

## RESEARCH ARTICLE

# Effects of Two Traditional Chinese Cooking Oils, Canola and Pork, on pH and Cholic Acid Content of Faeces and Colon Tumorigenesis in Kunming Mice

Xiao-Qiong He<sup>1\*</sup>, Jia-Li Duan<sup>1</sup>, Jin Zhou<sup>1</sup>, Zhong-Yu Song<sup>1</sup>, Simon Angelo Cichello<sup>1,2</sup>

## Abstract

Faecal pH and cholate are two important factors that can affect colon tumorigenesis, and can be modified by diet. In this study, the effects of two Chinese traditional cooking oils (pork oil and canola/rapeseed oil) on the pH and the cholic acid content in feces, in addition to colon tumorigenesis, were studied in mice. Kunming mice were randomized into various groups; negative control group (NCG), azoxymethane control group (ACG), pork oil group (POG), and canola oil Ggroup (COG). Mice in the ACG were fed a basic rodent chow; mice in POG and COG were given 10% cooking oil rodent chow with the respective oil type. All mice were given four weekly AOM (azoxymethane) i.p. injections (10mg/kg). The pH and cholic acid of the feces were examined every two weeks. Colon tumors, aberrant crypt foci and organ weights were examined 32 weeks following the final AOM injection. The results showed that canola oil significantly decreased faecal pH in female mice ( $P<0.05$ ), but had no influence on feces pH in male mice ( $P>0.05$ ). Pork oil significantly increased the feces pH in both male and female mice ( $P<0.05$ ). No significant change was found in feces cholic acid content when mice were fed 10% pork oil or canola oil compared with the ACG. Although Kunming mice were not susceptible to AOM-induced tumorigenesis in terms of colon tumor incidence, pork oil significantly increased the ACF number in male mice. Canola oil showed no influence on ACF in either male or female mice. Our results indicate that cooking oil effects faecal pH, but does not affect the faecal cholic acid content and thus AOM-induced colon neoplastic ACF is modified by dietary fat.

**Keywords:** Azoxymethane - feces pH - feces cholate - dietary fat - colon tumorigenesis - aberrant crypt foci

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## Introduction

Colorectal cancer is the formation of cancer in the colon or rectum (i.e. large intestine). Common symptoms include bloody stools (hematochezia), dysfunction of bowel movements, abrupt weight loss and fatigue (i.e. cancer cachexia). Colorectal cancer (CRC) is increasing in prevalence in Asia, in particular China (Sung et al., 2005). Colorectal cancer can be induced experimental using carcinogens such as Azoxymethane in rodents which alkylates DNA and initiate oncogenesis by forming DNA adducts (Rogers et al., 1977). The risk factors for development of CRC include co-morbidity with irritable bowel disease, increased duration of IBS, degree of colitis/ and inflammation, familial history of CRC, sclerosing cholangitis (Kim et al., 2014), lower fruit and vegetable intake and also dietary fiber, in combination with lifestyle influences such as tobacco smoking and physical inactivity. A number of dietary factors have been cited to reduce the risk of developing colon cancer and include; bioflavonoids (Pandurangan et al., 2014), Ganoderma lucidum via Fas/

Caspase dependent tumour apoptosis (Liang et al., 2014), demethoxycurcumin from *Curcuma longa* in intestinal cell culture via iNOS suppression (Somchit et al., 2014), but in particular, dietary oil i.e. vegetable, has a role in reduced tumorigenesis and inflammation (Quassinti et al., 2014, Cardeno et al., 2013) as opposed to animal fats (He et al., 2014).

The purpose of the present study was to determine whether two types of cooking oil (i.e. vegetable versus pork oil) could modulate the pH and cholic acid content of feces, and the occurrence of colorectal tumour induced by colon-selective carcinogen azoxymethane in mice. The following study aims to provide effective dietary therapy for the prevention ways for colon cancer.

## Materials and Methods

### Azoxymethane

AOM was purchased from Sigma (St. Louis, Mo). It was pre-dissolved in phosphate-buffered saline (10 mg/ml, PBS) and stored at  $-20^{\circ}\text{C}$  until use. AOM was diluted into

<sup>1</sup>School of Public Health, Kunming Medical University, Yunnan Province, China, <sup>2</sup>School of Life Sciences, La Trobe University, Australia \*For correspondence: [hexqcn@aliyun.com](mailto:hexqcn@aliyun.com)

1.5 mg/ml by physiological saline just before injection. AOM was given to mice by intra-peritoneal injection (ip). The dose of AOM was 10 mg per kg body weight each time.

