Food Sci. Biotechnol. Vol. 17, No. 6, pp. 1265 ~ 1271 (2008)

Food Science and Biotechnology

The Korean Society of Food Science and Technology

Anti-tumor and Anti-inflammatory Activity of the Methanol Extracts from Adlay Bran

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Abstract Adlay bran is a waste product previously thought to have no commercial value. Its methanolic extract was fractionated using *n*-hexane (ABM-Hex), ethyl acetate (ABM-EtOAc), 1-butanol (ABM-BuOH), and water (ABM-H_2O). The ABM-EtOAc fraction exhibited a strongest inhibition against growth of human lung cancer cell A549 and human colorectal carcinoma cells HT-29 and COLO 205. Inhibition of cell cycle progression at G_0/G_1 transition, increase of cells at the sub-G_1 phase, and DNA ladders were observed in cells treated with ABM-EtOAc. The ABM-BuOH fraction showed the strongest inhibition of proinflammatory cytokines tumor necrosis factor (TNF)-α and interlukin (IL)-1β in stimulated RAW 264.7 macrophages. Further, ABM-EtOAc and ABM-BuOH inhibited cyclooxygenase (COX)-2 expression in A549 and HT-29 carcinoma cells, while COX-1 expression was not affected. These results reveal that both ABM-EtOAc and ABM-BuOH may aid the prevention of cancers and the applications in cancer chemotherapy.

Keywords: A549 cell, COLO 205 cell, cyclooxygenase-2 (COX-2), cytotoxicity, HT-29 cell

Introduction

Adlay (soft-shelled Job's tears, *Coix lachryma-jobi* L. var. *ma-yuen* Stapf) is a grass crop that has long been used in traditional Chinese medicine as a 'nourishing' food. In the ancient Chinese medical text *Pen-Tsao-Kang-Mu* (1), adlay is prescribed for the treatment of warts, chapped skin, rheumatism, and neuralgia. Cereal wastes are particularly rich in polyphenols or bioactive compounds which were promising sources of interesting pharmacological properties (2). They may cause ground or water pollution if simply dumped and, in many cases, are unsuitable for animal feeding due to low digestibility or bitter taste. Even composting or incineration may cause problems due to the formation of unpleasant odor (3). Adlay seeds without the hull and testa are known as dehulled adlay. Removing the bran of dehulled adlay will make polished adlay. The adlay hull, testa, and bran are generally considered the waste products of adlay processing. Currently, adlay bran is mostly used as fertilizer or feed for the poultry industry.

A number of recent studies have shown that adlay may have anti-inflammatory and anti-tumor activities. The adlay seed has anti-inflammatory properties, which inhibited nitric oxide and superoxide anion radical production by activated macrophages (4). The anti-proliferative activity of adlay hull against human histolytic lymphoma U937 monocytic cells via apoptosis was reported (5). Moreover, the dehulled adlay has anti-proliferative and chemopreventive effects on lung cancer *in vivo* (6,7). In addition, rats were fed diets containing dehulled adlay significantly reduces tumor incidence, and cyclooxygenase-2 (COX-2) protein expression in the azoxymethane (AOM)-induced colon carcinogenesis rat model (8). COX-2, an inducible enzyme functionally related to inflammation and carcinogenesis, is the target of many chemopreventive agents. Tumor promoters and cytokines in cancer cells produce a large amount of prostaglandins and COX-2. Various studies report increased COX-2 expression as a consequence of carcinogenesis, angiogenesis, and tumor metastasis in gastric cancer, esophageal cancer, lung cancer, colon cancer, breast cancer, hypopharynx squamous cell carcinoma, and head and neck cancers. Inhibition of COX-2 by non-steroidal anti-inflammatory drugs (NSAIDs) has been shown to be effective in controlling carcinogenesis as well as in reducing the risk and metastasis of lung and colon cancers (9,10).

Recent studies have shown that duhulled adlay had a higher anti-inflammation and anti-tumor activity than polished adlay. However, the characteristics of different extracts of adlay bran are still unknown. Adlay bran is a cheap and abundant byproduct in the adlay processing industry. The objective of this study was to explore whether adlay bran extract has potential chemopreventive activity. Our investigation includes extracting adlay bran with methanol and further partitioning the methanolic extract with *n*-hexane, ethyl acetate, 1-butanol, and water. Finally, the anti-tumor and anti-inflammatory activities of these different polar subfractions were analyzed.

