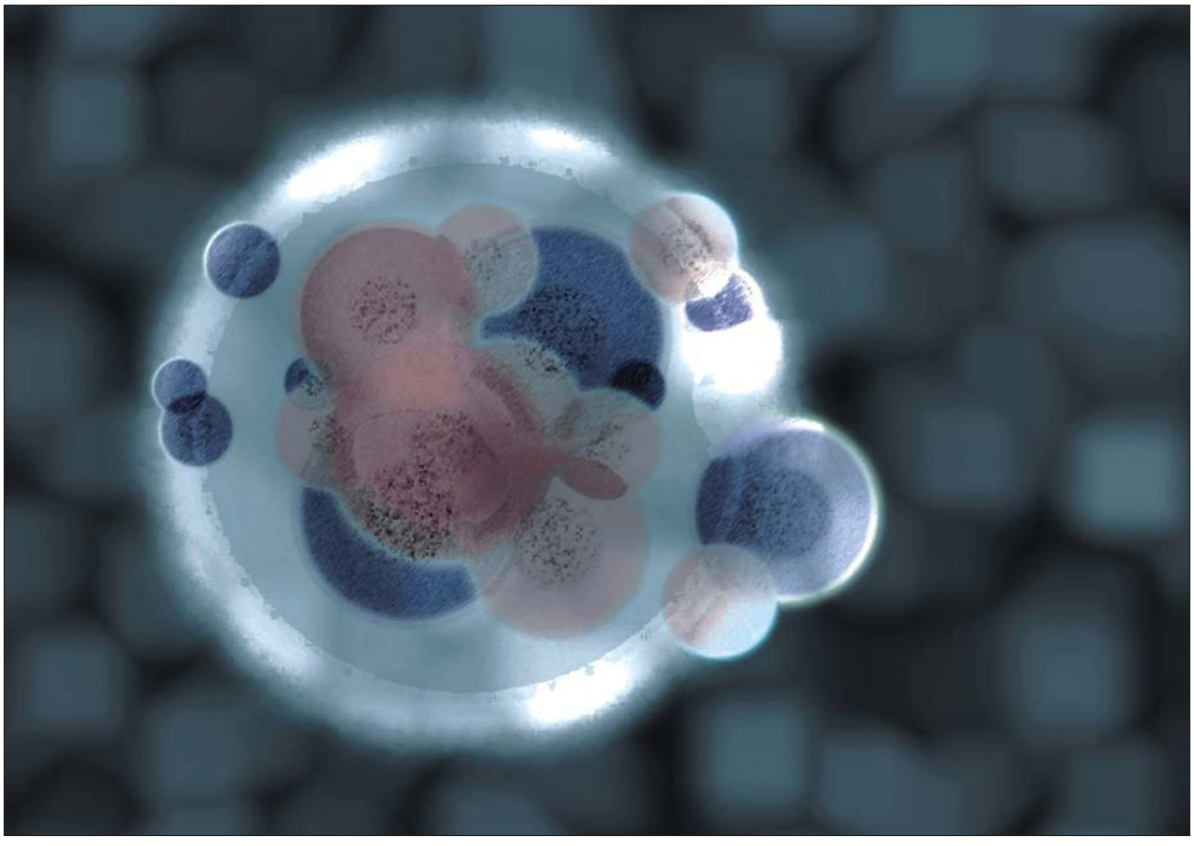

Journal of Biological Sciences

ISSN 1727-3048

science alert

ANSInet
an open access publisher
http://ansinet.com

Journal of Biological Sciences 13 (6): 436-451, 2013
ISSN 1727-3048 / DOI: 10.3923/jbs.2013.436.451

Preventive Potential of Cinnamon (*Cinnamomum cassia*) Extract on Aberrant Crypt Foci Formation in the Proximal and Distal Colon of Azoxymethane-induced Mice

[1]Aleli Jillian L. Sia, [2]Gliceria B. Ramos, [1]Carolyn Marie D. Legaspi, [1]John Edward O. Tanchuco, [1]Miriam P. De Vera, [2]Michael B. Ples and [1]Glenn L. Sia Su
[1]Department of Biology, College of Arts and Sciences, University of the Philippines, Manila, Philippines
[2]Biology Department, College of Science, De La Salle University, Manila, Philippines

Abstract: Cinnamon is known to contain primary polyphenolic antioxidants that have the preventive potential against Aberrant Crypt Foci (ACF) formation and subsequent colon cancer development. This study aims to assess the preventive potential of *Cinnamomum cassia* extract on the development of ACF in mice colon. ICR male mice were divided into six groups: one positive control group (AOM only), three negative control groups (Saline only, Saline only+low dose and Saline only+high dose Cinnamon extract) and two treatment groups (AOM+low dose and AOM+high dose Cinnamon extract). On days 21 and 35, mice were sacrificed for colon extraction and subsequent histological analyses. Results revealed an increasing trend for mean ACF number, mean tumor number, crypt multiplicity and degree of dysplasia in Saline groups treated with low and high doses of Cinnamon indicative of prooxidant activity. A decreasing trend was found for AOM groups with low and high doses of Cinnamon extract indicative of its antioxidant activity. The low dose of Cinnamon showed prevention among mice sacrificed on day 21, whereas the high dose showed prevention on day 35 of tumor progression. The distal colon was found to be more susceptible to tumorogenesis than the proximal colon. Significant correlation was found between the day of sacrifice and mean tumor number, day of sacrifice and crypt multiplicity and day of sacrifice and moderate dysplasia revealing how preventive potential is related to phases of tumor development. Cinnamon was thus found to exhibit both antioxidant and prooxidant activities which are time and dose dependent.

Key words: ACF, AOM-induced, cinnamon extract, distal colon, proximal colon, tumorogenesis

INTRODUCTION

There have been many studies correlating the appearance of Aberrant Crypt Foci (ACF) to colon cancer. The ACF are comprised of microscopically elevated crypts above the normal mucosa. These are excessively thickened epithelial cells and exhibit altered luminal openings clearly different from the normal adjacent crypts (Bird, 1995; Paulsen *et al*., 2005). The ACF develop by continuous fission of crypt stem cells via genetic alterations (Tanaka, 2009). These lesions are twice the size of normal crypts and easily visible. Lesions are continually observed in carcinogen-treated rodents and colon cancer patients and thus, ACF are considered biomarkers in determining the occurrence of colon cancer. For most studies on colon cancer, the carcinogenic substance used is azoxymethane (AOM). It induces the formation of tumors and preneoplastic lesions at the distal colon of rodents. These lesions share similar histopathological characteristics to human colorectal tumors (Kukitsu *et al*., 2008). The AOM-induced colon cancer in rodents thus serves as a model to facilitate further studies on colon cancer.

A wide range of chemotherapeutic drugs are available to cure colon cancer which is met with no response in human patients and the side effects remain devious. There has been a shift toward natural dietary supplementation that possesses chemopreventive effects on colon cancer with minimal toxicity (Singh *et al*., 2007). Plant-based dietary supplements are rich in phytochemicals which possibly mediate physiological functions related to suppression of cancer. Although it is implied that plant-based diet prevents colon cancer, there have been few studies on the identification of bioactive preventives (Volate *et al*., 2005).

One of the main classes of preventive drugs is the phenolic compounds, derived from natural sources and has potent antioxidant and anticarcinogenic activities. One such plant genus rich in phenolic compounds is *Cinnamomum* sp., which contains substantial amounts of

Corresponding Author: Gliceria B. Ramos, Department of Biology, College of Science, De La Salle University, Taft Avenue, Manila, Philippines, 1004 Office Tel: +63 2 5360228 Fax: 63 2 5360228

phenolic antioxidants to counteract the damaging effects of free radicals, thus preventing mutagenesis (Jayaprakasha *et al*., 2007).

Therefore, it is of interest to determine the possible chemopreventive function of cinnamon extracts in ACF formation in AOM-induced colon cancer in mice through its antioxidant activity. The study evaluated the possible preventive potential of *Cinnamomum cassia* extract on the development of ACF in mice colon based on the number, degree of dysplasia and crypt multiplicity of ACF. It included an estimation of the concentration at which the plant extract is most effective. This study would contribute additional knowledge to the study of herbal medicine as a preventive measure against various diseases in humans.

MATERIALS AND METHODS

Standardization of animals, extract and reagents: The ICR mice strain were all male, approximately 6-week-old with same vigour and body weight. Commercially available mice food pellets were given ad libitum. Filtered drinking water was changed once a day. The mice were acclimatized for 1 week in the laboratory with a maintained temperature of 27.30±2.06°C and exposed to a day-night cycle of 12 h (12:12).

Standard reagents used for the experimentation were prepared. AOM, Neutral Buffered Formalin (NBF) and 0.9% saline solution were commercially obtained, whereas the phosphate-buffered saline solution (PBS) with sodium chloride, sodium phosphate and potassium phosphate was prepared in the laboratory. Powdered bark extract of *Cinnamomum cassia* was purchased and the bark/extract ratio of 36:1 was made. The powder was extracted using supercritical CO_2 and water and mixed in the water bottles of the treatment group mice following the procedures of Suh *et al*. (2007).