#### *Dietary oil*

Two Chinese traditional cooking oils (Lard oil and Rapeseed/ Canola oil; interchangeable terms herein) were chosen to investigate the regulatory effect of dietary fat on AOM-induced diseases in this research. Lard oil was refined from the pork leaf fat by us in the lab. The rapeseed oil was made in China and was purchased from supermarket. The two cooking oils were commonly consumed by Chinese people. Lard oil has 10% of polyunsaturated fatty acid (PUFA), 48% monounsaturated fatty acid (MUFA), and 42% saturated fatty acid (SFA). Lard oil is the representation cooking oil of SFA in China. Rapeseed oil has 36% of PUFA (i.e. 21% of the total oil composition is the omega 6;  $\alpha$ -linoleic acid, omega 3;  $\alpha$ -linolenic acid (ALA)), 61% MUFA (i.e. primarily the omega 9; oleic acid) and 6% SFA. Rapeseed oil is the representation cooking oil of unsaturated fatty acid in China.

#### *Preparation of rodent chow*

Powdered basic mice feed was provided by the Experimental Animal Center, Kunming Medical College, Yunnan Province, P.R. China. The ratio of the oil in high fat feed is 10% of the total feed in weight, namely 1kg of cooking oil and 9kg of powdered basic mice feed were completely mixed and then made into rodent chow. The feed was freshly prepared every two weeks and stored in fridge for use.

#### *Animals*

Kunming mice (local crossbreed experimental animal) were obtained from the Experimental Animal Center, Kunming Medical University, Yunnan Province, China. They were maintained in a temperature- and humidity-controlled animal facility with a 12-hr light-dark cycle. Mice in each group were fed Ad lib rodent chow correspondingly according to research design and autoclaved distilled water. Mice were observed daily for clinical signs of illness, and body weight was measured every two weeks to reduce body weight change due to stress of handling.

#### *Experimental procedure*

Eighty 5-week-old Kunming mice (half for male and female) were randomized into 4 groups (20 mice/group, 5mice/cage). The groups were denoted as; Basic Control Group (BCG), AOM Control Group (ACG), Lard Oil Group (LOG), and Rapeseed Oil Group (ROG). The mice in BCG and ACG were fed the same basic rodent chow, mice in LOG were fed 10% lard oil rodent chow, and mice in ROG were fed 10% rapeseed oil rodent chow. Mice in ACG, LOG and ROG were given four weekly AOM i.p. injections (10 mg/kg, on day 7, 14, 21 and 28). Mice in BCG were given saline injection at the same time. Mice were killed 32 weeks later after the last AOM injection. Heart, liver, kidney, spleen and testis were isolated and

weighed. The organ co-efficient (OCE); organ weight divided by the body weight of the mouse and multiplied by 100.

#### *Faeces collection*

The abdomen of mice were gently pressured by fingers in the morning every two weeks, the secreted fresh feces was collected into corresponding ependorf tubes and then stored –at 80°C for pH and cholic acid determination.

#### *pH determination*

0.5g of thawed faeces was added into 100ml distilled water, and stirred until dissolved. The samples then were soaked for 30 minutes at room temperature. The supernatant was used for pH examination by a pH meter (Bench Top Sper Scientific 860031).

#### *Cholic acid determination*

The thawed feces was dried at 80°C stove until a constant moisture content was achieved. It was then powdered using a motor and pestle. 0.5g of faecal powder was added into 5ml ethanol and shaken for 30 minutes at 70°C in a water bath thrice. The ethanolic supernatant of 3 macerations with faeces was collected into a tube, and then the ethanol was evaporated at 80°C in a water bath. 5ml of petroleum was added into the tube to remove the fat and neutral-cholesterol. The deposit was dissolved into 5ml of 2% Triton X-100 ethanol, and then dried at 80°C in a water bath. The deposit was dissolved into 3 ml distilled water and then the cholic acid was determined at 605 nm using a spectrophotometer. Standard cholate was used as a reference for the standard curve.

#### *Measurement of tumor and aberrant crypt foci*

Distal colon was scored for the presence/ absence of colon tumorigenesis. Aberrant crypt foci (ACF) formation was assayed according to the method of McLellan and Bird (1991). Briefly, the remains of the colon after the removal of colon tumour were stained for 15 min with 0.2% methylene blue and the mucosal surface was examined for the presence of aberrant crypt (AC) under a dissecting microscope at a magnification of  $\times 40$ . The parameter used to assess the colons was occurrence of ACF. The occurrence was measured by quantifying the mean number of foci of AC per distal colon.

#### *Statistical analysis*

Results were expressed as means $\pm$ SEM. All data were analysed using SPSS version 13 statistical package. Differences were considered significant at  $p\leq 0.05$ .