Materials and Methods

Cell culture and reagents MRC-5 (human lung fibroblast),

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Received May 7, 2008; Revised July 1, 2008;
Accepted July 2, 2008

A549 (human lung cancer), HT-29 (human colon carcinoma), COLO 205 (human colon carcinoma), and RAW 264.7 (murine monocyte/macrophage) cells were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% heat-inactivated fetal bovine serum (FBS, Hyclone, Logan, UT, USA), 100 units/mL of penicillin (Sigma-Aldrich, St. Louis, MO, USA) and 100 μg/mL of streptomycin (Sigma-Aldrich), and were kept at 37°C in a 5% CO_2 incubator. The anti-COX-1 and anti-COX-2 antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). The extraction and partition procedure of the adlay bran was the same as described by Kuo *et al.* (5). Briefly, the adlay bran methanolic extract (ABM) was obtained and then divided into 4 subfractions denoted as the ABM-Hex (*n*-hexane fraction), ABM-EtOAc (ethyl acetate fraction), ABM-BuOH (1-butanol fraction), and ABM-H_2O (water fraction).

Cytotoxicity assay After 48 hr treatment with different concentrations of the fractions, the viability of MRC-5, A549, HT-29, and COLO 205 cells was determined by [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] (MTT) and lactate dehydrogenase (LDH) assays. The MTT assay was performed as described by Hansen *et al.* (11). LDH assay was performed using an LDH assay kit (Roche, Mannheim, Germany) (12). Briefly, cell medium was collected from each treatment and mixed with LDH substrate in a 96-well plate. After incubation for 30 min at room temperature, the absorbance was measured at 490 nm by a microplate reader (ThermoSpectronic, New York, NY, USA). The optical density measured was converted after standardization with low (no treatment; 0% toxicity) and high controls (1% Triton X-100; 100% toxicity), and the cytotoxicity was calculated using the following equation:

$$\text{Cytotoxicity (\%)} = [(\text{experimental value} - \text{low control})/(\text{high control} - \text{low control})] \times 100$$

DNA extraction and electrophoresis A549, HT-29, and COLO 205 cells (10^6 cells/mL) were harvested, washed with phosphate buffered saline (PBS), lysed in 100 mL of lysis buffer [50 mM Tris, pH 8.0; 10 mM ethylenediamine tetraacetic acid (EDTA), 0.5% sodium sarcosinate, and 1 mg/mL proteinase K] at 56°C overnight and treated with 0.5 μg/mL RNase A for 3 hr at 56°C. The DNA was extracted using the phenol:chloroform: isoamyl alcohol (25:24:1) method (13). DNA samples were loaded onto a presolidified 2% agarose gel containing 0.1 μg/mL ethidium bromide. The agarose gels were run at 50 V for 90 min in Tris-borate-EDTA (TBE) buffer, then observed and photographed under ultraviolet (UV) light.

Flow cytometry analysis Cells were cultured in the absence or presence of the test samples for 48 hr in 10% FBS medium. Cells were fixed in 100% ethanol and stained with propidium iodide. Cell cycle distribution was analyzed by fluorescence-activated cell sorting (FACS) flow cytometry (Becton Dickinson, San Jose, CA, USA) as previously described (14).

Nitric oxide (NO) quantification and analysis of cytokine production NO production in RAW 264.7 cell culture supernatant was determined using the Griess reaction by mixing 100 μL of culture supernatant with 100 μL of Griess reagent (15). Cytokines tumor mecrosis factor (TNF)-α and interlukin (IL)-1β in RAW 264.7 cell culture supernatant were determined using a commercial enzyme immunoassay kit (R&D Systems, Minneapolis, MN, USA) according to the manufacturer's protocol.

Western blot Western blot was performed as described by Chang *et al.* (6). Total cellular proteins derived from cells under different treatments were prepared and separated on 12% sodium dodecyl sulfate (SDS)-polyacrylamide gels for COX-1 and COX-2 detection. Expression of the indicated protein was analyzed with an ECL chemiluminescence system (Amersham Pharmacia, NJ, USA) and reported with actin antibody to confirm equal loading of proteins in each lane.

