Inhibitory effects of bitter melon (*Momordica charantia* Linn.) on bacterial mutagenesis and aberrant crypt focus formation in the rat colon

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Abstract: Antimutagenicity and chemopreventive activity of an 80%-ethanol extract of bitter melon (*Momordica charantia* Linn.) against the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) was investigated. The bitter melon extract was nonmutagenic and inhibited the mutagenicity of heterocyclic amines 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine, and aflatoxin B_1 in the *Salmonella* mutation assay. To examine the inhibitory effect of bitter melon on AOM-induced ACF formation, male F344 rats were fed various concentrations of the extract (0.1, 0.5, and 1.0 g/kg body weight) for five weeks during the initiation stage. One week after the administration of the plant extract, rats were subcutaneously given AOM at 15 mg/kg body weight once a week for two weeks. Three rats in each group were sacrificed 12 hr after the second AOM injection to analyze DNA adducts, O^6-methylguanine (O^6-meG) and N^7-methylguanine in the liver and colon. The remaining rats were sacrificed 3 weeks after the second AOM injection to observe ACF. To examine the inhibitory effect of the extract on ACF formation in the postinitiation stage, rats were fed the extract at 0.1 and 1.0 g/kg body weight for 12 weeks starting two weeks after the second AOM injection. Treatment with bitter melon extract significantly inhibited ACF formation in the colon during the initiation stage and dose-dependently decreased the average of O^6-meG DNA adduct in the colonic mucosa. During the postinitiation stage, bitter melon extract, at 1.0 g/kg body weight, significantly inhibited ACF formation in the colon, especially the formation of ACF with four or more crypts per focus. These findings suggest that bitter melon is a possible chemopreventive agent against colon carcinogenesis. J. Med. Invest. 48 : 88-96, 2001

Keywords **:** *bitter melon ; Momordica charantia* Linn.*; antimutagenicity ; azoxymethane-induced aberrant crypt foci ; O^6-methylguanine*

INTRODUCTION

Evidence from epidemiologic, clinical and laboratory studies suggests that cancer may be caused by complex interactions between genetic and environmental factors (1-3). The risks of colorectal cancer are likely to result from exposure to carcinogens associated with daily food intake, because contamination of carcinogenic substances in food is not always avoidable (4). Cancer prevention may be carried out, however, by reduction in the number of events which bring about exposure to colon carcinogens. This conceptual

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model leads to the notion that the occurrence of cancer may be reduced or prevented by administration of inhibitory compounds, either as individual drugs or as naturally occurring constituents of the daily diet.

Bitter melon (*Momordica charantia* Linn.) is a medicinal plant which has been therapeutically used in the treatment of diabetes mellitus (5). It may also have anti-viral (6-9), anti-tumor (10-12) and immunopotentiating qualities (13). The potentiation of antimutagenic and anti-viral activity of bitter melon has been correlated with the presence of specific acylglucosylsterols (14) and *Momordica* anti-HIV protein (MAP protein) (8), respectively. *In vitro* anti-tumor potential of multifunctional MAP 30 protein of bitter melon has also been observed with certain cell lines (15). A previous study indicated that the bitter melon extract is nonmutagenic in the Ames test and the alkaline single cell gel electrophoresis (COMET) assay (16). It also inhibits mouse skin papillomagenesis (17).

Aberrant crypt foci (ACF) are putative preneoplastic lesions in the colons of both rodents (18) and humans (19). Typical colon carcinogens azoxymethane (AOM) and some heterocyclic amines such as 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ) induce ACF in the rodent colon (20-22). ACF formation caused by AOM has been used as a biomarker for studying the protective effects of various agents against colon cancer development (23). We previously reported that Thai medicinal plants lemon grass and roselle are possible chemopreventive agents against colon cancer (24, 25). In the present study, we examined the chemopreventive activity of bitter melon for colon carcinogenesis using AOM-induced ACF formaion in the rat colons.

