of significance. As the outcomes for the ethanolic kava extract, polar kava fraction, and nonpolar kava fraction in no case differed significantly from one another, the value for each outcome is also shown for all kava groups combined. Rats given the sham injection (i.e., no carcinogen) displayed no ACF. Consequently, this group was excluded from the statistical analysis of ACF and ACF mucin staining pattern. Correlations between ACF-related parameters were calculated using the least squares method. All statistical analyses were carried out using the SAS System for Windows release 9.1 (SAS Institute, Cary, NC).

RESULTS

Composition of the Kava Extracts

As shown in Fig. 1, the ethanolic kava extract (Fig. 1B) differs somewhat from a kava extract prepared in the traditional fashion (Fig. 1A), in that the ethanolic extract contains nonpolar compounds not present in the traditional kava. Because of the low extraction efficiency of the traditional kava extraction protocol, which is only ∼5% of that of the ethanol extraction (Fig. 1), it was not feasible to obtain a sufficient amount of traditional kava for the animal studies proposed herein. We therefore fractionated the commercial kava extract into polar and nonpolar fractions with the polar fraction mimicking the traditional kava preparation in composition and the nonpolar fraction containing mostly the compounds not detectable in traditional kava preparation. The polar kava fraction (Fig. 1C), however, resembles the traditional kava, both in the presence of the same polar compounds and the absence of nonpolar compounds. The nonpolar kava fraction (Fig. 1D), however, contains mostly the compounds not detectable in the traditional kava extract, except for the components with a retention time of 31 min, which are dihydrokavain and desmethoxyyangonin (28).

Body Weight, Food Intake, and Liver Weight

Final body weights in animals fed the kava diets were significantly less than for saline and DMH-treated control groups by approximately 10% (Table 1). Average daily food intake was also slightly but significantly decreased in the kava groups relative to the DMH-treated control group, and food intake of the ethanolic extract group was significantly less than the saline-treated control group. The food intake of the combined kava

TABLE 1
Final body weight, average food intake, and liver weight in rats fed kava extracts[1]

Variable	Saline-treated control	DMH-treated control	Ethanolic kava extract	Polar kava fraction	Nonpolar kava fraction	Combined kava groups
Final body wt (g)	536 ± 15^{a}	550 ± 14^{a}	482 ± 7^{b}	498 ± 10^{b}	491 ± 12^{b}	$491 \pm 6^{\S}$
Average food intake (g/day)	21.5 ± 0.6^{ab}	22.3 ± 0.7^{a}	19.3 ± 0.3^{c}	20.5 ± 0.5^{bc}	19.8 ± 0.6^{bc}	$19.9 \pm 0.3^{\S}$
Liver weight (g)	19.08 ± 0.86	19.28 ± 0.84	20.16 ± 0.62	20.08 ± 0.81	19.88 ± 1.12	20.04 ± 0.49
Relative liver wt (g/100 g body wt)	3.56 ± 0.12^{b}	3.51 ± 0.13^{b}	4.19 ± 0.13^{a}	4.01 ± 0.12^{a}	4.05 ± 0.20^{a}	$4.08 \pm 0.57^{\S}$

[1]Values represent means ± SEM, $n = 10$ for saline-treated control group, $n = 13$–15 for all other groups. Values within a row that do not share a superscript are significantly different ($P < 0.05$).

[§]Significantly different from the DMH-treated control group ($P < 0.05$).

