Research Article

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Effects of adlay bran and its ethanolic extract and residue on preneoplastic lesions of the colon in rats

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Abstract

BACKGROUND: Adlay (*Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a cereal crop used in traditional Chinese medicine and as a nutritious food. Epidemiologists have suspected that the low cancer rates in southeastern China might be related to adlay. Previous studies have shown that adlay has anti-tumour and anti-inflammatory activity. This study investigated the effect of adlay bran and its fractions on chemically induced colon carcinogenesis in rats.

RESULTS: Adlay bran and its ethanolic extract and residue significantly reduced the number of preneoplastic aberrant crypt foci (ACF) and modified their mucin composition. The inhibitory effect of adlay bran ethanolic extract on ACF showed a dose dependence. Adlay bran and its ethanolic extract suppressed small ACF (one, two or three crypts) and ACF in the distal colon, while the residue suppressed large ACF (four or more crypts).

CONCLUSION: These findings suggest the possibility that adlay bran and its ethanolic extract and residue inhibit colonic preneoplastic lesions in an early stage. Adlay and its fractions may have the potential to be developed as chemopreventive cereal products.

Keywords: adlay bran; colon cancer; aberrant crypt foci; mucin

INTRODUCTION

Adlay (Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is an annual cereal crop that has long been used in traditional Chinese medicine and as a nutritious food in many Asian countries. The seed of adlay has been widely used as a diuretic, digestive tonic, analgesic, antispasmodic and anti-inflammatory agent from ancient times. Epidemiologists have suspected that the low cancer rates in southeastern China might be related to adlay.[1] Many recent studies have shown that adlay or its components may suppress the development of some cancers *in vitro*[2–7] and *in vivo*.[3,8–11]

Colorectal cancer is the third most common cancer worldwide.[12] The incidence rates of colorectal cancer are increasing rather rapidly in countries where the overall risk was formerly low, especially in Asia.[12] In Taiwan the incidence of colorectal cancer ranks first and the number of deaths from colorectal cancer ranks third among all cancers. Colorectal carcinogenesis is a multistep process. According to the widely accepted 'adenoma–carcinoma sequence' theory, colorectal cancer has a natural history of transition from normal crypts through adenoma to adenocarcinoma occurring over an average of 10–20 years.[13]

Several surrogate endpoint biomarkers (SEBs) of colorectal cancer (i.e. indicators of colorectal cancer risk) have been identified and classified into pathogenic, cellular, biochemical, molecular and genetic markers.[14,15] These SEBs can aid the development of novel therapeutic interventions, preventive strategies and chemopreventive agents. Much interest is currently being directed towards research in the use of SEBs that change early during colorectal carcinogenesis.[13] Preneoplastic lesions, representing an early stage of tumour development, are ideal biomarkers in short-term carcinogenesis studies.[16] Aberrant crypt foci (ACF) and mucin-depleted foci (MDF) are preneoplastic lesions of colorectal cancer in rodents and humans, and their characteristics have been clearly described.[16,17] Recent studies have found that MDF carry alterations in the Wnt signalling pathway and mutations in the β-catenin, *Apc* and *Kras* genes, with a frequency similar to that observed in tumours.[16] Therefore, although MDF have been used so far in a limited number of studies, they are a promising biomarker in colon carcinogenesis.

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Inflammation plays an important role in colon carcinogenesis, and dietary agents can modulate colon carcinogenesis through an anti-inflammatory mechanism.[18–20] Cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS), two markers associated with inflammation, have been shown to play a critical role in signal pathways of colorectal cancer.[21] The above-mentioned pathogenic markers (ACF and MDF) and biochemical markers (COX-2 and iNOS) are considered to be SEBs of colorectal carcinogenesis and widely used in chemopreventive studies.