Experiment proper: After 1 week of acclimatization, a total of 51 mice were randomly assigned into six groups, housed individually. The first group, Saline only group, served as the negative control that received an intraperitoneal dose of 15 mg kg^{-1} b.wt. of 0.9% NaCl solution once a week for 3 weeks. The second group, the Saline+low dose group which received an intraperitoneal dose of 15 mg kg^{-1} b.wt. of 0.9% NaCl solution once a week for 3 weeks and a daily dose of 50 mg mL^{-1} of extract dissolved in distilled water until the last day of the experiment. The third group, Saline+high dose group, received an intraperitoneal dose of 15 mg kg^{-1} body weight of 0.9% NaCl solution once a week for 3 weeks and a daily dose of 100 mg mL^{-1} of cinnamon extract dissolved in distilled water. The fourth group, AOM only group, received an intraperitoneal dose of 15 mg kg^{-1} b.wt. of AOM dissolved in 0.9% NaCl once a week for 3 weeks. The fifth group, AOM+Low Dose, received an intraperitoneal dose of 15 mg kg^{-1} body weight of AOM dissolved in 0.9% NaCl once a week for 3 weeks and a daily dose of 50 mg mL^{-1} cinnamon extract dissolved in distilled water. The last group, AOM+high dose group, received an intraperitoneal dose of 15 mg kg^{-1} b.wt. of AOM dissolved in 0.9% NaCl once a week for 3 weeks and daily dose of 100 mg mL^{-1} cinnamon extract dissolved in distilled water until the last day of the experiment (Kim *et al*., 2006).

Throughout the experiment, all the mice were weighed once a week and data recorded for 8 weeks. After the third injection (day 15), the mice were allowed to recover until day 21. On day 21, three mice from each group were sacrificed by cervical dislocation followed by immediate lobotomy and extraction of the proximal and distal colon. On the last day of the experiment (day 35), all remaining mice were sacrificed by cervical dislocation followed by the same extraction procedure done on day 21 (Ramos *et al*., 2010). The colon samples were sliced into two equal parts, the proximal and distal colon and then washed with cold PBS to flush out contents (Suh *et al*., 2007). The colon samples were then preserved in 10% NBF.

Histological ACF analysis: Preserved colon samples were histologically sectioned. Each slide contained five colon sections, 50 μm apart which were evaluated under compound light microscope (Papanikolaou *et al*., 2000). The samples were documented using True Vision compound light microscope and two camera models: Polaroidt 132 and Olympus fe-4020 digital cameras. The ACF number, tumor number and crypt multiplicity in the proximal and distal colon were counted via visual observation and values were written as actual count per area of colon (mm^2). Crypt multiplicity is defined as the number of crypts found per focus (Bird, 1995). The degree of dysplasia for each ACF formed was observed using the classification scheme of Paulsen *et al*. (2005).

Statistical analysis: Results were expressed as Mean±standard error of mean. Data on ACF number, tumor number, crypt multiplicity and degree of dysplasia were subjected to one-factor analysis of variance (ANOVA) and means were compared post hoc analysis using Duncan's Multiple Range Test (DMRT) using Statistical Package for the Social Sciences (SPSS) version 17. The comparison between day 21 and 35 mice in all criteria was computed via Spearman's rank

correlation test to determine the difference of day 21 and 35 groups, with critical value at 0.412 using Paleontological Statistics (PAST) Software. The p values of the results were determined for comparing the z statistic for Spearman's rank correlation with the normal distribution curve. The level of significance in all cases was $p<0.05$.

RESULTS

The mean number of ACF in the proximal colon of day 21 mice has a significant difference between groups at $p = 0.013$. The Saline+high group has the highest mean ACF number at 0.4 crypt mm^{-2}. AOM only and AOM+low groups both have the least number of ACF for the proximal colon at 0.1 crypt mm^{-2}. In the distal colon, a significant difference was also observed between groups at $p = 0.013$. The Saline+high group has the highest ACF number at 0.7 crypt mm^{-2}, whereas AOM only group has the least number of ACF at 0.1 crypt mm^{-2}. Generally, an increasing number of ACF was observed among Saline-treated groups with a trend of Saline only<Saline+low<Saline+high for the proximal colon, whereas for the distal colon, a trend of Saline+low<Saline only<Saline+high was observed. Among AOM-treated groups in the proximal and distal colon, the mean ACF number for AOM only and AOM+low groups were approximately similar but the AOM+high group is significantly higher (Table 1).

The mean number of ACF for proximal and distal colon sections of day 35 mice did not significantly vary among the treatment groups. For both the proximal and distal colon sections, the highest mean ACF number is found in the AOM+low group at 0.3 and 0.5 crypt mm^{-2}, respectively. The lowest mean ACF number for both the proximal and distal colon sections was observed in the Saline+low group at 0 and 0.1 crypt mm^{-2}, respectively. Among Saline-treated groups, Saline only and Saline+low groups have approximately similar mean ACF number but Saline+high group is comparatively higher. The AOM-treated groups have generally higher mean values at the AOM+low group (Table 2).

Among the six treatment groups, the number of tumors of day 21 mice varied in the distal colon at $p = 0.001$ but not for the proximal colon. In both the proximal and distal colon sections, the AOM only group has the highest mean tumor number at 0.5 and 0.7 crypt mm^{-2}, respectively. For both the proximal and distal colon sections, an increasing number of tumors were observed in Saline-treated groups with a trend of Saline only<Saline+low<Saline+high. However, in AOM-treated groups, AOM+low group has shown a comparable preventive effect in the proximal colon only. The distal colon has shown a decreasing mean tumor number with a trend of AOM only>AOM+low>AOM+high (Table 3). The mean number of tumors in the proximal and distal colon sections of day 35 mice have shown significant differences between treatment groups at $p = 0.011$ and $p = 0.009$, respectively. The AOM+low group has the highest mean tumor number for the proximal colon at 0.8 crypt mm^{-2}. In the distal colon, the highest mean tumor number was found in the AOM only group at 1.0 crypt mm^{-2}. For both the proximal and distal colon sections, it was observed that an increasing number was found in Saline-treated groups with a trend of Saline only<Saline+low<Saline+high. However, a decreasing

Table 1: Mean aberrant crypt foci (ACF) number (±S.E.M.) in the proximal and distal colon of day 21 mice

	Mean ACF No.	
Treatment groups	Proximal colon	Distal colon
Saline only	0.11±0.04^{a}	0.34±0.05^{a}
Saline+low dose†	0.30±0.07^{b}	0.32±0.16^{a}
Saline+high dose‡	0.36±0.05^{b}	0.65±0.05^{b}
AOM only	0.10±0.05^{a}	0.09±0.01^{a}
AOM+low dose†	0.10±0.08^{a}	0.15±0.03^{a}
AOM+high dose‡	0.34±0.06^{b}	0.29±0.12^{a}

Values with a common letter notation are significant at $p<0.05$, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Table 2: Mean aberrant crypt foci (ACF) number (±SEM) in the proximal and distal colon of day 35 mice

	Mean ACF No.	
Treatment groups	Proximal colon	Distal colon
Saline only	0.08±0.02	0.10±0.02^{a}
Saline+low dose†	0.04±0.02	0.07±0.03^{a}
Saline+high dose‡	0.19±0.11	0.44±0.14^{ab}
AOM only	0.24±0.13	0.17±0.05^{ab}
AOM+low dose†	0.27±0.03	0.54±0.24^{b}
AOM+high dose‡	0.13±0.08	0.28±0.06^{ab}

Values with a common letter notation are significant at $p<0.05$, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Table 3: Mean tumor number (±SEM) in the proximal and distal colon of day 21 mice

	Mean tumor No.	
Treatment groups	Proximal colon	Distal colon
Saline only	0.02±0.02	0.04±0.04^{a}
Saline+low dose†	0.09±0.06	0.07±0.07^{a}
Saline+high dose‡	0.28±0.04	0.40±0.05^{b}
AOM only	0.52±0.21	0.73±0.18^{c}
AOM+low dose†	0.23±0.11	0.56±0.07^{bc}
AOM+high dose‡	0.38±0.13	0.45±0.10^{bc}

Values with a common letter notation are significant at $p<0.05$, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

number of mean tumor number was observed in AOM-treated groups for the distal colon only with a trend of AOM only>AOM+low>AOM+high. In the proximal colon, a comparable preventive effect was observed at the AOM+low group (Table 4).

Crypt multiplicity in the proximal colon and distal colon of Day 21 mice have shown significant differences between groups at p = 0.028 and p = 0.015, respectively. The AOM only group has the highest mean crypt multiplicity for both proximal and distal colon at 42.8 and 70.9 crypts mm^{-2}, respectively. Similar to day 21 mean tumor number, increasing mean number of crypt multiplicity was observed in Saline-treated groups with a trend of Saline only<Saline+low<Saline+high. Among AOM-treated groups, AOM+low group has shown a comparable preventive effect for the proximal colon only (Table 5).

For the mean crypt multiplicity of day 35 mice in the proximal colon, the AOM+low group has the highest mean crypt multiplicity at 76.4 crypts mm^{-2} but observed at the AOM only group at 83.7 crypts mm^{-2} for the distal colon (Table 6). A significant difference between groups was exhibited in both the proximal and distal colon sections at p = 0.001 and p = 0.016, respectively. It was observed that an increasing mean crypt multiplicity was found in Saline-treated groups with a trend of Saline only<Saline+low<Saline+high for both the proximal colon. However, decreasing mean crypt multiplicity was observed in AOM-treated groups in the distal colon with a trend of AOM only>AOM+low>AOM+high but for the proximal colon, the highest mean crypt multiplicity was found at the AOM+low group.