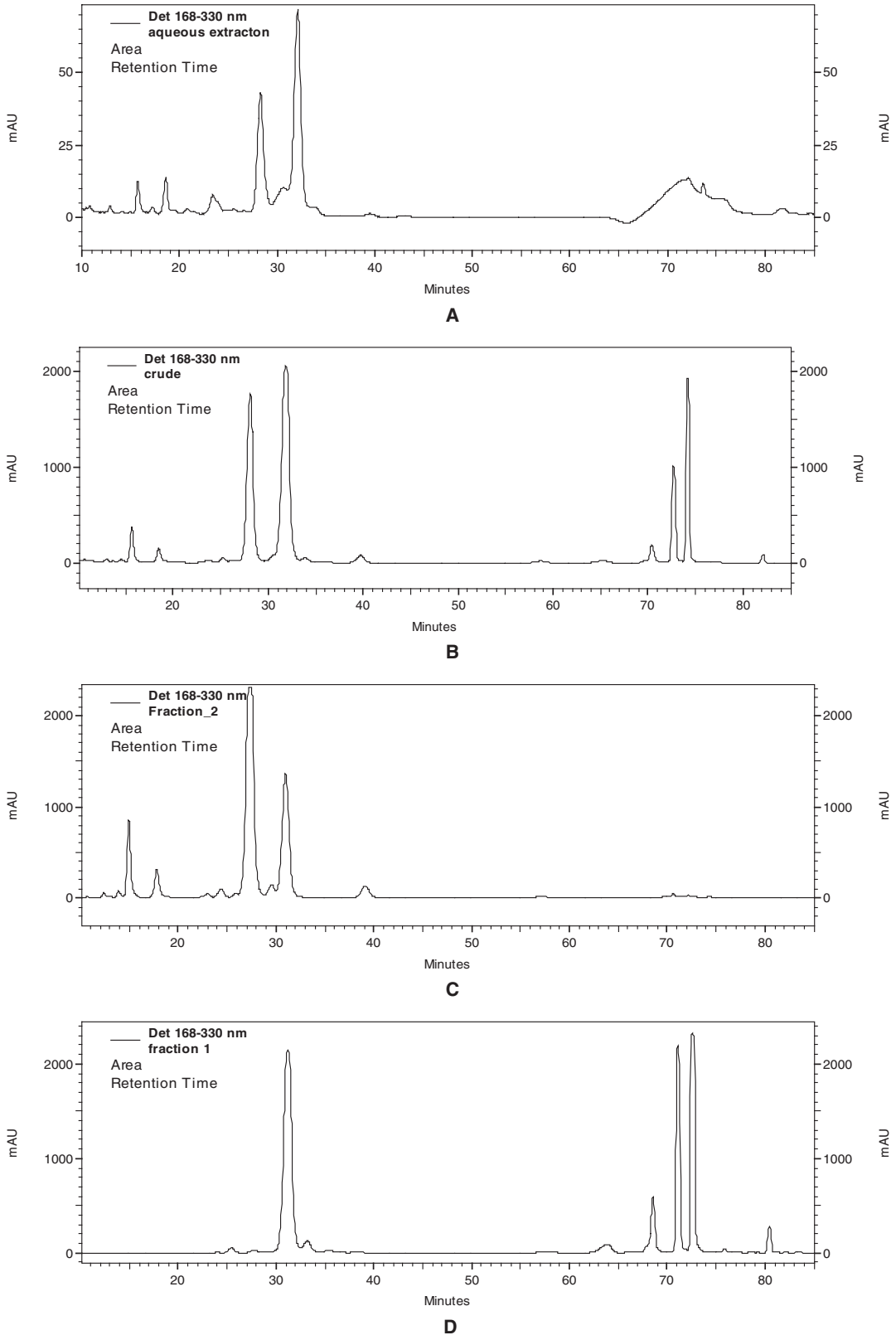

FIG. 1. HPLC chromatograms of kava extracts and fractions. A: aqueous extract; B: ethanolic extract; C: polar fraction; and D: nonpolar fraction. See text for details of the fractionation and analysis.