Our previous study found that dietary dehulled adlay suppressed preneoplastic ACF in colons and COX-2 expression in colon tumours using an animal model of chemically induced colon cancer; however, colon tumours were not inhibited.[11] We concluded that dehulled adlay may suppress early events in colon carcinogenesis, but the active component and its mechanism of action remained unknown. A recent study indicated that adlay bran had high levels of bioactive phytochemicals,[22] suggesting that bran may be the fraction of adlay that acts against colon cancer. To investigate the role of adlay bran in colon carcinogenesis and to elucidate the active fraction, this study examined the effect of adlay bran and its ethanolic extract and residue on preneoplastic lesions of colon cancer.

MATERIALS AND METHODS

Adlay preparation

Adlay was purchased from a local farmer who planted Taichung Shenyu No. 4 (TCS4) variety of *C. lachryma-jobi* L. var. *ma-yuen* Stapf in Taichung, Taiwan. Adlay seeds were dried at ambient temperature with ventilation, dehulled by a grinder and divided into hull, testa and dehulled adlay by gentle blowing using an electric fan. Adlay bran was separated from the dehulled adlay, blended into powder and screened through a 20-mesh sieve (aperture 0.94 mm). The yield of adlay bran from dehulled adlay was 8%. Adlay bran powder was extracted with 950 g kg^{-1} ethanol at room temperature for 24 h. The ethanolic extract was filtered and concentrated under reduced pressure by a rotary vacuum evaporator. The ethanolic residue was freeze-dried and powdered. The yield of ethanolic extract of adlay bran was 18%. The proximate composition of adlay bran and its ethanolic extract and residue was analysed according to the AOAC method[23] and the method of Prosky *et al.*[24] The analytical results are shown in Table 1.

Table 1. Proximate composition of adlay bran, ethanolic extract of adlay bran and residue from ethanolic extraction of adlay bran (wet weight basis)

	Content (g kg^{-1})		
Component	Adlay bran	Ethanolic extract	Residue
Moisture	95	46	64
Ash	68	42	133
Crude fat	286	652	207
Crude protein	142	60	185
Total dietary fibre	337	65	375
NFE[a]	73	135	37

[a] Nitrogen-free extract, 1000 − (moisture + ash + crude fat + crude protein + total dietary fibre).

Animals and diets

The study protocol was approved by the Institutional Animal Care and Use Committee of Taipei Medical University. Male F344 rats were obtained at age 5 weeks from the National Laboratory Animal Center (Taipei, Taiwan). Animals were housed in plastic cages (three or four rats per cage) in a room under controlled conditions of 21 ± 2 °C and 40–60% relative humidity, with a 12/12 h light/dark cycle. They were allowed free access to food and water and fed an American Institute of Nutrition (AIN)-93G diet.[25] After 2 weeks of acclimatisation, animals were assigned to one of eight groups (11 or 12 rats per group). The control group (group C) received an AIN-93G diet. The adlay bran group (group B) received an AIN-93G diet containing 16 g kg^{-1} adlay bran (the bran content of a 200 g kg^{-1} dehulled adlay diet, which was shown to be effective in our previous study).[11] Three groups received an AIN-93G diet with 2.9 g kg^{-1} (group LBE), 8.7 g kg^{-1} (group MBE) and 14.4 g kg^{-1} (group HBE) ethanolic extract of adlay bran respectively. Ethanolic extract at the 2.9 g kg^{-1} level was equal to 16 g kg^{-1} adlay bran. Three groups received an AIN-93G diet with 13.1 g kg^{-1} (group LBR), 39.3 g kg^{-1} (group MBR) and 65.6 g kg^{-1} (group HBR) ethanolic residue of adlay bran respectively. Ethanolic residue at the 13.1 g kg^{-1} level was equal to 16 g kg^{-1} adlay bran. The compositions of the experimental diets are shown in Table 2.

Experimental procedures

Animals were fed the experimental diets beginning 1 week prior to the first carcinogen treatment (intraperitoneal injection of 1,2-dimethylhydrazine (DMH, Acros Organics, Morris Plains, NJ, USA) once weekly for 4 weeks at a dose of 40 mg kg^{-1} body weight). After 10 weeks of feeding, all rats were killed and their colons were removed for evaluation. Body weight and food intake were recorded weekly.