Statistical analysis The results were statistically evaluated using Student's *t* test. Comparison was made between the control and the experimental values.

Results and Discussion

Cytotoxic effect of different ABM fractions on MRC-5, A549, HT-29, and COLO 205 carcinoma cells After 48 hr treatment with different concentrations of the fractions, the viability of MRC-5, A549, HT-29, and COLO 205 cells was determined by MTT and LDH assay. The results of the MTT assay indicated that the ABM-EtOAc had a higher suppressive proliferration effect on the carcinoma cells tested than ABM-Hex, ABM-BuOH, and ABM-H_2O samples (Table 1). ABM-EtOAc (200 μg/mL), however, showed relatively little cytotoxicity on the MRC-5 cells decreasing 23% cell viability. ABM-EtOAc inhibited A549 cell viability with an IC_{50} of 82 μg/mL, and this effect was dose-dependent. However, COLO 205 and HT-29 cells were more resistant to ABM-EtOAc by showing the IC_{50} values 131 and 153 μg/mL, respectively. MTT test revealed that cells cultured with ABM-EtOAc at lower than 100 μg/mL and ABM-BuOH at lower than 200 μg/mL, for 48 hr, did not alter MRC-5 cell viability compared with the group cultured with vehicle. LDH release from MRC-5 cells in the presence of ABM-EtOAc was negligible up to 200 μg/mL. A549 cells displayed the strongest cytotoxicity, 28% with ABM-EtOAc (200 μg/mL), while the HT-29, COLO 205, and MRC-5 cells displayed 22, 12, and 5% cytotoxicity, respectively, under the same conditions based on LDH assay. ABM-EtOAc below 100 μg/mL inhibited the cancer cell growth, but LDH release caused by cell membrane damage did not occur, suggesting that the decreasing of cell numbers was not related to necrosis (16). When the cancer cells were treated with a high dose of ABM-EtOAc (200 μg/mL) after 48 hr, the apoptotic time was too long to allow the release of LDH due to partial membrane damage turned into necrosis. Fadok *et al.* (17) indicated that the apoptotic cell will turn to necrosis at last, and it was also found in this study.

Table 1. Effect of ABM fractions on cell viability[1)] and cytotoxicity[2)] on MRC-5, A549, HT-29, and COLO 205 cells

Sample	Conc. (μg/mL)	MRC-5		A549		HT-29		COLO 205	
		MTT[1)]	LDH[2)]	MTT	LDH	MTT	LDH	MTT	LDH
ABM-H_2O	25	1.00±0.07[3)]	0.04±0.06	0.99±0.06	0.06±0.06	1.08±0.05	0.02±0.04	0.98±0.06	0.04±0.04
	50	1.02±0.08	0.04±0.06	1.00±0.09	0.03±0.05	1.00±0.07	0.02±0.04	0.99±0.05	0.02±0.04
	100	1.00±0.08	0.06±0.04	0.97±0.06	0.06±0.05	1.00±0.09	0.05±0.07	0.97±0.06	0.05±0.04
	200	1.08±0.07	0.05±0.05	0.90±0.06	0.06±0.04	0.94±0.05	0.08±0.05	0.92±0.06	0.07±0.06
ABM-BuOH	25	0.96±0.06	0.04±0.05	0.91±0.07	0.03±0.03	0.97±0.06	0.02±0.05	0.99±0.08	0.02±0.05
	50	1.01±0.08	0.05±0.05	0.94±0.05	0.03±0.06	1.00±0.04	0.08±0.05	1.02±0.07	0.01±0.05
	100	1.00±0.04	0.08±0.06	0.89±0.07	0.08±0.05	0.97±0.07	0.06±0.08	0.95±0.07	0.06±0.06
	200	0.93±0.05	0.06±0.06	0.74±0.08**	0.09±0.06	0.88±0.07*	0.08±0.07	0.89±0.08	0.07±0.08
ABM-EtOAc	25	1.01±0.08	0.02±0.04	0.88±0.07*	0.05±0.05	0.92±0.07	0.04±0.04	0.98±0.11	0.04±0.04
	50	1.04±0.07	0.02±0.07	0.64±0.05**	0.03±0.04	0.83±0.06*	0.06±0.06	0.91±0.06	0.01±0.02
	100	0.93±0.06	0.06±0.06	0.39±0.07**	0.09±0.07	0.62±0.07**	0.08±0.06	0.68±0.06*	0.08±0.08
	200	0.77±0.05**	0.05±0.06	0.12±0.07**	0.28±0.05**	0.23±0.03**	0.22±0.09*	0.31±0.06**	0.12±0.07*
ABM-Hex	25	1.01±0.07	0.05±0.05	1.00±0.06	0.05±0.06	1.03±0.06	0.03±0.05	0.98±0.11	0.02±0.07
	50	1.06±0.06	0.03±0.03	0.93±0.05	0.05±0.06	1.02±0.06	0.04±0.03	0.91±0.06	0.03±0.04
	100	0.99±0.07	0.05±0.08	0.86±0.06*	0.05±0.07	0.95±0.04	0.07±0.05	0.68±0.06	0.05±0.05
	200	0.95±0.05	0.07±0.05	0.71±0.06**	0.12±0.08	0.85±0.06*	0.08±0.06	0.31±0.06**	0.08±0.06