TABLE 2
Aberrant crypt (AC) foci (ACF), sialomucin-expressing ACF, and mucin-depleted ACF in rats fed kava extracts[1]

Number/cm^2 of distal colon	DMH-treated control ($n = 13$)	Ethanolic kava extract ($n = 15$)	Polar kava fraction ($n = 15$)	Nonpolar kava fraction ($n = 15$)	Combined kava groups ($n = 45$)
AC	17.57 ± 2.71^{a}	12.89 ± 1.84^{ab}	12.53 ± 1.46^{ab}	11.00 ± 1.62^{b}	$12.12 \pm 0.94^{\S}$
ACF	6.57 ± 1.01^{a}	5.25 ± 0.77^{ab}	4.75 ± 0.54^{ab}	4.33 ± 0.60^{b}	$4.78 \pm 0.37^{\S}$
≤3 AC/ACF	4.98 ± 0.77	4.31 ± 0.66	3.66 ± 0.47	3.42 ± 0.47	3.80 ± 0.31
≥4 AC/ACF (as% of total ACF)	1.59 ± 0.26^{a} (23.4)	0.93 ± 0.16^{b} (19.6)	1.09 ± 0.16^{ab} (23.8)	0.91 ± 0.19^{b} (21.0)	$0.98 \pm 0.10^{\S}$ (21.5)
Sulfomucin-only expressing ACF	0.49 ± 0.16	1.07 ± 0.32	1.02 ± 0.31	1.01 ± 0.21	1.04 ± 0.16
≥4 Sulfomucin-expressing ACF	0.07 ± 0.03	0.10 ± 0.04	0.20 ± 0.09	0.16 ± 0.05	0.15 ± 0.04
Sialomucin-only expressing ACF	1.25 ± 0.45	0.71 ± 0.22	0.77 ± 0.26	0.71 ± 0.16	$0.73 \pm 0.05^{\#}$
≥4 Sialomucin-only expressing AC/ACF	0.46 ± 0.18^{a}	0.26 ± 0.11^{ab}	0.25 ± 0.09^{ab}	0.18 ± 0.05^{b}	$0.18 \pm 0.02^{\S}$
Mucin-depleted ACF	0.03 ± 0.02	0.02 ± 0.01	0.01 ± 0.01	0.01 ± 0.01	0.01 ± 0.00

[1]Values represent means ± SEM. Values within a row that do not share a superscript are significantly different ($P < 0.05$).
[§]Significantly different from the DMH-treated control group ($P < 0.05$).
[#]Trend for difference from the DMH-treated control group ($P = 0.10$).

groups was significantly less than the DMH-treated control group. Liver weight did not differ among the groups. However, relative liver weight (i.e., liver weight/100 g body weight) was significantly greater in the kava groups than in the saline- and DMH-treated control groups, and relative liver weight in the combined kava groups was significantly greater than the DMH-treated control group.

ACF and Mucin Staining Pattern

All groups fed diets containing kava had fewer AC and ACF than the DMH-treated control group (Table 2). However, these differences were only statistically significant for the nonpolar kava fraction. The combined kava groups were significantly different from the DMH-treated control group for both AC and ACF number, showing a 31% and 27% inhibition, respectively. Small ACF (≤3 AC/ACF) did not differ among the groups. However, both the ethanolic extract and nonpolar fraction had fewer large ACF (≥4 AC/ACF) than the DMH-treated control, and the polar fraction showed a trend toward fewer large ACF. Furthermore, the combined kava groups had fewer large ACF than the DMH-treated control.

Groups fed the kava diets had greater numbers of ACF expressing only sulfomucin (Table 2), although these differences were not statistically significant. Similarly, there were no significant differences among any of the groups in sulfomucin-expressing large ACF (≥4 AC/ACF). However, there was a tendency for groups fed kava diets to have fewer ACF expressing only sialomucin, and there was a trend ($P = 0.10$) for fewer sialomucin-only expressing ACF in the combined kava extract groups relative to the DMH-treated control group. There were also fewer large sialomucin-only expressing ACF (≥4 AC/ACF) in the kava extract fed groups compared to the DMH-treated control group, although this difference only achieved statistical significance for the nonpolar kava fraction. The combined kava groups had significantly fewer large sialomucin-only ACF than the DMH-treated control group. Overall, very few mucin-depleted foci were found. Although the number were fewer in the kava groups, these differences were not statistically significant.