ACF assay

ACF were assessed by the method of Bird.[26] Colons were removed, cut along the longitudinal axis and flushed with phosphate-buffered saline (PBS). Each colon was cut into three (proximal, middle and distal) equal lengths and fixed flat between filter papers in 100 g kg^{-1} buffered formalin (Mallinckrodt Specialty Chemicals Co., Paris, KY, USA) for at least 24 h. The fixed colon sections were stained with a 2 g kg^{-1} solution of methylene blue (Showa Chemicals Co., Tokyo, Japan) and placed on microscopic slides with the mucosal side up. ACF were examined under 40× magnification using a light microscope (Nikon Corp., Tokyo, Japan) and distinguished from normal crypts by their increased size, irregular and dilated luminal opening and thicker epithelial lining and pericryptal zone. The number of ACF per colon, the number of aberrant crypts observed in each focus and the location of each focus were recorded.

Determination of MDF and mucin-producing ACF

MDF and mucin-producing ACF were visualised by staining with high iron diamine alcian blue (HIDAB).[27] A high iron diamine solution was prepared by dissolving 20 mg of *N,N′*-dimethyl-*p*-phenylene diamine and 120 mg of *N,N′*-dimethyl-*m*-phenylene diamine (both from Sigma Chemical Co., St Louis, MO, USA) in 50 mL of distilled water plus 1.4 mL of 600 g kg^{-1} ferric chloride (Nacalai Tesque, Inc., Tokyo, Japan). The methylene blue-stained colons were immersed in the high iron diamine solution in a Petri dish protected from light for 50 min at room temperature,

Table 2. Experimental diets

Ingredient (g kg^{-1})	Group[a] C	B	LBE	MBE	HBE	LBR	MBR	HBR
Adlay bran	–	16.0	–	–	–	–	–	–
Ethanolic extract of adlay bran	–	–	2.9	8.7	14.4	–	–	–
Residue from ethanolic extraction of adlay bran	–	–	–	–	–	13.1	39.3	65.6
Cornstarch	397.5	393.7	396.5	395.6	394.3	394.4	388.3	382.2
Casein	200.0	197.7	199.8	199.5	199.1	197.6	192.7	187.9
Dextrinised cornstarch	132.0	132.0	132.0	132.0	132.0	132.0	132.0	132.0
Sucrose	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Soybean oil	70.0	65.4	68.1	64.4	60.6	67.3	61.9	56.5
α-Cellulose	50.0	44.6	49.8	49.4	49.1	45.1	35.2	25.4
AIN-93G-MX mineral mix	35.0	35.0	35.0	35.0	35.0	35.0	35.0	35.0
AIN-93-VX vitamin mix	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
L-Cystine	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Choline bitartrate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
tert-Butylhydroquinone (mg kg^{-1})	14.0	14.0	14.0	14.0	14.0	14.0	14.0	14.0

[a] C, AIN-93G diet; B, 16 g kg^{-1} adlay bran; LBE, 2.9 g kg^{-1} ethanolic extract of adlay bran; MBE, 8.7 g kg^{-1} ethanolic extract of adlay bran; HBE, 14.4 g kg^{-1} ethanolic extract of adlay bran; LBR, 13.1 g kg^{-1} residue from ethanolic extraction of adlay bran; MBR, 39.3 g kg^{-1} residue from ethanolic extraction of adlay bran; HBR, 65.6 g kg^{-1} residue from ethanolic extraction of adlay bran.