The degree of dysplasia observed in the proximal colon of day 21 mice has shown no significant difference among groups for hyperplastic, moderately dysplastic and severely dysplastic crypts. Most hyperplastic crypts were found in Saline+high group at 0.1 crypt mm^{-2} and the least in the Saline+low group at 0 crypt mm^{-2}. It was observed that the highest mean number of moderately dysplastic crypts was found in the AOM only group at 0.5 crypt mm^{-2} (Table 7) and was least at the Saline+low group at 0.1 crypt mm^{-2}. The largest number of mean severely dysplastic crypts was found in the AOM+high group at 0.2 crypt mm^{-2} and was least in the Saline+high group at 0 crypt mm^{-2} (Table 7). For mildly dysplastic crypts, a significant difference between treatment groups was observed at p = 0.030. The highest mean number of mildly dysplastic crypts was found in the Saline+low group at 0.3 crypt mm^{-2} and was least in the AOM only group at 0 crypt mm^{-2} (Table 7).

In the proximal colon of day 35 mice, no significant difference among treatment groups was observed in all groups. The highest mean number of mildly dysplastic crypts was found in the AOM only group at 0.2 crypt mm^{-2}. Moderately dysplastic crypts

Table 4: Mean tumor number (±SEM) in the proximal and distal colon of day 35 mice

	Mean tumor No.	
Treatment groups	Proximal colon	Distal colon
Saline only	0.06±0.05^{a}	0.06±0.03^{a}
Saline+low dose†	0.18±0.04^{ab}	0.28±0.05^{ab}
Saline+high dose‡	0.32±0.07^{ab}	0.36±0.10^{ab}
AOM only	0.55±0.23^{bc}	1.03±0.03^{c}
AOM+low dose†	0.81±0.08^{c}	0.74±0.34^{bc}
AOM+high dose‡	0.57±0.25^{bc}	0.46±0.20^{ab}

Values with a common letter notation are significant at p<0.05, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Table 5: Mean crypt multiplicity (±SEM) of ACF in the colon of day 21 mice

	Mean crypt multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.26±1.40^{a}	6.55±1.61^{a}
Saline+low dose†	9.31±1.71^{a}	9.96±6.11^{ab}
Saline+high dose‡	23.33±3.78^{ab}	41.68±10.74^{abc}
AOM only	42.75±11.33^{b}	70.91±16.91^{b}
AOM+low dose†	17.71±10.68^{ab}	47.47±13.85^{bc}
AOM+high dose‡	37.22±11.43^{b}	50.57±6.84^{b}

Values with a common letter notation are significant at p<0.05, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Table 6: Mean crypt multiplicity (±SEM) of ACF in the colon of day 35 mice

	Mean crypt multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.78±0.86^{a}	5.74±1.76^{a}
Saline+low dose†	24.69±6.94^{ab}	24.92±4.62^{ab}
Saline+high dose‡	40.34±10.89^{bc}	44.69±2.84^{abc}
AOM only	42.22±11.35^{bc}	83.72±13.85^{c}
AOM+low dose†	76.44±8.66^{d}	70.22±29.13^{bc}
AOM+high dose‡	61.70±18.07^{cd}	38.56±8.64^{abc}

Values with a common letter notation are significant at p<0.05, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Table 7: Mean±SEM for histological ACF analysis of the proximal colon of day 21 mice

	Mean crypt multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.78±0.86^{a}	5.74±1.76^{a}
Saline+low dose†	24.69±6.94^{ab}	24.92±4.62^{ab}
Saline+high dose‡	40.34±10.89^{bc}	44.69±2.84^{abc}
AOM only	42.22±11.35^{bc}	83.72±13.85^{c}
AOM+low dose†	76.44±8.66^{d}	70.22±29.13^{bc}
AOM+high dose‡	61.70±18.07^{cd}	38.56±8.64^{abc}

Values with a common letter notation are significant at p<0.05, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

were highest in the AOM+low group at 0.8 crypt mm^{-2} and equally low in the Saline only and Saline+low groups at 0.1 crypt mm^{-2} (Table 8). The highest mean number of severely dysplastic crypts was found in the Saline+high group at 0.1 crypt mm^{-2} and least in the AOM+high group at 0.1 crypt mm^{-2} (Table 8). No hyperplastic crypts were found in the proximal colon of day 35 mice.

For the distal colon of day 21 mice, no significant difference exists between groups for hyperplastic and mild dysplastic crypts. The highest mean number of hyperplastic crypts was found in the Saline only group at 0.2 crypt mm^{-2} and least in the AOM+high group in which no crypts were observed. The highest mean number of mildly dysplastic crypts was found in the Saline+high group at 0.3 crypt mm^{-2} and least in AOM+low group at 0.1 crypt mm^{-2}. The highest mean number of moderately dysplastic crypts were found in the Saline+high group at 0.6 crypt mm^{-2} and was least in the Saline only group at 0.1 crypt mm^{-2}. Severely dysplastic crypts have the highest mean number at the AOM only group at 0.4 crypt mm^{-2} and were least in the Saline+high group at 0.1 crypt mm^{-2} (Table 9). Moderate dysplasia and severe dysplasia have shown significant differences between treatment groups at p = 0.012 and p = 0.010, respectively.

In the distal colon of day 35 mice, the highest mean mildly dysplastic crypts were found in AOM+low group at 0.3 crypt mm^{-2} and was least in the Saline only group at 0 crypt mm^{-2}. Moderately dysplastic crypts were highest in the AOM only group at 1.0 crypt mm^{-2} and were least in the Saline only group at 0.1 crypt mm^{-2}. Severely dysplastic crypts were found to be highest in the Saline+high group at 0.1 crypt mm^{-2} and were least for Saline only group at 0 crypt mm^{-2} (Table 10). No hyperplastic crypts were found in the distal colon of day 35 mice. Only moderately dysplastic crypts have exhibited a significant difference among classification groups in the distal colon of day 35 mice at p = 0.001.

Representative photomicrographs of Normal proximal and distal colon are shown in Fig. 1. Aberrant Crypt Foci (ACF)-tumor formation are shown in Fig. 2-6.

Table 8: Mean±SEM for histological ACF analysis of the proximal colon of day 35 mice

	Mean crypt multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.78±0.86^{a}	5.74±1.76^{a}
Saline+low dose†	24.69±6.94^{ab}	24.92±4.62^{ab}
Saline+high dose‡	40.34±10.89^{bc}	44.69±2.84^{abc}
AOM only	42.22±11.35^{bc}	83.72±13.85^{c}
AOM+low dose†	76.44±8.66^{d}	70.22±29.13^{bc}
AOM+high dose‡	61.70±18.07^{cd}	38.56±8.64^{abc}

Values with a common letter notation are significant at p<0.05, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Table 9: Mean±SEM for histological ACF analysis of the distal colon of day 21 mice

	Mean crypt multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.78±0.86^{a}	5.74±1.76^{a}
Saline+low dose†	24.69±6.94^{ab}	24.92±4.62^{ab}
Saline+high dose‡	40.34±10.89^{bc}	44.69±2.84^{abc}
AOM only	42.22±11.35^{bc}	83.72±13.85^{c}
AOM+low dose†	76.44±8.66^{d}	70.22±29.13^{bc}
AOM+high dose‡	61.70±18.07^{cd}	38.56±8.64^{abc}

Values with a common letter notation are significant at p<0.05, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

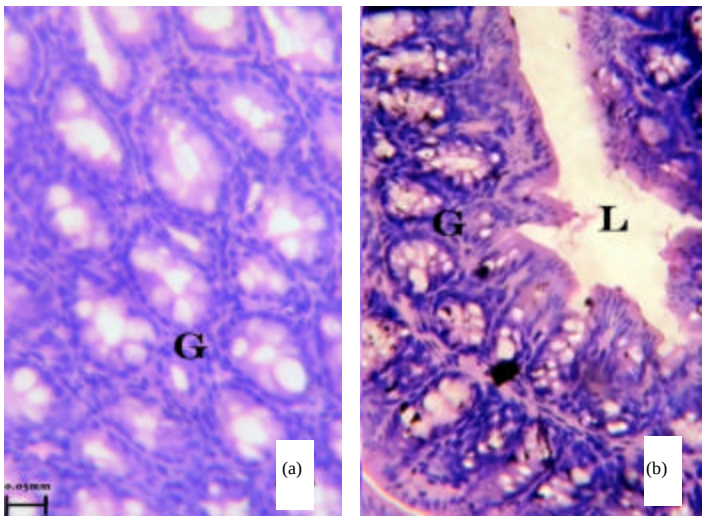

Fig. 1(a-b): Normal colon mucosa in the (a) proximal and (b) distal colon (HPO, 400X), L: Luminal side, G: Goblet cells