[1)]Obtained from MTT assay was expressed as a % control values.
[2)]Obtained from LDH assay was expressed as a % extracellular LDH.
[3)]Values are presented as mean±SD of 3 independent experiments, each using 6 replicate wells/concentration (*p< 0.05, **p< 0.01).

Effect of ABM-EtOAc on apoptosis-inducing activity in A549, HT-29, and COLO 205 carcinoma cells In order to elucidate the type of cell death induced by ABM-EtOAc, DNA integrity, and cell cycle distribution were analyzed. A common feature of apoptosis is the activation of certain endonucleotidases leading to a DNA ladder. Our results indicate that ABM-EtOAc induced typical apoptosis in COLO 205 cells (Fig. 1). On the other hand, the A549 and HT-29 cancer cells treated with ABM-EtOAc did not showed an obvious dose-dependent fragmentation pattern after 48 hr. Therefore, the cell death process may occur via necrosis, because A549 and HT-29 are sensitive to ABM-EtOAc. This suggests that if the treatment time is long, the cell will turn to necrosis. This hypothesis is supported by the results of the LDH assay. A549, HT-29, and COLO 205 treated with ABM-EtOAc were analyzed by flow cytometry using propidium iodide (PI) staining (Table 2). An increased sub-G_1 peak, which has been suggested to be the apoptotic DNA, was observed. The percentages of apoptotic COLO 205 cells were 0.1, 3.46, 18.47, 25.81, and 36.86% of control values for 25, 50, 100, and 200 μg/mL ABM-EtOAc treatment, respectively (Table 2). In A549 and HT-29 cells, ABM-EtOAc inhibited the cell cycle progression at the G_1/S transition, by preventing S-phase entry. Cell cycle arrest at the G_0/G_1 phase and inhibition of cell cycle progression at the G_1/S transition was dose-dependent. ABM-EtOAc treatment induced a concentration-dependent G_0/G_1 phase cell cycle arrest with an accompanying decrease in the G_2/M phase in A549 and HT-29 cells (Table 2). A similar result from another report demonstrated that the methanolic extract of adlay seed inhibited cell cycle progression at the G_0/G_1 transition, and found that adlay seed extract suppressed cyclin A expression but did not affect cyclin D1 and E expressions (6). Many reports have indicated that the extended cell cycle arrest will induce the cell to gradually turn into apoptosis (18,19). Additionally, in COLO 205 cells, we found a sub-G_1 peak, which is characteristic of typical apoptosis. This apoptotic phenomenon is consistent with the appearance of the DNA ladder. Apoptosis plays an important role in many biological processes including carcinogenesis, tumorigenesis, and the removal of damaged precancerous cells (20). The induction of apoptosis or terminal differentiation of malignant cells provides an important viable strategy for the management of cancer (21). This is the first report on ABM-EtOAc-induced apoptosis in human lung and colorectal carcinoma cells. Many natural products used in cancer chemotherapy act through the induction of cell cycle arrest and apoptosis to prevent tumor promotion and progression (22-25). ABM-EtOAc with its ability to induce apoptosis in tumor has the potential for use in anti-tumor therapy.