rinsed in distilled water, stained with 10 g kg^{-1} alcian blue (Sigma Chemical Co.) in 30 g kg^{-1} acetic acid (Nacalai Tesque, Inc.) for 30 min, rinsed in 800 g kg^{-1} ethanol (Taiwan Tobacco and Liquor Co., Taipei, Taiwan), rinsed in distilled water and observed under 40× magnification using a light microscope (Nikon Corp.). MDF were identified using the criteria established by Caderni *et al*.[28] Briefly, to be considered mucin-depleted, a focus had to show no or very little production of mucins in addition to fulfilling two of the following: (1) distortion of the opening of the lumen compared with normal surrounding crypts; (2) elevation of the lesion above the surface of the colon; (3) crypt multiplicity >3. The distal colons were also examined for mucin-producing ACF using the criteria established by Jenab *et al*.[29] Dark brown staining of ACF by HIDAB implied sulfomucin (SUM) production, while bright or dark blue staining indicated sialomucin (SIM) production. ACF with more than 85% SUM-producing cells were considered as SUM-ACF, those with more than 85% SIM-producing cells were considered as SIM-ACF and those with a smaller percentage of both were considered as mixed-type ACF (MIX-ACF).

Statistical analysis

Data are expressed as mean ± standard deviation (SD). Differences in data between the experimental groups were assessed using the Mann–Whitney–Wilcoxon (MWW) test and SAS Version 8.1 (SAS Institute, Cary, NC, USA). Means were considered significantly different at $P < 0.05$.

RESULTS

There were no significant differences in initial body weight, final body weight, body weight gain, feed intake and feed efficiency in all groups (data not shown). As shown in Table 3, adlay bran (group B) and its ethanolic extract (groups LBE, MBE and HBE) and residue (groups LBR, MBR and HBR) significantly reduced the number of ACF as compared with the control group (group C) ($P < 0.05$). The inhibitory effect of adlay bran ethanolic extract on ACF and aberrant crypts showed a dose dependence. The crypt multiplicity (number of aberrant crypts/focus) was not affected by adlay bran and its ethanolic extract and residue, except for MBE, which resulted in a significantly higher crypt multiplicity than did C ($P < 0.05$).

Table 3. Effect of adlay bran, ethanolic extract of adlay bran and residue from ethanolic extraction of adlay bran on DMH-induced ACF in colon of male F344 rats[a]

Experimental group[b]	Number of ACF/colon	Number of aberrant crypts/colon	Crypt multiplicity (number of aberrant crypts/focus)
Control (C)	186 ± 26	405 ± 55	2.2 ± 0.1
Bran (B)	113 ± 22[c]	265 ± 52[c]	2.3 ± 0.2
Low bran extract (LBE)	148 ± 16[c]	342 ± 49	2.3 ± 0.1
Medium bran extract (MBE)	135 ± 27[c]	329 ± 72	2.4 ± 0.2[c]
High bran extract (HBE)	117 ± 18[c,d]	267 ± 37[c]	2.3 ± 0.1
Low bran residue (LBR)	143 ± 12[c]	313 ± 36[c]	2.2 ± 0.1
Medium bran residue (MBR)	130 ± 41[c]	285 ± 85[c]	2.2 ± 0.2
High bran residue (HBR)	136 ± 33[c]	286 ± 66[c]	2.1 ± 0.2

[a] All values are mean ± SD ($n = 8$). The ACF incidence is 100% in each group.
[b] C, AIN-93G diet; B, 16 g kg^{-1} adlay bran; LBE, 2.9 g kg^{-1} ethanolic extract of adlay bran; MBE, 8.7 g kg^{-1} ethanolic extract of adlay bran; HBE, 14.4 g kg^{-1} ethanolic extract of adlay bran; LBR, 13.1 g kg^{-1} residue from ethanolic extraction of adlay bran; MBR, 39.3 g kg^{-1} residue from ethanolic extraction of adlay bran; HBR, 65.6 g kg^{-1} residue from ethanolic extraction of adlay bran.
[c] Significantly different from group C (MWW test, $P < 0.05$).
[d] Significantly different from group LBE (MWW test, $P < 0.05$).