The size distribution of ACF for the DMH-treated control group and the combined kava groups is shown in Fig. 2. There were fewer ACF for every size of ACF except 6 AC/ACF in the combined kava group compared to the DMH-treated control. These differences were statistically significant for ACF containing 4, 5, 7, or 8 AC per ACF.

The size distribution of ACF staining for either sialomucin or sulfomucin is show in Fig. 3. As seen in Fig. 3A, for

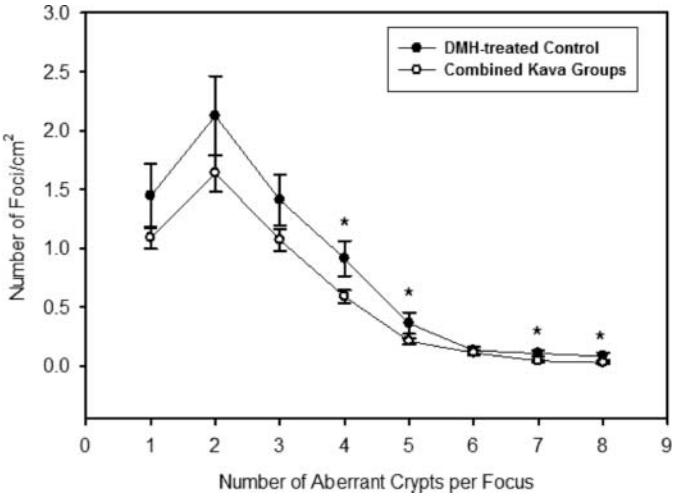

FIG. 2. Size distribution of colonic aberrant crypt foci in the distal colon in the DMH-treated control and combined kava groups. *$P < 0.05$.

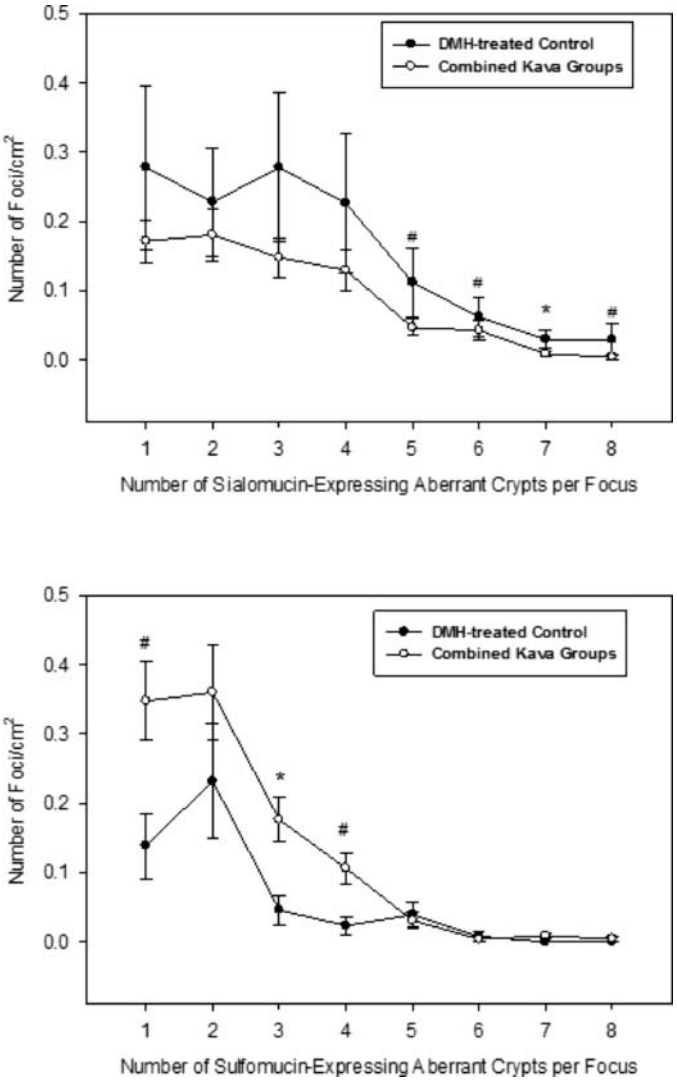

FIG. 3. Size distribution of colonic aberrant crypt foci expressing only sialomucin (A) or sulfomucin (B) in the distal colon in the DMH-treated control and combined kava groups. $^{*}P < 0.05$; $^{\#}P < 0.1$.