Effect on nitrite and proinflammatory cytokine production in RAW 264.7 macrophages RAW 264.7 cells were cultured either in medium alone or in a medium containing interferon (IFN)-γ (50 U/mL) and lipopolysaccharide (LPS) (1 μg/mL). The cells were then treated with the 4 fractions of ABM, and NO release was measured using Griess reagent. Among the four samples, ABM-BuOH and ABM-EtOAc significantly reduced nitrite accumulation and showed concentration-dependent inhibition. At 200 μg/mL concentration, the inhibitory effect of the ABM-BuOH and ABM-EtOAc fractions were 42.8 and 31.6%, respectively. To evaluate the effect of the test treatments on proinflammatory cytokine production, LPS stimulated release of TNF-α and IL-1β was investigated using RAW 264.7 cells. LPS remarkably increased production of TNF-α and IL-1β from 66±33 and 50±34 pg/mL to 1036±56

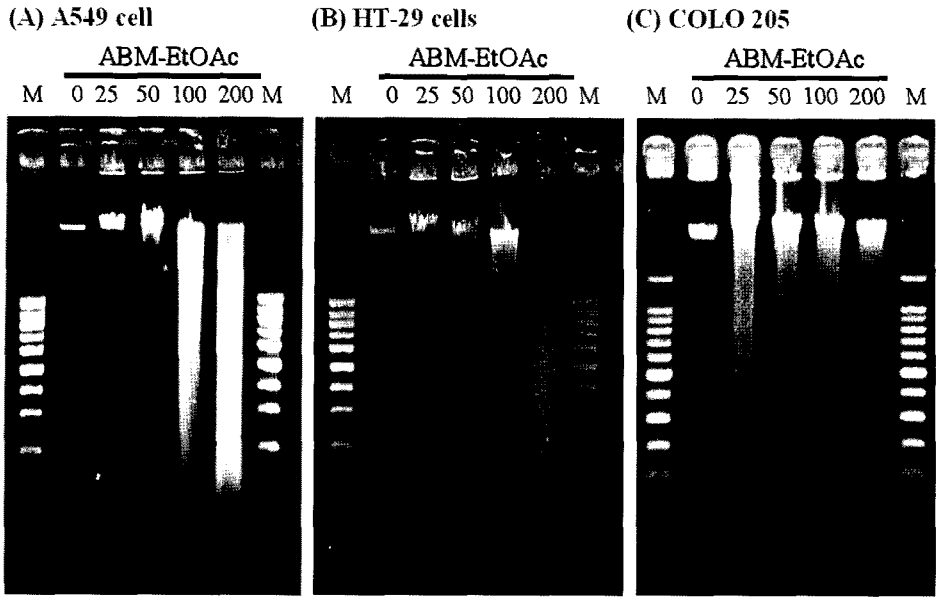

Fig. 1. Effect of ABM-EtOAc on DNA fragmentation of A549, HT-29, and COLO 205 cells. Cells were treated with 25, 50, 100, and 200 μg/mL of ABM-EtOAc for 48 hr.

and 488±38 pg/mL, respectively. ABM did not enhance TNF-α and IL-1β production in unstimulated macrophages (data not shown) whereas in LPS (1 μg/mL) induced cells ABM-BuOH and ABM-EtOAc at 200 μg/mL, significantly inhibited the release of TNF-α (46.0 and 54.0%, respectively) and IL-1β (53.5 and 50.2%, respectively). Macrophages can be induced to secrete a series of cytokines such as TNF-α and IL-1β, which play important roles in immune and inflammatory responses, and are considered proinflammatory cytokines (26-28). It was found that ABM-EtOAc and ABM-BuOH were able to restrain these proinflammatory cytokines. These results imply that inhibition of TNF-α and IL-1β release from macrophages by ABM-BuOH was not due to cell death. On the other hand, the ABM-EtOAc also displayed significant inhibition of TNF-α and IL-1β release, but viability decreased at a higher dose. Similar results have been observed in a diterpene tanshinone IIA, which was isolated from the medicinal herb *Salvia miltiorrhiza*. Tanshinone IIA markedly inhibited TNF-α and IL-1β production in murine macrophages (28). The results showed that ABM-EtOAc and ABM-BuOH have anti-inflammatory potential and that the amount of ABM-EtOAc and ABM-BuOH compound used, as well as the contents of the total phenolic compound, are strongly correlated with its activities (data not shown).