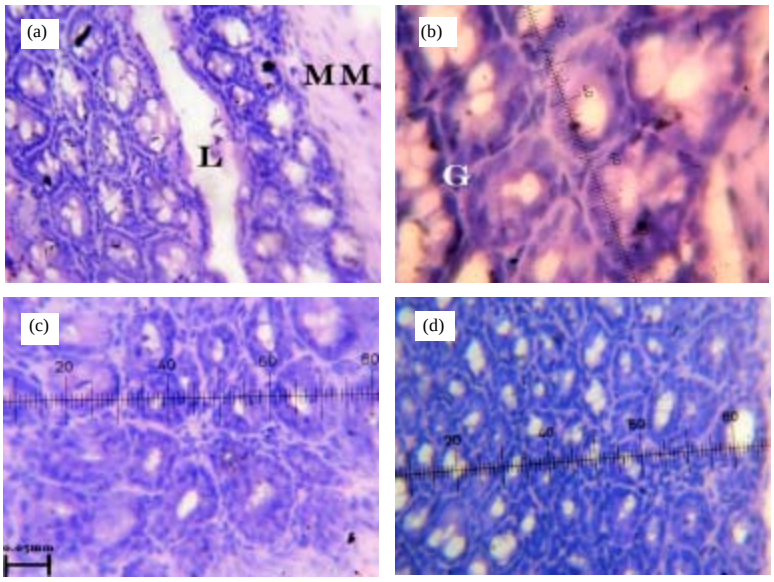

Fig. 2(a-d): Proximal colon of day 21 mice with aberrant crypt foci (ACF)-tumor formation. From A to D, non-filled arrows show (a) hyperplastic crypts with normal epithelium and dilation of goblet cells, (b) Mildly dysplastic crypts which show reduction in goblet cell number, slightly crowded nuclei with polarity well preserved, (c) Moderately dysplastic crypts which show much reduction of goblet cells and pseudostratified appearance and thickened basement membrane and (d) Severely dysplastic crypts which show complete loss of goblet cells, numerous mitoses and loss of nuclear polarity (HPO, 400 X), L: Luminal side, MM: Muscularis mucosa, G: Goblet cells

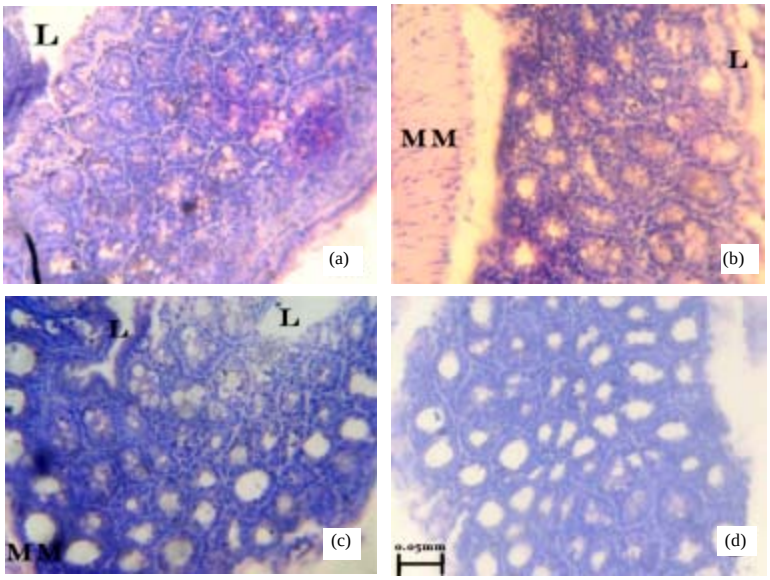

Fig. 3(a-d): Proximal colon of ay 35 mice with ACF-tumor formation. From A-D, non-filled arrows show (a-b) Mildly dysplastic crypts which show reduction ingoblet cell number, slightly crowded nuclei with polarity well preserved, (c) moderately dysplastic crypts show much reduction of goblet cells, increased crowding, pseudostratified appearance and thickened basement membrane and (d) severely dysplastic crypts show complete loss of goblet cells, numerous mitoses and loss of nuclear polarity. The basement membrane is very prominent (HPO, 400 X), L: Luminal side, MM: Muscularis mucosa

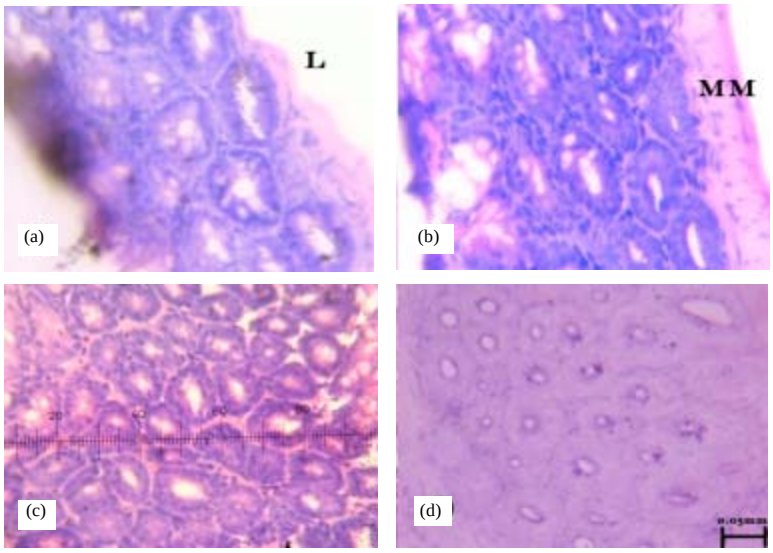

Fig. 4(a-d): Distal colon of day 21 mice with ACF-tumor formation. From A-D, non-filled arrows show (a) Hyperplastic crypts with normal epithelium and slight dilation of goblet cells, (b) Mildly dysplastic crypts which show reduction in goblet cell number, slightly crowded nuclei with polarity well preserved, (c) Moderately dysplastic crypts show much reduction of goblet cells, increased crowding, pseudostratified appearance and thickened basement membrane and (d) severely dysplastic crypts which show complete loss of goblet cells, apparent thickening of foci and loss of nuclear polarity. (HPO, 400 X), L: Luminal side, MM: muscularis mucosa

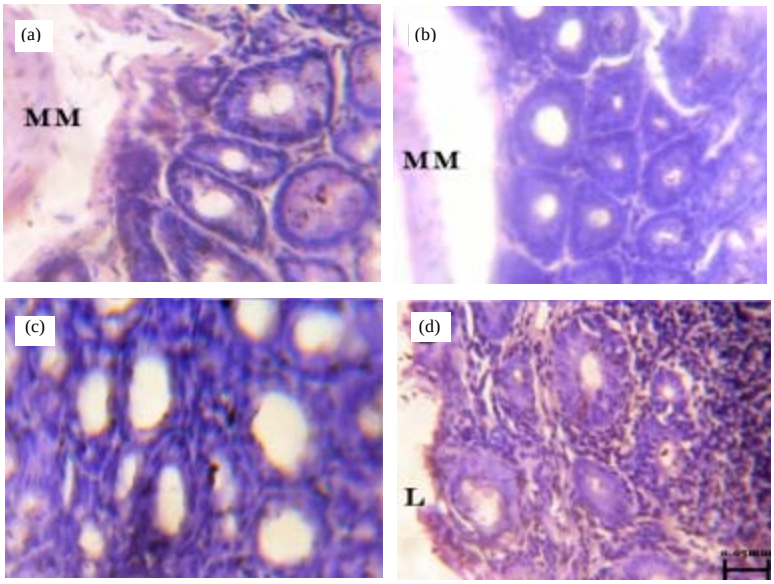

Fig. 5(a-d): Distal colon of day 35 mice with aberrant crypt foci (ACF)-tumor formation. From A-D, non-filled arrows show (a) Mildly dysplastic crypts show reduction in goblet cell number, crowded nuclei with polarity well preserved, (b) moderately dysplastic crypts show much reduction of goblet cells, increased crowding, pseudostratified appearance and nuclei elongation, (c) severely dysplastic crypts show complete loss of goblet cells, eularged foci and loss of nuclear polarity and (d) moderately dysplastic crypts were observed to invade into gut associated lymphoid tissues (GALT) (HPO, 400 X), L: Luminal side, MM: Muscularis mucosa

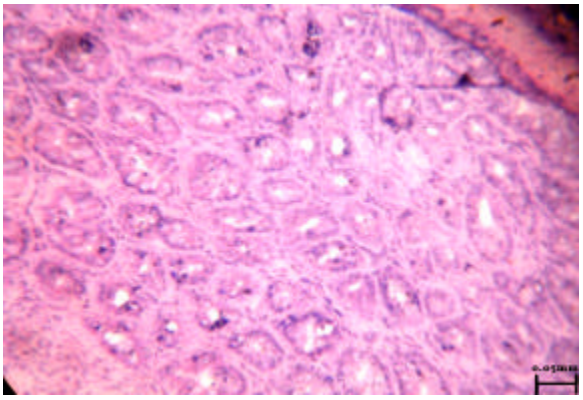

Fig. 6: Progress of dysplasia in the distal colon of day 35 mouse. The circle-marked bracket exhibits hyperplastic crypts, with normal epithelium and slight dilation of goblet cells. The star-marked bracket shows mildly dysplastic crypts with a marked reduction in goblet cells, preserved polarity and crowding and elongation of nuclei. The unmarked bracket demonstrates moderately dysplastic crypts with focal enlargement, pseudostratified appearance, increased loss of goblet cells and increased crowding