Table 4. Effect of adlay bran, ethanolic extract of adlay bran and residue from ethanolic extraction of adlay bran on number of DMH-induced ACF according to number of crypts in colon of male F344 rats[a]

Experimental group[b]	Number of foci containing			
	1 crypt	2 crypts	3 crypts	4 or more crypts
Control (C)	47 ± 16	82 ± 10	40 ± 9	17 ± 8
Bran (B)	23 ± 10[c]	47 ± 8[c]	27 ± 7[c]	16 ± 8
Low bran extract (LBE)	33 ± 4[c]	61 ± 8[c]	36 ± 7	19 ± 7
Medium bran extract (MBE)	26 ± 7[c]	62 ± 13[c]	33 ± 9	16 ± 7
High bran extract (HBE)	22 ± 7[c]	53 ± 12[c]	29 ± 4[c]	12 ± 4
Low bran residue (LBR)	33 ± 3[c]	67 ± 10[c]	32 ± 5	12 ± 5
Medium bran residue (MBR)	30 ± 15[c]	57 ± 19[c]	32 ± 9	11 ± 4
High bran residue (HBR)	30 ± 12[c]	61 ± 13[c]	36 ± 9	9 ± 4[c]

[a] All values are mean ± SD ($n = 8$).
[b] C, AIN-93G diet; B, 16 g kg^{-1} adlay bran; LBE, 2.9 g kg^{-1} ethanolic extract of adlay bran; MBE, 8.7 g kg^{-1} ethanolic extract of adlay bran; HBE, 14.4 g kg^{-1} ethanolic extract of adlay bran; LBR, 13.1 g kg^{-1} residue from ethanolic extraction of adlay bran; MBR, 39.3 g kg^{-1} residue from ethanolic extraction of adlay bran; HBR, 65.6 g kg^{-1} residue from ethanolic extraction of adlay bran.
[c] Significantly different from group C (MWW test, $P < 0.05$).

Table 5. Effect of adlay bran, ethanolic extract of adlay bran and residue from ethanolic extraction of adlay bran on distribution of DMH-induced ACF in colon of male F344 rats[a]

Experimental group[b]	ACF distribution		
	Proximal colon	Middle colon	Distal colon
Control (C)	12 ± 11	71 ± 26	104 ± 19
Bran (B)	7 ± 4	53 ± 22	53 ± 7[c]
Low bran extract (LBE)	14 ± 6	66 ± 23	69 ± 18[c]
Medium bran extract (MBE)	11 ± 8	57 ± 17	66 ± 24[c]
High bran extract (HBE)	8 ± 5	49 ± 11	61 ± 16[c]
Low bran residue (LBR)	10 ± 8	58 ± 26	76 ± 27
Medium bran residue (MBR)	7 ± 4	55 ± 27	68 ± 12[c]
High bran residue (HBR)	5 ± 4	53 ± 13	78 ± 28

[a] All values are mean ± SD ($n = 8$).
[b] C, AIN-93G diet; B, 16 g kg^{-1} adlay bran; LBE, 2.9 g kg^{-1} ethanolic extract of adlay bran; MBE, 8.7 g kg^{-1} ethanolic extract of adlay bran; HBE, 14.4 g kg^{-1} ethanolic extract of adlay bran; LBR, 13.1 g kg^{-1} residue from ethanolic extraction of adlay bran; MBR, 39.3 g kg^{-1} residue from ethanolic extraction of adlay bran; HBR, 65.6 g kg^{-1} residue from ethanolic extraction of adlay bran.
[c] Significantly different from group C (MWW test, $P < 0.05$).

As shown in Table 4, adlay bran and its ethanolic extract and residue significantly reduced the numbers of one- and two-crypt ACF as compared with group C ($P < 0.05$). In addition, B and HBE significantly reduced the number of three-crypt ACF ($P < 0.05$), whereas HBR reduced the number of ACF consisting of four or more crypts ($P < 0.05$). ACF were mainly observed in the distal colon, and their numbers were significantly decreased in all experimental groups except for LBR and HBR as compared with group C ($P < 0.05$) (Table 5).