Table 2. Effect of ABM-EtOAc on cell cycle distribution of A549, HT-29, and COLO 205

Cell	Conc. (μg/mL)	Cell cycle distribution[1] (%)			
		Sub G_1	G_0/G_1	S	G_2/M
A549	Control	0.33±0.02	56.28±2.51	31.80±1.49	11.58±0.70
	25	0.26±0.01	60.20±3.04	29.62±1.63	9.91±0.48
	50	1.44±0.07	64.45±3.36	28.40±1.37	5.73±0.31
	100	3.95±0.20	69.33±3.47	25.47±1.39	1.24±0.05
	200	5.06±0.25	45.54±2.25	44.66±2.15	4.74±0.15
HT-29	Control	0.28±0.01	46.11±2.17	39.66±1.86	13.95±0.63
	25	0.48±0.02	49.31±2.32	42.27±2.03	7.94±0.38
	50	0	51.03±2.47	43.10±2.07	5.87±0.31
	100	1.30±0.07	68.25±3.38	27.14±1.46	3.28±0.15
	200	1.50±0.08	68.67±3.52	26.61±1.56	3.20±0.14
COLO 205	Control	0.10±0.01	66.01±3.17	24.52±1.53	9.38±0.52
	25	3.46±0.17	66.66±3.28	23.55±1.26	6.32±0.38
	50	18.47±0.92	54.37±2.58	23.45±1.32	3.71±0.25
	100	25.81±1.29	46.80±2.04	25.49±1.15	1.89±0.15
	200	36.86±1.84	41.30±2.11	18.20±0.88	3.64±0.21

[1]Results are expressed as mean±SD (n=3).

Table 3. Effect of the different fractions of ABM on cell viability, LPS/IFN-γ-induced nitrite accumulation, LPS-induced TNF-α, and IL-1β of RAW 264.7 cell

Sample	Conc. (μg/mL)	Cell viability	NO[1] (μM)	TNF-α[2] (pg/ mL)	IL-1β[2] (pg/mL)
Control		1.00±0.05[3]	5.0±3.4	66±33	50±34
Positive control		1.02±0.05	63.6±2.5	1036±56	488±38
ABM- H_2O	25	1.01±0.09	58.2±4.7	1026±83	472±35
	50	0.98±0.07	60.4±4.5	999±44	485±41
	100	0.95±0.05	55.7±4.2	1073±87	501±44
	200	1.08±0.09	52.7±4.7*	951±45	476±39
ABM-BuOH	25	0.95±0.05	59.8±4.4	1034±93	491±31
	50	0.95±0.09	48.7±4.9**	955±51	471±51
	100	1.08±0.09	35.6±4.2**	767±36**	381±39*
	200	0.99±0.06	27.2±3.0**	477±51**	261±45**
ABM-EtOAc	25	0.99±0.09	58.0±3.6*	1015±61	500±43
	50	0.94±0.07	45.1±3.3**	1042±57	446±31
	100	0.87±0.06*	31.2±3.5**	856±53*	373±38*
	200	0.71±0.05**	20.1±1.3**	560±36**	245±35**
ABM-Hex	25	1.06±0.07	60.8±2.7	1005±77	512±43
	50	1.05±0.07	55.2±3.4*	983±26	471±33
	100	1.03±0.06	46.5±3.0**	993±62	452±46
	200	0.97±0.03	45.6±4.1**	871±97*	389±38*

[1]For nitrite accumulation, cells were exposed to LPS (1 μg/mL) in the presence or absence of the test samples for 24 hr. Positive control group was RAW 264.7 treated with LPS.
[2]For TNF-α and IL-1β, cells were exposed to LPS (1 μg/mL) plus IFN-γ (50 U/mL) in the presence or absence of the test samples for 24 hr. Positive control group was RAW 264.7 treated with LPS plus IFN-γ.
[3]Results are expressed as mean±SD of a quadruplicated experiment; *significant difference was compared with control (*$p<0.05$, **$p<0.01$).