Table 10: Mean±SEM for histological ACF analysis of the distal colon of day 35 mice

	Mean crypt multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.78±0.86^{a}	5.74±1.76^{a}
Saline+low dose†	24.69±6.94^{ab}	24.92±4.62^{ab}
Saline+high dose‡	40.34±10.89^{bc}	44.69±2.84^{abc}
AOM only	42.22±11.35^{bc}	83.72±13.85^{c}
AOM+low dose†	76.44±8.66^{d}	70.22±29.13^{bc}
AOM+high dose‡	61.70±18.07^{cd}	38.56±8.64^{abc}

Values with a common letter notation are significant at $p<0.05$, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Table 11: Spearman's rank correlation coefficient (r_s) and p values for the proximal colon of day 21 vs. 35 mice

	Mean Crypt Multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.78±0.86^{a}	5.74±1.76^{a}
Saline+low dose†	24.69±6.94^{ab}	24.92±4.62^{ab}
Saline+high dose‡	40.34±10.89^{bc}	44.69±2.84^{abc}
AOM only	42.22±11.35^{bc}	83.72±13.85^{c}
AOM+low dose†	76.44±8.66^{d}	70.22±29.13^{bc}
AOM+high dose‡	61.70±18.07^{cd}	38.56±8.64^{abc}

Values with a common letter notation are significant at $p<0.05$, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

Spearman's rank correlation coefficient was used to compare the effect of day of sacrifice (day 21 vs. 35) to characters being investigated. For the proximal colon, it was shown that a significant correlation was found between day of sacrifice and tumor number at $r_s = 0.56$, day of sacrifice and crypt multiplicity at $r_s = 0.56$ and day of sacrifice and moderately dysplastic crypts at $r_s = 0.54$ (Table 11). For the distal colon, the same characters were found to be significantly correlated with day of sacrifice, namely tumor number, crypt multiplicity and moderate dysplasia with the r_s values at 0.74, 0.69 and 0.64, respectively (Table 12).

Table 12: Spearman's rank correlation coefficient (r_s) and p values for the distal colon of day 21 vs. 35 mice

	Mean crypt multiplicity	
Treatment groups	Proximal colon	Distal colon
Saline only	2.78±0.86^{a}	5.74±1.76^{a}
Saline+low dose†	24.69±6.94^{ab}	24.92±4.62^{ab}
Saline+high dose‡	40.34±10.89^{bc}	44.69±2.84^{abc}
AOM only	42.22±11.35^{bc}	83.72±13.85^{c}
AOM+low dose†	76.44±8.66^{d}	70.22±29.13^{bc}
AOM+high dose‡	61.70±18.07^{cd}	38.56±8.64^{abc}

Values with a common letter notation are significant at $p<0.05$, †Low dose treatment consists of 50 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water, ‡High dose treatment consists of 100 mg of *Cinnamomum cassia* powder extract/100 mL of filtered water

DISCUSSION

The cinnamon extract exhibited an antioxidant effect on the development of ACF and showed a decrease in the incidence of ACF on both the low and high doses of cinnamon extract. The number of ACF was found to increase most noticeably in the distal colonic segment. Some groups in the proximal segment exhibited an increased in the number of ACF and tumors and increased dysplasia when sacrificed on day 35. Even mice which did not receive AOM injections but were treated with cinnamon extract exhibited ACF and tumor development particularly mice given high doses of cinnamon extract. However, some preventive activity-such as a lower

number of ACF, tumors and crypt multiplicity-were observed in groups particularly those mice that received a low dose of cinnamon extract.

ACF number: The proximal and distal colon segments of day 21 mice exhibited a significant difference with a high number of ACF when supplied with a high dose of cinnamon extract. It is possible that the high dosage of cinnamon caused prooxidation. A trend of increasing ACF number was observed in both AOM and Saline groups of day 21 mice as the dose of cinnamon was increased (Fig. 1). This is an indication of a possible prooxidant activity caused by the cinnamon extract. Prooxidant activity allows the development of oxidative stress, leading to the development of cancer. This suggests the direct proportionality between cinnamon extract concentration and prooxidant activity. Comparing both the proximal and distal AOM+low groups to the AOM+high groups, some preventive activity may be inferred. This may suggest that only a low dose of cinnamon is necessary for prevention at the induction phase of carcinogenesis (day 21).

For day 35 mice, the observed trend for both proximal and distal colon segments is less consistent as there is no significant difference between groups indicating same rate of tumorigenesis across groups. For the proximal colon, an increasing number of ACF was observed in Saline-treated groups but decreasing in AOM-treated groups (Fig. 2). This suggests possible prooxidant activity where colon cancer is not evident but preventive activity can be inferred in AOM-treated mice. For the distal colon, only the Saline-treated groups exhibited a similar trend to proximal colon: increasing ACF number as cinnamon extract is increased, indicating prooxidant activity as well. The AOM-treated groups exhibited a different trend, where AOM+low group had the highest number of ACF present and low ACF number for AOM+high group, indicating a possible preventive effect at a high dose of cinnamon extract on the latent phase of cancer (Fig. 2). This difference may also be caused by the difference in properties of the proximal and distal colon segments mainly absorption rate, subsequently affecting the absorption of cinnamon extract.

Tumor number: In day 21 mice, there is a general trend of increasing tumor number for both the Saline-treated groups of proximal and distal colon segments (Fig. 3). This supports the proposed prooxidant activity of cinnamon. However, the AOM-treated groups of both proximal and distal colon show differences: AOM-treated groups of the distal colon segment simply show a decreasing tumor number but among the proximal colon segment, AOM+low group has the lowest count. This may indicate that a low dose of cinnamon can perhaps have more preventive potential than the high dose during the induction phase of cancer, suggesting that cinnamon may be more effective in preventing early tumor progression than ACF development.

Comparing tumor number for the proximal and distal colon, the distal colon generally has a higher number of tumors present (Fig. 3). This may be an indication of the susceptibility of the distal colon to carcinogenic factors in comparison to the proximal colon. This susceptibility may be caused by differences of absorption and other properties between the colonic segments.

In day 35 mice, both the proximal and distal groups exhibit significant differences (Fig. 4). The Saline-treated groups for both proximal and distal segments show an increasing trend of tumor number indicating prooxidant activity. The AOM-treated groups exhibit a decreasing trend of tumor number, suggesting that a high dose of cinnamon is needed for prevention to occur. It must be noted that the AOM only and AOM+high groups of the proximal segment have similar values. It may be inferred that the AOM+low group exhibited prooxidant activity. Comparing the values of the proximal and distal segments, the latter is generally higher and thus more susceptible to carcinogenic factors.

Crypt multiplicity: Among the day 21 colon samples, the AOM only group exhibited the highest value for crypt multiplicity which is theoretically accurate and thus has the highest counts of ACF and tumors. The trend observed in Fig. 5 is increasing among Saline-treated groups, indicating the prooxidant effect of cinnamon. However, among AOM-treated groups, the AOM+low group show a preventive effect since the crypt multiplicity is relatively small compared to other groups.

Among day 35 samples, the proximal and distal Saline-treated groups exhibit the increasing trend indicating prooxidant activity due to cinnamon extract. Distal segments exhibit preventive effect, particularly the AOM+high group (Fig. 6). It must be noted that the AOM only group of the proximal segment had a lower value of crypt multiplicity than the AOM+low and AOM+high groups. This indicates less susceptibility to cancer development in the proximal segment than the distal segment. This difference in crypt multiplicity may be due to difference in properties of each segment affecting the absorption of cinnamon extract.

Degree of dysplasia: The proximal colon segments of day 21 mice exhibited mostly mildly dysplastic crypts having a significant difference to other groups (Fig. 7). This is

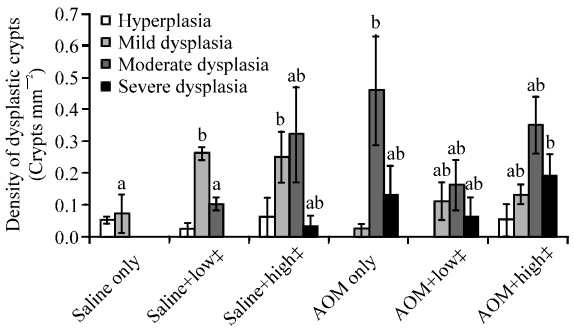

Fig. 7: Degree of dysplasia for the proximal colon of day 21 mice, Values with a common letter notation are significant at $p<0.05$

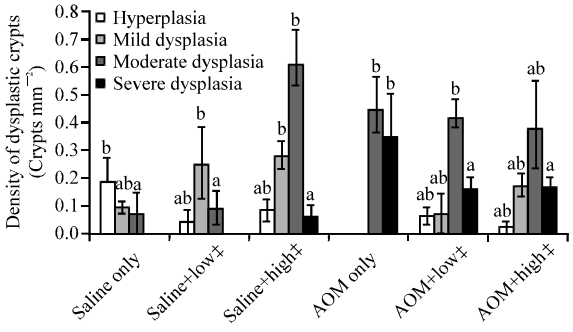

Fig. 9: Degree of dysplasia for the distal colon of day 21 mice, Values with a common letter notation are significant at $p<0.05$