## **Results**

From Table 1, results suggest that pork oil could significantly promote an increase in faecal pH, whereas the opposite effect was observed in the canola oil group. Deducting from the pH change it is inferred that increased bile excretion occurred in the pork oil group and thus changing the intestine environment. However, Table 2 indicates no change in cholic acid release and thus not correlated with bile release nor pH change. This result

**Table 1. Faecal pH Value (Mean±Standard Deviation)**

Group	Male	Female	Total
Control	8.61±0.28	8.58±0.27	8.59±0.27
COG	8.55±0.27	8.44±0.28*	8.50±0.25
POG	8.65±0.24#	8.67±0.23*#	8.67±0.23*#

\*Compared with the control,  $p<0.05$ ; #: compared with the COG,  $p<0.05$

**Table 2. Cholic Acid Content in Faeces (mg/ml) (Mean±Standard Deviation)**

Group	Male	Female	Total
Control	0.33±0.16	0.34±0.15	0.34±0.15
COG	0.32±0.15	0.34±0.14	0.33±0.14
POG	0.32±0.16	0.37±0.15	0.35±0.15

**Table 3. Aberrant Crypt Foci (ACF) in Different Groups**

Group	Mice No.	Mice bearing ACF	ACF Incidence
Control	20	19	95.00%
COG	19	17	89.47%
POG	17	17	100.00%

**Table 4. Aberrant Crypt Foci (ACF) and Colon Tumour Induced by AOM in Kunming Mice**

Group	Mice n =	ACF		Colon Tumour	
		Mice	Incidence	Mice	Incidence
Negative Control	17	12	70.59%	0	0.00%
AOM Control	20	19	95.00%	1	5.00%

**Table 5. ACF Number/Mouse in Different Groups by Gender**

Group	Male	Female	Total
Control	2.90±1.97	4.80±1.40	3.85±1.93
COG	3.78±3.23	5.10±2.85	4.42±3.01
POG	4.88±2.94*	5.40±2.32	5.35±2.42*

\*Compared with the control,  $p<0.05$

**Table 6. Colon Tumorigenesis in Different Groups**

Group	Male		Female		Tumor Incidence
	Mice	Tumour	Mice	Tumour	
Control	10	0	10	1	5.00%
COG	9	0	10	1	5.26%
POG	7	1	10	1	11.76%

indicates that the food oil type changes pH but does not change the cholic acid content of the in faeces.

As depicted in Table 3, pork oil appears to increase the incidence of aberrant crypt foci (ACF), and canola oil reduces the ACF below the control group. The results suggest that the food oil affect ACF formation. Further, in Table 4, the results show that the Kunming mice were not sensitive to AOM-induced colon tumorigenesis, the observation is solely due to AOM injection.

The results showed pork oil could significantly promote ACF formation (pre-neoplastic foci) in both the male and total population, although this increase was not

significant in the female group. In Table 6, the results showed pork oil could enhance colon tumorigenesis in male mice, which was consistent with the results of ACF (Tables 4 and 5).

## Discussion

There are many factors contributing to colon tumourgenensis including obesity (Kaneko et al., 2014), smoking, inadequate exercise, but specific dietary components such as oil, sugar, herbs, vitamin D, selenium, iron, selenium, folate may influence colon tumorigenensis, mainly via inflammation and oxidative stress (Slattery et al., 2013). He et al., 2014 observed that in Kunming mice exposed to AOM plus canola oil had a strong inhibitory effect of AOM-induced colorectal carcinogenesis. On the other hand, in an 8-year epidemiological study, olive oil (contained in fried foods) was found to protect against the development of colon cancer (Galeone et al., 2007). Similarly to canola oil composition of fatty acids, olive oil also contains 60-80% oleic acid (omega 9 fatty acid) and 21% linoleic acid (omega 6). However, lard (pork oil) contains 45% oleic acid, but contains half the linoleic acid in comparison with canola oil. Further, ALA is found at 11% concentration in canola, and in pork loin (muscle/lard mix) lard it is <0.5%. Thus, both a lower  $\alpha$ -linoleic, little or the absence of ALA in pork lard versus canola oil, suggests that it may be the presence and percentage of fatty acid, combinations and types either promotes or inhibits colon tumor genesis. This hypothesis is supported by the observations by Shinohara et al. 2012, who showed that jacaric acid (conjugated linolenic acid isomer) displayed a potent anti-tumor effect in nude mice with transplanted DLD-1 cells (colorectal adenocarcinoma cell lines). Moreover, Luqman Hakim et al 2014 observed that gelam honey and ginger potentiate the anti-tumor effect (i.e. elevated apoptosis) of 5-FU FU (5-fluorouracil) in an *in vitro* study of HCT 116 colorectal cancer cells. Thus, the consumption of pork oil with phytonutrients such as the numerous compounds found in honey and ginger (i.e. gingerols, shogaols) may act like ALA to have tumor protective properties with a diet high in omega 9, but not omega 6 nor omega 3 fatty acids.