Effect of the ABM on TPA- and TNF-α-stimulated COX-1 and COX-2 expression in A549 and HT-29 carcinoma cells Exponentially growing A549 and HT-29 cancer cells were cultured in 10% FBS medium containing different fractions of ABM at 50 μg/mL and stimulated with TPA (for A549) or TNF-α (for HT-29) for 12 and 6 hr, respectively. After incubation, whole cell lysates were subjected to SDS-polyacrylamide gel electrophoresis (PAGE) and immunoblot analysis to examine COX-1 and COX-2 protein levels. As demonstrated shown in Fig. 2, ABM-BuOH and ABM-EtOAc inhibited the level of COX-2 expression of A549 and HT-29 cells at 50 μg/mL but did not show any inhibitory effect on the COX-1 protein level. The effects of the ABM-BuOH and ABM-EtOAc treatment were significantly distinguished from the vehicle control. Further, A549 and HT-29 carcinoma cells were treated with different concentrations of the ABM-EtOAc or ABM-BuOH (Fig. 3). ABM-EtOAc and ABM-BuOH suppressed COX-2 expression in a dose-dependent manner. In contrast, none of the test samples affected COX-1 expression under similar experimental conditions. These results suggest that ABM-EtOAc and ABM-BuOH fractions inhibit the growth of lung cancer cells by suppressing the expression of the proinflammatory protein COX-2.

In this study, we demonstrated for the first time that the ABM-EtOAc and ABM-BuOH inhibit COX-2 expression in human lung and colon cancer cells. COX-2 is an inflammatory mediator that is thought to play a critical role in the initiation and maintenance of cancer cell survival and growth (29). COX-2 expression may provide a number of growth advantages, including stimulation of proliferation, inhibition of apoptosis, and enhancement of angiogenesis for cancer cells. The results suggest that inhibition of COX-2 expression is one of the mechanisms of anti-tumor activity of ABM-EtOAc and anti-inflammatory activity of ABM-EtOAc and ABM-BuOH.

NSAIDs have been shown to exert anti-proliferative and pro-apoptotic effects on a variety of cancer cell lines (30), suggesting a possible mechanism for their chemopreventive action on cancer. Classic NSAIDs inhibit not only COX-2 but also COX-1, resulting in the common side effect of gastric mucosal damage. To reduce the gastrointestinal side effects of NSAIDs, selective COX-2 inhibitors were developed (31). In the present study, it was found that ABM-EtOAc and ABM-BuOH suppressed COX-2 expression but did not affect COX-1. These results suggest that ABM-BuOH and ABM-EtOAc are highly specific to COX-2 inhibitors, and thus would avoid side effects in the digestive tract.

The use of ABM-EtOAc and ABM-BuOH either as a daily food or as an ingredient in food preparation may have potential benefits for health, including reducing pain related to inflammation, reducing the incident of cardiovascular disease, and preventing cancer by acting as anti-cancer and anti-inflammation agents. However, detailed information about the structure of active compounds requires further investigation.

ABM-EtOAc and ABM-BuOH suppressed the secretion of pro-inflammatory cytokines and reduced COX-2 expression.

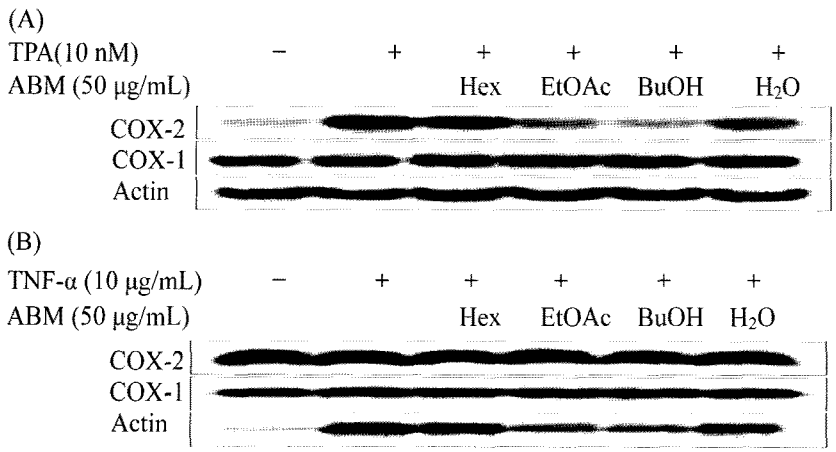

Fig. 2. The effect of 4 fractions of adlay bran methanolic extract (ABM) on TPA-stimulated COX-2 expression in A549 lung cancer cells (A), and TNF-stimulated COX-2 expression in HT-29 colorectal cacinoma cells (B). A549 and HT-28 cells were treated with 50 μg/mL of different fractions of ABM for 12 hr followed by treatment of 10 nM TPA for another 2 hr or followed with TNF-α (10 μg/mL) for 6 hr, respectively.