every size of ACF there were a greater number of ACF staining only for sialomucin in the DMH-treated control group than the combined kava groups. At 7 AC/ACF this difference was statistically significant, and at 5, 6, and 8 AC per ACF, there was a trend for a difference ($0.10 \leq P \geq 0.05$). Fig. 3B indicates that this pattern was generally reversed for sulfomucin staining. That is, that there were a greater number of ACF staining only for sulfomucin in the combined kava groups than the DMH-treated control group. This difference was statistically significant for 3 AC per ACF, and there was a trend for a difference at 1 and 4 AC per ACF ($0.10 \leq P \geq 0.05$).

As shown in Table 3, the morphological markers significantly correlated with one another, to varying degrees. The strongest correlations were between the total ACF and large ACF ($r = 0.783$) and total ACF and MDF ($r = 0.539$). Correlations between total or large ACF and sialomucin-only expressing ACF were intermediate in strength ($r = 0.388$ and $r = 0.354$, respectively). The weakest correlation was between sialomucin-only expressing ACF and MDF ($r = 0.258$).

TABLE 3
Correlation matrix of morphological markers of colon cancer risk in rats given the chemical carcinogen

Variable	1	2	3	4
1. Total aberrant crypt foci				
2. ≥4 Aberrant crypt/aberrant crypt foci	0.783 <0.0001			
3. Sialomucin-only expressing aberrant crypt foci	0.388 0.003	0.354 0.007		
4. Mucin-depleted aberrant crypt foci	0.539 <0.0001	0.428 <0.001	0.258 0.050	

The upper value represents the correlation coefficient and the lower value the probability of statistical significance.

Liver Histopathology

Animals in all groups had similar background lesions in the liver. These lesions consisted of diffuse, mild to moderate hepatocellular vacuolar degeneration and multifocal mild hepatic lipidosis. The hepatocellular vacuolar degeneration is considered a relatively nonspecific histologic finding associated with induction of hepatic enzymes or indicative of an underlying metabolic derangement or alteration in nutritional status. Animal groups that received the carcinogen (i.e., DMH-treated control, ethanolic extract group, and polar and nonpolar fraction groups) had a few scattered areas of individual hepatocellular necrosis and mild biliary hyperplasia. The extent and severity of these lesions were similar throughout all carcinogen-treated groups but were absent in the saline-treated control group. These lesions, therefore, were likely secondary to the carcinogen treatment. No unique histologic lesions were noted in the ethanolic extract group, or polar or nonpolar fraction groups.

DISCUSSION

Epidemiological studies conducted in the South Pacific have noted a surprisingly low incidence of a number of cancers (2). Kava consumption has been suggested as the chemopreventive agent responsible for this finding, based on observations that cancer incidence varies inversely with kava consumption among South Pacific Islands (5). However, reports of hepatotoxicity by kava have led to cautions regarding its use.

There has been only one experimental investigation of the chemopreventive effect of kava. The study of Johnson et al. (29) examined the effect of kava on lung carcinogenesis in a mouse

model and demonstrated that an ethanolic kava extract reduced tumor multiplicity. In this 30-wk study, no evidence of liver toxicity was found, as determined by liver weight, by enzymatic markers for liver damage, and by pathology. The current study was undertaken to extend the investigation of the chemopreventive properties of kava to colon cancer, the third most common form of cancer in the United States. In addition to examining kava in the form of an ethanolic extract, 2 kava fractions were also investigated as a beginning step in the identification of the chemopreventive agent(s) in kava.