From Table 1, results suggest that pork oil could significantly promote an increase in faecal pH, whereas the opposite effect was observed in the canola oil group. Deducting from the pH change it is inferred that there was an increased bile excretion occurred in the pork oil group and thus changing the intestine environment. However, Table 2 indicates no change in cholic acid release and thus not correlated with bile release nor pH change. This result indicates that the food oil type does not change the cholic acid content of the in faeces, although faecal pH changed. In contrary, fish oil has been shown to reduce bile acid excretion (and the secondary bile acid; lithocholic acid 'LA') and LA being a promoter of colon carcinogenesis suggests a bile acid lowering effect of benefit (Bartram et al., 2007), as see in the canola oil group versus pork oil group. Thus, the discrepancy in the pH between the canola (decreased) and pork oil (increased) may be explained by LA instead of the measured cholic acid. Interestingly, other

plant oil's and fish oil as opposed to animal oils have been shown to reduce the incidence of colon tumorigenesis. It appears that plant oil; flaxseed (Williams et al., 2007), corn oil (Wu et al., 2004), fish oil and or  $\alpha$ -cellulose (colon butyrate formation) (Coleman et al., 2002), olive oil (arachidonic acid metabolism and local PGE (2) synthesis) (Bartola et al., 2000) induce colon cancer protection via different mechanisms to the reduction in the formation of aberrant crypt foci. Again, as previously hypothesized, these observations maybe related to omega 3 fatty acid content of the oil (i.e. anti-inflammatory effect and thus tumor genesis inhibitory effect).

In wistar rats, a diet of corn oil and olive oil plus dimethylhydrazine (carcinogen; DNA methylating agent), had the lowest level of ACF/field and cell proliferation when compared with a standard diet (França Fda et al., 2014), also using Egyptian flaxseed oil (Salim et al., 2011). Fish oil appears to have a protect role for the incidence of pre-cancerous lesions via greater transforming growth factor  $\beta$  expression and lower interleukin-8 expression, but its anti-tumor effect seemed linked to inflammatory modulatory effect via increased accumulation of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in hepatic tissue (Rosa et al., 2012). Further, this observation of a high PUFA content of oil with tumorigenesis inhibitory has also been seen in microalgal oils (van Beelen et al., 2008).

As depicted in Table 3, pork oil appears to increase the incidence of ACF. The results suggest that the food oil affects ACF formation, and possibly a degree of saturation maybe related to degree of ACF formation, as fish oil (polyunsaturated) reduced ACF formation (Moreira et al., 2009), possibly via reduced COX-2 expression (i.e. reduced inflammation) (Rao et al., 2001). ACF formation is related to oil type (i.e. animal versus plant based) rather than related to the percentage of animal fat in the diet (Geter et al., 2004). Almonds and almond oil appear to reduce ACF in azoxymethane (15 mg/kg body weight)-treated F344 male rats when investigated (Davis et al., 2001). As mentioned the higher degree of PUFA content (i.e. fish, flaxseed oils) inhibit ACF formation via a reduction in Wnt/ $\beta$ -catenin signalling (oncogenic pathway) (Fujise et al., 2006), which may also be reduced in omega 3/ omega 6 oil fed rats or mice such as per this study and exposure to mutagens such as AOM. Further, ACF formation may also be related to the haeme content of animal protein (i.e. beef), with calcium possibly protecting against colon tumorigenesis (Pierre et al., 2007). This may explain the result by Parnaud et al. 1998 who fed whole bacon that was salted, and resulted in increased water intake in the rat with reduced colon carcinogenesis.

The results showed pork oil could significantly promote ACF formation (pre-neoplastic foci) in both the male and total population, although this increase was not significant in the female group. In Table 6, the results showed pork oil could enhance colon tumorigenesis in male mice, which was consistent with the results of ACF (Tables 4 and 5). Interestingly, linoleic acid appears to be important in preventing azoxymethane-induced rat colon carcinogenesis through elevation of colonic peroxisome proliferator-activated receptor (PPAR- $\gamma$ ) expression and

alteration of lipid composition in bitter melon extract fed rats (Kohno et al., 2004b) and also pomegranate seed oil (conjugated  $\alpha$ -linolenic acid) fed rats (Kohno et al., 2004a), via reduced PPAR- $\gamma$  protein in the non-tumor mucosa. In conclusion, canola oil, which contains 11% ALA protects Kunming mice against tumorigenesis, via reduced intestinal pH and intestinal inflammation. It is concluded that canola oil may be proposed as a dietary therapy to reduce the relative risk of colon cancer when compared with pork oil.

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