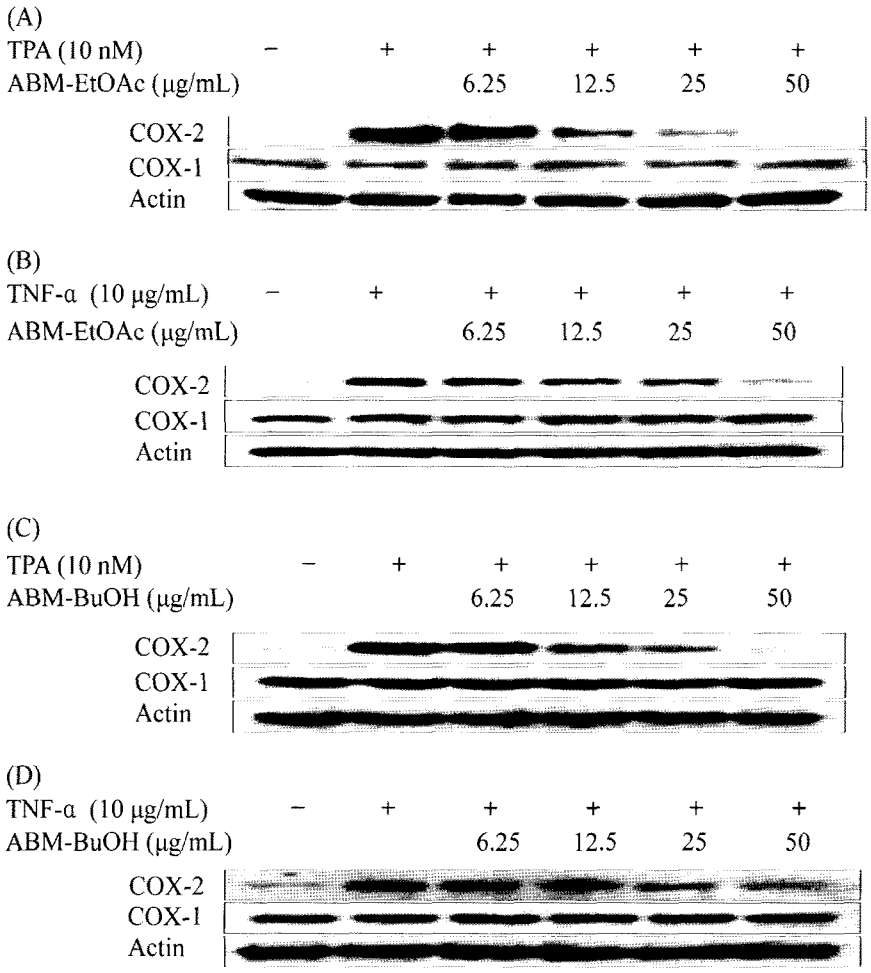

Fig. 3. The effect of ABM-EtOAc and ABM-BuOH on COX-2 expression in A549 and HT-29 cells. (A) (B), The effect of ABM-EtOAc; (C) (D), the effect of ABM-BuOH in A549 and HT-29 cells. Cells were treated with 6.25, 15.5, 25.0, and 50.0 μg/mL concentrations of ABM-EtOAc and ABM-BuOH fractions of ABM for 12 hr followed by treatment of 10 nM TPA for another 2 hr (A 549 cells) or followed with TNF-α (10 μg/mL) for 6 hr, respectively.

ABM-EtOAc suppressed the proliferation of human lung cancer cells and human colorectal carcinoma cells through induction of apoptosis and cell cycle arrest at the G_0/G_1 phase. South eastern Asia is the major location of adlay plantation. Adlay brans are easily available in this area and thus their exploitation may contribute to reducing disposal costs in the adlay agroindustry. This study can serve as a reference in developing ABM-EtOAc and ABM-BuOH as

anti-cancer and anti-inflammatory drugs which utilize the cheap and abundant RAW material, adlay bran waste.

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