MDF appeared in all groups. The MDF incidences were slightly but not significantly lower in groups HBR (25.0%) and HBE (37.5%) than in group C (62.5%) (data not shown). Adlay bran and its ethanolic extract and residue did not affect the number and crypt multiplicity of MDF (data not shown); however, they significantly reduced the number of ACF producing sulfomucin (SUM-ACF) ($P < 0.05$) (Table 6). B, LBE, HBE, MBR and HBR also significantly reduced the number of ACF producing both sulfomucin and sialomucin (MIX-ACF) ($P < 0.05$), whereas B significantly reduced the number of ACF producing sialomucin (SIM-ACF) ($P < 0.05$).

DISCUSSION

Our previous study found that dehulled adlay suppressed the development of preneoplastic ACF in the colon of carcinogen-treated rats.[11] The present study further demonstrated that adlay bran may be the milling fraction active against colon carcinogenesis. We have also shown that both the ethanolic extract and the residue from ethanolic extraction of adlay bran suppressed the development of ACF and that the inhibitory effect of adlay bran ethanolic extract on ACF and aberrant crypts showed a dose dependence. These results suggest the possibility that some of the active components of adlay exist in the bran ethanolic extract and others exist in the residue.

It has been proposed that ACF develop via a fission mechanism of crypt growth.[30] Briefly, an ACF with a single crypt and round bottom becomes (via proliferation) an ACF with bifurcating crypts and V-shaped clefts at the bottom and then becomes a two-crypt ACF. ACF with more crypts are more advanced. The results of this study indicated that adlay bran and its ethanolic extract mainly reduce the number of small ACF (one, two or three crypts), whereas the residue from ethanolic extraction of adlay bran mainly reduces the number of large ACF (with four or more crypts), suggesting that adlay bran and its ethanolic extract and the residue from ethanolic extraction may intervene in an early and a later stage of ACF development respectively.

ACF develop as early as 2–4 weeks after carcinogen administration and appear predominantly in the distal colon during early time points; in time, ACF appear in the proximal colon and some of them begin to expand.[26,27] In the present study, adlay bran and its ethanolic extract and residue reduced the number of ACF in the distal colon. The residue reduced the number of ACF in the proximal colon, although the reduction was not statistically significant, because the numbers of ACF were small and the inter-individual differences in the number of ACF were large. Analysis of the ACF distribution also indicates that adlay bran and its ethanolic extract may suppress the development of ACF mainly in an early stage and that the residue may suppress the development of ACF mainly in a later stage.

The distal colon of rats shows a pattern of mucus production similar to that of the normal human colorectal mucosa in which SUM secretion predominates.[27] Preneoplastic alterations in the distal colon of rats are accompanied by a shift from SUM to SIM secretion, suggesting that SIM-dominant ACF have the potential to progress to colon tumours.[31,32] Caderni *et al.*[27] found that when ACF multiplicity increases, the number of ACF producing SUM decreases rapidly, whereas the number of ACF containing both SUM and SIM increases progressively. The number of ACF containing only SIM increases slowly with increasing ACF

Table 6. Effect of adlay bran, ethanolic extract of adlay bran and residue from ethanolic extraction of adlay bran on number of DMH-induced ACF according to type of mucin produced by foci in distal colon of male F344 rats[a]

Experimental group[b]	Number of ACF producing[c] SUM	MIX	SIM
Control (C)	49 ± 13	35 ± 13	20 ± 8
Bran (B)	26 ± 7[d]	16 ± 6[d]	11 ± 4[d]
Low bran extract (LBE)	26 ± 7[d]	23 ± 6[d]	20 ± 8
Medium bran extract (MBE)	21 ± 8[d]	27 ± 1	17 ± 6
High bran extract (HBE)	20 ± 5[d]	22 ± 7[d]	19 ± 6
Low bran residue (LBR)	27 ± 10[d]	29 ± 9	21 ± 10
Medium bran residue (MBR)	25 ± 6[d]	24 ± 5[d]	20 ± 7
High bran residue (HBR)	32 ± 15[d]	25 ± 8[d]	21 ± 9