Two morphological endpoints were employed in this study to examine the chemopreventive effect of kava on colon cancer, the number of AC and ACF, respectively, and the mucin staining pattern of the ACF. AC and ACF number are frequently used measures of the risk of colon cancer in chemoprevention studies in animal models and, more recently, in human magnifying endoscopic studies. Although the relevance of AC and ACF as a marker for colon cancer risk has been questioned, based on a lack of correlation between ACF number and tumor number (30), the current evidence strongly supports their utility to predict eventual tumor formation when examined in the early stage of carcinogenesis (31), such as was done in the current study. ACF vary in their histopathological presentation but can be broadly classified as either dysplastic or heteroplastic. A strong association has been found between dysplastic ACF and tumor susceptibility after carcinogen treatment in mice (32), and a dysplastic ACF–adenoma–adenocarcinoma sequence is now well accepted (33). As sialomucin expression is associated with the degree of dysplasia (34,26), the number of ACF expressing sialomucin provides an additional indicator of colon cancer risk.

Kava consumption, as either an ethanolic extract, or as polar or nonpolar fractions of this extract, slightly but significantly reduced final body weight and food intake when fed diets containing 6 g/kg diet. Although Johnson et al. (29) found no effect on food intake, they also noted a slight but significant decrease in body weight in mice fed an ethanolic kava extract. Caloric restriction has been known for decades to reduce tumor incidence in experimental animal models (35), and a 20% caloric reduction has been shown to reduce ACF approximately 20% (36). However, the effect of a 20% caloric restriction on ACF number is influenced by the level of fat in the diet and the length of time the diets were fed, such that 4 wk of this caloric restriction had no effect, whereas after 12 wk, the caloric restriction reduced ACF number by approximately 20% (37). In the present experiment, the combined kava extract groups had an 11% decrease in food intake, compared to the positive control group, but had 27% fewer ACF. Thus, the reduction in ACF found in the kava groups exceeds what would be expected by their reduced energy intake, and thus much or most of the effect of kava on ACF formation is likely attributable to compounds within the kava itself. This degree of inhibition of ACF number is comparable to that found with other compounds demonstrating chemopreventive activity, including the NSAIDs indometracin (38) and sulindac (39), as well as curcumin (40) and ginseng (41).

Large ACF, usually defined as $\geq$4 AC per ACF in rodent studies, are suggested to have greater tumorigenic potential than small ACF (42). Both the ethanolic kava extract and the nonpolar fraction, as well as the combined kava groups, had significantly fewer large ACF than the positive control group, with a trend toward fewer large ACF in the polar kava fraction. Large ACF as a proportion of the total ACF did not differ among the groups, suggesting that the chemopreventive agent(s) in kava are not acting selectively on the more advanced ACF. This also implies that kava may be acting at a very early stage to limit ACF development.

Changes in the mucin expression pattern of ACF appear to be useful indicators of colon cancer risk. As described above, sialomucin-producing ACF are more dysplastic (26), which indicates a state more advanced toward carcinogenesis. Consistent with this concept, a strong trend between the presence of sialomucin-producing ACF and probability of bearing a tumor in carcinogen-treated rats has been reported (24). In addition to reducing AC formation, kava altered the expression pattern of mucin within the ACF toward less dysplasia, as demonstrated by fewer ACF expressing only sialomucin. Although this difference occurred at every crypt multiplicity, the differences reached statistical significance only at multiplicities $\geq$5. The number of ACF expressing only sulfomucins declined overall as the crypt multiplicity increased, as noted by others (24). However, at multiplicities of $\leq$4, the combined kava groups had greater number of sulfomucin-only expressing ACF than the positive control, again suggesting less ACF dysplasia in the kava groups.

MDF are characterized by an absence or very limited expression of mucin. MDF are more dysplastic than sialomucin expressing ACF, occur in approximately the same number as tumors (43), and show the same proportion of β-catenin gene mutations as do colonic tumors (44), all evidence suggesting that MDF may be more specific precancerous lesions than ACF. Overall, however, very few MDF were detected in the present study. In a previous study using a similar experimental design, we found a greater number of MDF, approximately 0.12 per cm^2 (45), than the value of 0.03 per cm^2 in the present study. Others have also reported a greater number of MDF (43,44). However, this difference in number of MDF may be due, at least in some cases, to differences in the dose of carcinogen, as other studies have used higher doses than that used in the present study (44). Although there were fewer MDF in the kava groups compared to the positive control group, these differences did not achieve statistical significance.