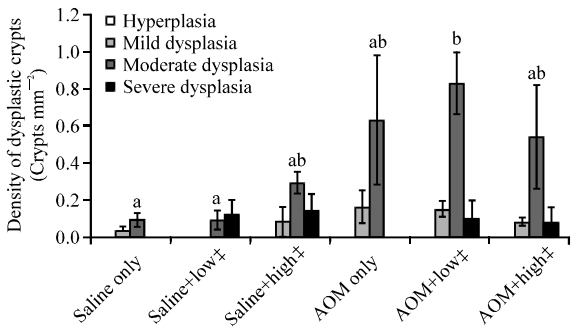

Fig. 8: Degree of dysplasia for the proximal colon of day 35 mice, Values with a common letter notation are significant at $p<0.05$

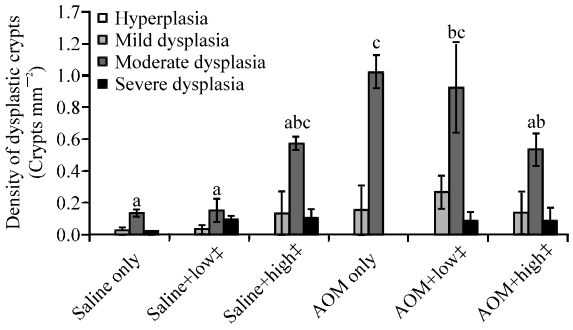

Fig. 10: Degree of dysplasia for the distal colon of day 35 mice, Values with a common letter notation are significant at $p<0.05$

expected since day 21 falls at the initiation phase and degree of dysplasia may change as ACF progress, eventually becoming severe as more mutations are induced by the carcinogen (AOM) which ensures further progression. AOM-treated groups generally exhibited high numbers of moderate to severely dysplastic crypts. The AOM+low group exhibited smallest values for dysplasia among AOM-treated groups suggests preventive activity.

No hyperplasia was observed in the proximal segment of day 35 mice (Fig. 8). For the proximal segment of day 35 mice, all groups did not exhibit any significant differences from one another indicating similar frequencies degrees of dysplasia present. This result is expected since the latent phase is evident and the carcinogen has induced numerous mutations to facilitate genomic instability in the colon. It must be noted that AOM+high groups exhibited mild preventive activity since its values were lower compared to the other AOM-treated groups.

In the distal colon segments of day 21 mice, there was significantly more moderate to severely dysplastic crypts observed but there no hyperplastic crypts observed among the 6 groups (Fig. 9). This result is expected since the absorption properties of the distal colon differ from the proximal colon. It is possible that the distal colon is more susceptible and thus progress further in the development of cancer than the proximal segment.

The expected result for the distal colon of day 35 mice should have been similar to the distal segment of day 21 mice, where there is an increase in the number of moderate to severe dysplastic crypts. However, the results show that only moderate dysplasia was significantly different to other treatment groups (Fig. 10). It was earlier mentioned that the distal colon could be more susceptible to the carcinogen compared to the proximal segment; however, if this was true, then the number of severely dysplastic crypts should have also been significantly different. This difference from the result of day 21 distal segments may be due to the day of sacrifice and properties of the colon. During the latent

phase, the effects of the mutations may not be as severe as when it was first initiated attributed to the large accumulation of mutations.

Spearman's rank correlation revealed that tumor number, crypt multiplicity and moderate dysplasia are the parameters strongly correlated with the day of sacrifice for both proximal and distal colon segments. A significant correlation to tumor number and crypt multiplicity may be explained by the continued cancer progression and accumulation of mutations as the days pass between the induction and latent phases of cancer. It may be possible that prooxidation, attributed to the cinnamon extract, contributed to cancer development. It is evident from Fig. 7-10 that moderately dysplastic crypts increase as time progresses. This supports the significant correlation found between day of sacrifice and moderately dysplastic crypts. It may be hypothesized that moderate crypts became numerous due to the increased rate of mutation occurring in the colon as time progresses. Again, prooxidation may have also contributed to the rapid mutation of the aberrant crypts.

Generally, the low-dose cinnamon extract shows more preventive action on the proximal and distal colon of day 21 mice, whereas the high-dose cinnamon shows more preventive action on the proximal and distal colon of day 35 mice. This suggests that the preventive action of cinnamon may be dose dependent.

Prooxidant activity and preventive effect: Prooxidant activity was observed in mice colon samples, especially the samples in the Saline-treated groups. Generally, the number of ACF and tumors found in the Saline+low and Saline+high groups of all mice were significantly larger than the Saline only group (Fig. 1-4). It is possible to develop some numbers of ACF and tumors due to environmental stress and other external factors; however, a significant difference was not expected to ensue. Prooxidant activity was therefore suspected as the primary cause for this significant difference. Prooxidant activity may also explain why some mice that were given AOM but treated with cinnamon extract had an even higher number of ACF compared to the AOM only groups (Table 1 and 2, Fig. 1 and 2) particularly mice given high doses of cinnamon extract.

The prooxidant activity of cinnamon may be attributed to cinnamaldehyde, a cinnamon polyphenol. Cinnamaldehyde was found in the study to induce carcinogenesis in a time- and dose-dependent manner. Therefore, it is probable that the Saline groups treated with low and high doses of cinnamon were adversely affected, especially those mice that were given a high dose of cinnamon extract since the reaction is concentration dependent.

Cinnamaldehyde is a known polyphenol and several studies have shown that polyphenols have both an antioxidant and prooxidant effect. Studies explain that the prooxidant effect is actually a result of the antioxidant. This occurs when there is only one source for an antioxidant substance. Different antioxidants use different chemical pathways to exert their effects. When there is only one source of antioxidant, there is only one chemical pathway used and excess products from a single pathway may be detrimental to the health of the organism. The excess products cause the prooxidant reaction to occur. In contrast, anticarcinogenic effect occurs best when there are several sources of antioxidants that prevent the accumulation of a single product and thus, less toxicity occurs (Boudet, 2007).

Several studies explain the mechanism by which polyphenolic prooxidation occurs. One process is known as the oxidation-reduction system of phenol quinones. Reactive oxygen species form during the futile redox cycling of quinones which causes oxidative stress in the organism (Chedea *et al*., 2010). Another possible mechanism of reaction is via the interaction of polyphenols with copper which results to copper-mediated DNA damage. The polyphenolic compounds reduce copper from Cu 2-1. The copper readily reacts with hydrogen peroxide or other reactive oxygen species that form hydroxide radicals in the body. The polyphenols may also regenerate the production of hydrogen peroxide or react with ascorbate to form more polyphenolic compounds. This causes a copper redox cycle wherein many free radicals are generated, inducing oxidative stress and DNA damage (Perron *et al*., 2011). The copper redox cycle was also found to induce the degradation of certain tissues and cells such as human lymphocytes (Hadi *et al*., 2007).

Another source of prooxidant activity is from flavonoids. The cinnamon extract used has 20% cinnamon flavones which are dependent on the number of hydroxyl groups in the flavonoid molecule. If more than two hydroxyl groups are present, there is a high occurrence of prooxidant activity. The mechanism of flavonoid prooxidation is similar to polyphenolic prooxidation which is copper-mediated DNA damage. Prooxidant activity of flavonoids was also found to be concentration dependent (Prochazkova *et al*., 2011).

Although, many figures and tables testify to the prooxidant activity of the cinnamon extract, some preventive activity was also observed. Based on the results from Table 3, 4 and from Fig. 3, 4, it can be hypothesized that the number of tumors for AOM-treated mice with cinnamon extract is less than the number of tumors found in the AOM only group mice. Furthermore, crypt multiplicity of AOM+low and AOM+high groups of

mice is also generally less than the mice found in the AOM only group (Fig. 5 and 6). It can also be deduced that the preventive activity occurs only in groups of mice given AOM injections whereas prooxidant activity occurs in mice from Saline-treated groups. A possible hypothesis is that the antioxidant activity of cinnamon can only occur when there are substrates from severe oxidation activities already present in the organism with which cinnamaldehyde can react with. If the substrates from oxidative stress reactions are not present, then it may be possible for cinnamaldehyde to react with other substrates in the body causing polyphenolic prooxidation to occur and the subsequent development of mutations leading to genomic instability and further progression of carcinogenesis.

The effect of cyclooxygenase-2 expression on malignancy: As ACF are putative preneoplastic lesions, the mutation of cyclooxygenase-2 (COX-2) activity may primarily lead to the initiation of ACF formation until its further progression to carcinogenesis. COX-2 may promote carcinogenesis by the following modes: affecting pathways of carcinogenesis and inhibiting apoptosis (Zyada *et al*., 2009). In gastrointestinal cancers, COX-2 inhibitors are claimed to be epithelial cancer chemopreventives. COX-2 is an inducible enzyme affected by pathophysiological conditions such as growth factors, inflammatory stimuli, oncogenes and tumor promoters. Prostaglandins (especially PGI-2) produced by COX-2 promotes PPAR family of nuclear hormone receptors, changing calcium ion levels, activates the RXR receptor, leading to accelerated cellular proliferation-a hallmark of malignant transformation. It was shown that colorectal cancer shows expression of COX-2 via the Familial Adenomatous Polyposis (FAP) gene and the COX-2 gene (Mohan and Epstein, 2003).