[a] All values are mean ± SD ($n = 8$).
[b] C, AIN-93G diet; B, 16 g kg^{-1} adlay bran; LBE, 2.9 g kg^{-1} ethanolic extract of adlay bran; MBE, 8.7 g kg^{-1} ethanolic extract of adlay bran; HBE, 14.4 g kg^{-1} ethanolic extract of adlay bran; LBR, 13.1 g kg^{-1} residue from ethanolic extraction of adlay bran; MBR, 39.3 g kg^{-1} residue from ethanolic extraction of adlay bran; HBR, 65.6 g kg^{-1} residue from ethanolic extraction of adlay bran.
[c] SUM, sulfomucin; MIX, mixed sulfomucin and sialomucin; SIM, sialomucin.
[d] Significantly different from group C (MWW test, $P < 0.05$).

multiplicity. Based on their higher rate of cell proliferation, higher degree of dysplasia, greater size and greater degree of luminal change relative to SUM-producing ACF, it has been proposed that SIM-producing ACF are more advanced preneoplastic lesions of the colon mucosa.[29] In the present study, adlay bran ethanolic extract and residue reduced the numbers of SUM-ACF and MIX-ACF but not the number of SIM-ACF. In contrast, adlay bran was able to suppress the formation of SIM-ACF. It seems that adlay bran and its ethanolic extract and residue all play a role in early change in the mucin composition of ACF; adlay bran was most efficacious in preventing later change in the mucin composition of ACF.

MDF appear about 7 weeks after carcinogen administration, and their multiplicity increases with time.[28] MDF are believed to be a promising biomarker for studying the effect of chemopreventive agents on colon carcinogenesis.[16] In this study, adlay bran and its ethanolic extract and residue had no effect on MDF formation, although high levels of both adlay ethanolic extract and residue slightly decreased the incidence of MDF. The colons of rats in this study were observed 9 weeks after the first DMH injection; the average MDF number per rat was only 1.4–2.7, which is lower than that observed in other studies.[33,34] Presumably, the low number of MDF due to low carcinogen dosage and/or short induction time may explain the absence of an effect on MDF formation.

Several compounds in adlay have been proven to have anti-cancer activity. These compounds include coixenolide, α-monolinolein, fatty acids and phytosterols, which are all in the lipid-associated fraction of adlay.[8,9,22,35] Recently, we isolated five active lactam compounds from adlay bran methanolic extract and found that they strongly inhibited proliferation of human lung and colorectal cancer cell lines.[6] Lactams that are widely used as antibacterial agents with no significant toxicity also possess anti-cancer activity.[36] We expect that the lactam compounds obtained from adlay bran will be developed into novel drugs for cancer chemoprevention. In addition, we have isolated several phenolic acids from ethanolic extracts of adlay testa (the outermost layer of bran) and identified chlorogenic, caffeic and ferulic acids as the major components responsible for the antioxidant and anti-inflammatory effects of adlay testa extract in RAW 264.7 macrophages.[37] These results suggest that components of adlay, especially lipid-soluble components, have anti-cancer potential *in vitro*. However, the *in vivo* anti-cancer effects of these components remain to be elucidated.

CONCLUSION

The present study demonstrated that adlay and its fractions interfered with DMH-induced colon carcinogenesis in rats. Adlay bran and its ethanolic extract and residue suppressed the formation of preneoplastic ACF and modified their mucin composition but did not significantly affect MDF. These results suggest that adlay bran and its ethanolic extract and residue may inhibit early during colon carcinogenesis. Adlay and its fractions may have the potential to be developed as chemopreventive cereal products.

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