That changes in large ACF tend to parallel changes in total ACF can be inferred from several studies in which differences in total ACF occurred among groups (32,45). Thus the high correlation between total ACF and large ACF (defined as $\geq$4 AC/ACF) found in the present study is expected. A general proportionality between total ACF, sialomucin-only expressing ACF, and MDF has also been found (38,45,46,47), similar to our findings. The low correlation between the number of MDF and sialomucin-only expressing ACF may seem somewhat

surprising, given the view that they may represent a progression of dysplasia, and therefore might be expected to be highly correlated. However, because of the small numbers of sialomucin-only expressing ACF and, in particular, MDF in our study, the degree of association between these 2 morphological markers is less certain.

How kava may act to reduce colon cancer risk is unknown. Given that kava was fed both before and after carcinogen administration, it cannot be determined whether kava acted at the initiation or postinitiation phase of carcinogenesis. Dimethylhydrazine, the carcinogen used in the present study, is metabolized to azoxymethane, and then subsequently activated to methylazoxymethane, primarily by CYP2E1 (48,49), ultimately forming the methyldiazonium ion that reacts to form DNA adducts. However, as CYP2E1 activity was unaffected by a kava extract in human liver microsomes (6), it seems unlikely that kava influenced carcinogen activation, and therefore may not act at the initiation phase of carcinogenesis. Compounds extracted from kava have shown significant inhibitory activity toward COX-1 and COX-2 enzymes in vitro (9) and an ethanol extract of kava inhibited release of TNF-α in cell culture and in vivo (10). COX-2 inhibitors are well established to reduce colon carcinogenesis in animals, and considerable evidence supports their chemopreventive effect in humans (12). TNF-α is a proinflammatory cytokine that, when elevated, is well established to be associated with increased colon cancer risk (50). Thus, reduction in either COX-2 activity or TNF-α secretion is a potential mechanism by which kava may reduce colon cancer risk in the post-initiation phase.

Although kava has been consumed as an infusion made from the root for many years by South Pacific populations, concerns about the potential for hepatotoxicity have limited its adoption in the United States. This seems particularly true of commercial kava, which is prepared as an ethanolic extract of the kava root. There are few long-term animal studies of the risk of hepatotoxicity. In the most recent study, Behl et al. (20) examined kava toxicity in mice and rats at doses ranging from 0.125 to 2 g/kg body weight. In male rats administered kava for 3 mo at 0.25 g/kg body weight, the dose most comparable to the present study, there was no significant effect on body weight, but there was a significant increase in liver weight, both absolute and relative. There was no difference in hepatocellular hypertrophy between the control group and group administered 0.25 g/kg kava. In the present study, animals fed kava for 4 mo displayed a slight but statistically significant lower body weight and greater relative liver weight than the control group. Histologically, although some animals receiving the carcinogen showed evidence of hepatocellular necrosis and mild biliary hyperplasia, no differences in liver histology were noted between the control group receiving the carcinogen and any of the kava groups, indicating no histological evidence of damage beyond that induced by the carcinogen. Thus, the present study and that of Behl et al. (20) are consistent in finding that kava increases relative liver weight but leads to no histological evidence of hepatic damage.

In conclusion, ethanolic extracts of kava, as well as either polar or nonpolar fractions of this extract, showed evidence of reduction of morphological markers of colon cancer risk in carcinogen-treated animals. There was a slight tendency for the nonpolar fraction to be more effective than the polar fraction or the unfractionated ethanolic extract, but the groups did not differ from one another statistically. No histological evidence of hepatotoxicity was noted due to consumption of kava. Further investigations of the chemopreventive effects of kava in colon cancer seem warranted.

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