Multiple mechanisms of COX-2 activity may be found at different stages of malignancy. E-series prostaglandins are noted to induce cell proliferation, angiogenesis, invasion and metastasis, down-regulation of signals cell stage maintenance and cell-to-cell communication, inhibition of Tumor Necrosis Factor (TNF) and promotion of interleukin 10 (IL-10), a cytokine-promoting immunosuppression. Overexpression of COX-2 gene modifies cell adhesion, inhibits apoptosis and alters the response to growth regulatory signals. However, an indirect proportionality was found between antiapoptotic protein (Bcl-2) expression and apoptosis (Mohan and Epstein, 2003). A study by Zyada *et al*., 2009) attempted to correlate expression of COX-2 and Bcl-2 to clinicopathological features in the mucoepidermoid carcinoma (MEC). It was shown that COX-2 is a good predictor for lymph node metastasis, evaluation of borderline malignancy and an ideal therapeutic target for the prevention of MEC. Therefore, COX-2 and Bcl-2 are usable prognosis markers for MEC.

A correlation between p53 and COX-2 expression was found wherein p53 mutations, especially when found in dysplastic lesions and high levels of COX-2 expression were observed. COX-2 upregulation is also linked with the increased production of Vascular Endothelial Growth Factor (VEGF) by the epithelial cell and increased vascularization of tumors, thus increased angiogenesis. A correlation by COX-2 and VEGF does not prove a causal relationship but rather a simultaneous parallelism. This, however, can be a result of multiple pathways and thus, single enzyme inhibition is insufficient (Toomey *et al*., 2009). Elevated VEGF levels were found early in the progression from dysplasia to carcinoma (Mohan and Epstein, 2003).

Role of secondary metabolites in the cinnamon extract: Essential oils have two main components: terpenes and aromatic compounds. Cinnamaldehyde is an aromatic aldehyde found in essential oils. In a study by Smid *et al*. (1995), cinnamaldehyde was mentioned as a component of essential oil to inhibit fungal growth in tulips. Cinnamaldehyde has shown strong, irreversible antifungal activity against *Penicillium hirsutum in vitro*, specifically when administered via water phase. This supports its role in the plant as a secondary metabolite-defending the plant from bacterial infections and phytopathogenic microorganisms. Furthermore, the compound did not have any phytotoxic effects to tulip growth and flowering. This suggests that prooxidation may not be the only cause for the development of cancer in saline and AOM groups but its chemical nature.

The components of essential oils have no specific cellular targets and are lipophillic. Thus, these components such as cinnamaldehyde may pass through the cytoplasmic membrane, disrupt the structure of their different layers of polysaccharides, fatty acids and phospholipids and permeabilize them, demonstrating cytotoxicity. In bacteria, these compounds were found to cause the loss of ions, collapse of proton pumps, depletion of ATP and reduction of membrane potential. Furthermore, essential oils may coagulate the cytoplasm and damage lipids and proteins, ultimately leading to cellular lysis. In eukaryotes, essential oils were found to provoke depolarization of mitochondrial membranes by decreasing the membrane potential, affecting ionic calcium cycling and ionic channels, reducing pH gradient and affecting the proton pump and ATP pool. Due to the lipophillic nature of essential oils, the membrane

permeability changes and causes leakage of radicals, cytochrome c, calcium ions and proteins leading to oxidative stress and subsequent bioenergetic failure. The cell thus dies by apoptosis and necrosis which is similar to the effects of phenolic prooxidation (Bakkali *et al*., 2008). These cytotoxic effects caused by cinnamaldehyde may have contributed to colonic carcinogenesis in mice.

The same study also mentions that cytotoxic effects were observed in *Saccharomyces cerevisiae* and mammalian cells. The degree of cytotoxicity was found to depend on the state of the cell-where dividing cells were found to be more sensitive (Bakkali *et al*., 2008). It is possible that the loss of cell division control, a result of AOM treatment and proodixant activity, increased the rate of the carcinogenesis in the mouse colon.

Additional factors: A study by Walker (1996) stated that diet has been found to be the most influential factor in determining toxicity within an organism. ACF formation can be induced at much smaller numbers in rats via heterocyclic amines which form when meat and fish are cooked and are possible colon carcinogens encountered in the human diet (Pretlow and Pretlow, 2005). Therefore, it may be inferred that the kind of feeds (high-protein feeds) administered to the mice may have had an effect on the development of colon cancer, especially among the Saline only group, leading to the formation of background ACF.

The results obtained for each parameter were different between the proximal and distal colon segments. From all the tables and figures, there is a general trend where the proximal colon has less number of ACF and tumors, smaller crypt multiplicity and less severely dysplastic crypts whereas the distal colon generally exhibits the opposite characteristics. This was most probably attributable to many innate differences between the proximal and distal colon segments.

It is known that there are differences in the absorption of water and other substances along the colonic length. This implies that the AOM and cinnamon treatment administered to the mice could have been absorbed at different rates, resulting in varying degrees of ACF and tumor development along the colonic tract. It was found by Finkel and Larsson (1987) that the length of the proximal colon of young rats is longer than those of older rats. Increased length would indicate an increased area for absorption of substances. However, the results obtained show the distal colon had generally more moderate and severely dysplastic crypts compared to the proximal segment. Other factors beside absorption rates are responsible for the development of ACF along the colon.

It has been shown by Albuquerque *et al*. (2011) that the proximal and distal colon differ in bile acid metabolism, water resorption, luminal content, bacterial colonization and short-chain fatty acid production. A factor also attributing to the differences between the segments may be their embryonic origin-where the proximal colon originates from the midgut whereas the distal colon originates from the hindgut. Another characteristic that may contribute to the differences of the results from the proximal and distal colon segments is the kind of antioxidant source used. Annema *et al*. (2011) found that certain fruits or vegetables act on different areas along the colon, specifically that brassica vegetables decreased the risk of proximal colon cancer while apples and dark yellow vegetables decreased the risk of distal colon cancer. Correlating the results of the present study to this previous statement, it can be hypothesized that perhaps cinnamon decreases the risk of proximal colon cancer since the general observation is that the development of cancer is not as aberrant as compared to the distal colon. However, since there is some preventive action observed in the distal colon, it is also possible that cinnamon can decrease the risk for distal colon cancer as well. The reason why certain sources of antioxidants act on different parts of the colon is because there are several pathways for cancer to develop along the length of the colon (Annema *et al*., 2011). However, a clear understanding between the relationship of different cancer pathways and environmental factors, such as intake of certain fruits or vegetables, has not yet been achieved. There is a lack for a unified theory to explain colon carcinogenesis and preventive treatment.

As mentioned previously, the different characteristics of each colon segment may lead to different pathways of cancer development and have subsequently caused several studies to suggest that colon cancer be treated as two separate illnesses, depending on the location whether it is proximal or distal. There is a marked difference in the development of tumors in the proximal colon from the distal colon due to the difference in expression of tumor-related genes and level of genomic aberration which results to clinical and biological variances (McKay *et al*., 2001). Moreover, studies revealed that colorectal cancer should not be treated as a homogenous type of cancer due to the varying genetic pathways in which tumorigenesis may occur. The study explains that several mutations may occur in different introns of a single gene, resulting in polyclonal variations among colon cancer types (Guebel and Torres, 2008). At present, there is no official statement indicating that colon cancer should be treated as two different types of cancer. However, it was

suggested the treatment of colon cancer is as two discrete illnesses. Proximal colon cancer, also known as right-sided colon cancer, has been suggested to originate from the caecum, ascending colon, hepatic flexure, or transverse colon. Distal colon cancer, also known as left-sided colon cancer, originates from splenic flexure, descending colon and sigmoid colon (Annema *et al*., 2011).

It was investigated that the different pathways for colon cancer and the areas of the colon affected among mice and found that microsatellite instability (MSI) was the main cause for the development of ACF and tumors in the proximal region of the colon. MSI occurs by a defect in the DNA mismatch repair machinery and this subsequently affects cell metabolism and control of the cell cycle. Cancer which develops through MSI, is characterized by elevated mutation rates caused by repeating nucleotide sequences. The study found MSI as a molecular marker of proximal colon cancer (Jernvall *et al*., 1999). However, the reason why MSI affects only the proximal region of the colon is still unknown and insufficient.

The other more common pathways for colon cancer is caused by chromosomal instability (CIN) which is the activation of proto-oncogenes, such as K-ras mutations and the deactivation of tumor suppressor genes, such as p53 and APC (Cho *et al*., 2010). Carcinogenesis by this pathway is found all over the length of the colon with MSI mostly found at the proximal colonic segment. Reversing the statement, almost no MSI-induced carcinogenesis is found at the distal area of the colon and this helps to differentiate the two segments. The distal colon is said to undergo a p53 mutation more frequently than the proximal colon (Horii *et al*., 2008).

A study has found that distal colorectal cancer makes up around 60% of all cases of colorectal cancer in comparison to proximal colorectal cancer (Deng *et al*., 2008). This may be an indication that the distal colon is more susceptible to carcinogenic factors compared to the proximal colon. This statement supports the general trend observed, wherein more aberrant growth of crypts are found at the distal area of the colon samples. Susceptibility to carcinogenic factors may be linked to the difference in methylation occurring at the proximal or distal colon segment. This property is particularly important since methylation is the mechanism by which AOM induces cancer development.

A study by Horii *et al*. (2008) found that methylation occurs differently depending on the segments of the colon which is caused by the different carcinogenic pathways by which carcinogenesis occurs along the colon length. Hypermethylation was found to occur more often at the proximal colon, whereas hypomethylation occurred at the distal colon. Hypermethylation is associated with the silencing of tumor suppressor genes, whereas hypomethylation was found to induce genomic instability and thus increased gene mutation rates. It is known that, in order to induce carcinogenesis, many genes must have aberrant functions. Since, hypomethylation increases the rate of gene mutation, it is more probable that the distal segments of the colon samples generally have more severely dysplastic crypts compared to the proximal segment. The increased rate of mutation may have caused the rapid tumorigenesis to occur and thus the results of having more aberrant crypt growth in the distal colon samples of the mice.

CONCLUSION

There are several discoveries made in the study of the preventive potential of cinnamon in ACF formation in the colon. First, it was found that cinnamon had a double effect on the colon samples obtained: on one hand, it induced prooxidation in the Saline-treated groups of mice and on the other hand, it prevented the development of ACF and tumors on the AOM-treated groups of mice. Second, it was also found that cinnamon was more effective at preventing the development of proximal colon cancer than distal colon cancer since the parameters measured were generally found to be less severe for the proximal segment compared to the distal segment. A significant correlation was also found between the day of sacrifice and tumor number, day of sacrifice and crypt multiplicity and day of sacrifice and moderate dysplasia, showing the increased susceptibility of the distal colon segment. Lastly, preventive action is observed generally in groups treated with low-dose cinnamon sacrificed at day 21 but groups treated with high-dose cinnamon showed preventive action for mice sacrificed at day 35, demonstrating that the preventive action of cinnamon is time and dose dependent.

REFERENCES

Albuquerque, C., E.R. Bakker, W. van Veelen and R. Smits, 2011. Colorectal cancers choosing sides. Biochim. Biophys. Acta-Rev. Cancer, 1816: 219-231.

Annema, N., J.S. Heyworth, S.A. McNaughton, B. Iacopetta and L. Fritschi, 2011. Fruit and vegetable consumption and the risk of proximal colon, distal colon and rectal cancers in a case-control study in Western Australia. J. Am. Dietetic Assoc., 111: 1479-1490.

Bakkali, F., S. Averbeck, D. Averbeck and M. Idaomar, 2008. Biological effects of essential oils: A review. Food Chem. Toxicol., 46: 446-475.

Bird, R.P., 1995. Cancer letters of colon cancer. Cancer Lett., 93: 55-71.

Boudet, A.M., 2007. Evolution and current status of research in phenolic compounds. Phytochemistry, 68: 2722-2735.

Chedea, V.S., C. Braicu and C. Socaciu, 2010. Antioxidant/prooxidant activity of a polyphenolic grape seed extract. Food Chem., 121: 132-139.

Cho, Y.K., H.C. Kim, S.H. Kim, J.H. Park and H.R. Yun *et al*., 2010. Location-related differences in sporadic microsatellite unstable colorectal cancer. Digestive Liver Dis., 42: 611-615.

Deng, G., S. Kakar, H. Tanaka, K. Matsuzaki, S. Miura, M.H. Sleisenger and Y.S. Kim, 2008. Proximal and distal colorectal cancers show distinct gene-specific methylation profiles and clinical and molecular characteristics. Eur. J. Cancer, 44: 1290-1301.

Finkel, Y. and L. Larsson, 1987. Morphometric and functional studies of proximal and distal colon in young and adult rats. J. Pediatr. Gastroenterol. Nutr., 6: 454-459.

Guebel, D.V. and N.V. Torres, 2008. A computer model of oxygen dynamics in human colon mucosa: Implications in normal physiology and early tumor development. J. Theor. Biol., 250: 389-409.

Hadi, S.M., S.H. Bhat, A.S. Azmi, S. Hanif, U. Shamim and M.F. Ullah, 2007. Oxidative breakage of cellular DNA by plant polyphenols: A putative mechanism for anticancer properties. Semin. Cancer Biol., 17: 370-376.

Horii, J., S. Hiraoka, J. Kato, K. Harada and K. Kuwaki *et al*., 2008. Age-related methylation in normal colon mucosa differs between the proximal and distal colon in patients who underwent colonoscopy. Clin. Biochem., 41: 1440-1448.

Jayaprakasha G.K., P.S. Negi, B.S.Jena and L.J.M. Rao, 2007. Antioxidant and antimutagenic activities of *Cinnamomum zeylanicum* fruit extracts. J. Food Compos. Anal., 20: 330-336.

Jernvall, P., M.J. Makinen, T.J. Karttunen, J. Makela and P. Vihko, 1999. Microsatellite instability: Impact on cancer progression in proximal and distal colorectal cancers. Eur. J. Cancer, 35: 197-201.

Kim, S.H., S.H. Hyun and S.Y. Choung, 2006. Anti-diabetic effect of cinnamon extract on blood glucose in db/db mice. J. Ethnopharmacol., 104: 119-123.

Kukitsu, T., T. Takayama, K. Miyamishi, A. Nobuoka and S. Katsuki *et al*., 2008. Aberrant crypt foci as precursors of the dysplasia-carcinoma sequence in patients with ulcerative colitis. Clin. Cancer Res., 14: 48-54.

McKay, J.A., P.H. Rooney, V.G. Ross, J.J. Douglas and L.J. Murray *et al*., 2001. Marked differences in tumor associated protein expression and genetic stability between proximal and distal colon tumors. Eur. J. Cancer, 37: S272-S272.

Mohan, S. and J.B. Epstein, 2003. Carcinogenesis and cyclooxygenase: The potential role of COX-2 inhibition in upper aerodigestive tract cancer. Oral Oncol., 39: 537-546.

Papanikolaou, A., Q.S. Wang, D. Papanikolaou, H.E. Whiteley and D.W. Rosenberg, 2000. Sequential and morphological analyses of aberrant crypt foci formation in mice of differing susceptibility to azoxymethane-induced colon carcinogenesis. Carcinogenesis, 21: 1567-1572.

Paulsen, J.E., E.M. Loberg, H.B. Olstorn, H. Knutsen, I.L. Steffensen and J. Alexander, 2005. Flat dysplastic aberrant crypt foci are related to tumorigenesis in the colon of azoxymethane-treated rat. Cancer Res., 65: 121-129.

Perron, N.R., C.R. Garcia, J.R. Pinzon, M.N. Chaur and J.L. Brumaghim, 2011. Antioxidant and prooxidant effects of polyphenol compounds on copper-mediated DNA damage. J. Inorganic Biochem., 105: 745-753.

Pretlow, T. and T. Pretlow, 2005. Mutant KRAS in Aberrant Crypt Foci (ACF): Initiation of colorectal cancer. Biochimica Biophysica Acta, 1756: 83-96.

Prochazkova, D., I. Bousova and N. Wilhelmova, 2011. Antioxidant and prooxidant properties of flavonoids. Fitoterapia, 82: 513-523.

Ramos, G.B., M.P. De Vera, S.B. Tanaeli, K.V. Bayot and J.R. Caling *et al*., 2010. Celecoxib and refecoxib in the prevention of azoxymethane-induced formation of aberrant crypt foci in mice. Asia Life Sci., 19: 419-427.

Singh, G., S. Maurya, M.P. de Lampasona and C.A.N. Catalan, 2007. A comparison of chemical, antioxidant and antimicrobial studies of cinnamon leaf and bark volatile oils, oleoresins and their constituents. Food Chem. Toxicol., 45: 1650-1661.

Smid, E.J., Y. de Witte and L.G.M. Gorris, 1995. Secondary plant metabolites as control agents of postharvest *Penicillum* rot on tulip bulbs. Postharvest Biol. Technol., 6: 303-312.

Suh, N., S. Paul, X. Hao, B. Simi, H. Xiao, A.M. Rimando and B.S. Reddy, 2007. Pterostilbene, an active constituent of blueberries, suppresses aberrant crypt foci formation in the azoxymethane-induced colon carcinogenesis model in rats. Clin. Cancer Res., 13: 350-355.

Tanaka, T., 2009. Colorectal carcinogenesis: Review of human and experimental animal studies. J. Carcinogen., Vol. 8.

Toomey, D.P., J.F. Murphy and K.C. Conlon, 2009. COX-2, VEGF and tumour angiogenesis. Surgeon, 7: 174-180.

Volate, S.R., D.M. Davenport, S.J. Muga and M.J. Wargovich, 2005. Modulation of aberrant crypt foci and apoptosis by dietary herbal supplements (quercetin, curcumin, silymarin, ginseng and rutin). Carcinogenesis, 26: 1450-1456.

Walker, R., 1996. Modulation of toxicity by dietary and environmental factors. Environ. Toxicol. Pharmacol., 2: 181-188.

Zyada, M.M., M.E. Grawish and H.M. Elsabaa, 2009. Predictive value of cyclooxygenase 2 and Bcl-2 for cervical lymph node metastasis in mucoepidermoid carcinoma. Ann. Diagn. Pathol., 13: 313-321.