MATERIALS AND METHODS

1) Chemicals

AOM, proteinase K, and N^7-methylguanine (N^7-meG) were purchased from Sigma Chemical Co. (St. Louis, MO). O^6-methylguanine (O^6-meG) was supplied by Drs K. Ishizaki and M. Ikenaga, Kyoto University. Ribonuclease T_1 was obtained from Worthington Diagnostics (Freehold, NJ). Heterocyclic amines 2-amino-9*H*-pyrido[2,3-*b*]indole (AαC), 2-amino-6-methyldipyrido[1,2-*a*:3',2'-*d*]imidazole (Glu-P-1), 2-aminodipyrido[1,2-*a* : 3',2'-*d*]imidazole (Glu-P-2), 2-amino-3-methylimidazo[4,5-*f*]quinoline (IQ), 2-amino-3-methyl-9*H*-pyrido[2,3-*b*]indole (MeAαC), 2-amino-3,4-dimethylimidazo[4,5-*f*]quinoline (MeIQ), 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx), 2-amino-1-methyl-6-phenylimidazo[4,5-*b*] pyridine (PhIP), 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1), and 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) were supplied by Dr. K. Wakabayashi, National Cancer Center Research Institute. Phenol, folin-phenol reagent, sodium cacodylate, sodium dodecylsulfate and other chemicals of reagent grade or higher were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

2) Extraction of bitter melon

Bitter melon was purchased from a local market in Chiang Mai, Thailand. Ripe and un-ripe samples of bitter melon were washed with tap water, chopped into small pieces, homogenized with 80% ethanol (weight/volume) and then stirred at room temperature for 24 hr. The extract was filtered though a filter paper by suction and the residue was re-extracted with 80% ethanol. After filtration, the combined filtrates were evaporated to dryness with a vacuum rotatory evaporator at 50°C and then freeze-dried in a lyophilizer. The dried residue was used as bitter melon extract that is abbreviated to BM in Tables and Figures. The extract was weighed, dissolved in 25% dimethyl sulfoxide (DMSO), and kept at 4°C. The extract was passed through a Millipore filter membrane to obtain a sterile solution for mutagenicity and antimutagenicity tests.

3) Animals

Four-week-old male F344 rats were purchased from Shizuoka Laboratory Animal Center Japan (Hamamatsu, Japan). Animals were housed in plastic cages, three or four rats per cage in an experimental room (at the Institute for Animal Experimentation, The University of Tokushima School of Medicine) under controlled conditions of 23±2°C, 55±10% humidity, and a 13-hr light/11-hr dark cycle. The rats were fed laboratory chow (Oriental Yeast Co., Ltd., Tokyo, Japan) and provided with tap water *ad libitum*.

4) Antimutagenicity test

The antimutagenic activity of bitter melon extract was examined by the *Salmonella* mutation assay, using the preincubation technique (26) and *Salmonella typhimurium* strains TA98 and TA100. In the case of AOM, O^6-methylguanine-DNA methyltransferase-deficient strain YG7108 (*ada*$^-$*ogt*$^-$) constructed by Yamada *et al*. (24) was used as a tester strain. The test sample (50 μl) was added to 50 μl of a known mutagen. This

was followed by the addition of 0.5 ml of freshly prepared rat liver extract (S-9) mix or 0.1 M phosphate buffer (pH 7.4) and 0.1 ml of an overnight bacterial culture (2-3×10^9 colony-forming units/ml). The mixture was then shaken in a water bath at 37°C for 20 min. After shaking, 2 ml of molten agar containing 90.9 nmol of histidine/biotin were added and mixed. The mixture was poured on a minimal glucose agar plate and incubated at 37°C for 48 hr. His^+ revertant colonies on the incubated plate were then counted.

5) ACF analysis

After quarantine for one week, rats were divided into eight groups for ACF determination for the initiation stage as shown in Fig. 1. Rats in groups 1 through 5 were subcutaneously injected with AOM (15 mg/kg body weight) once a week for two weeks. Groups 6, 7 and 8 were injected with saline as vehicle controls. Groups 2, 3 and 4 were administered bitter melon extracts (1.0, 0.5 or 0.1 g/kg body weight, respectively) once a day by intragastric gavage. Groups 1 and 6 received 25% DMSO as a control. Group 5 was administered 1.0 g/kg bitter melon extract for only 1 week and on the next day they received the first AOM injection. All rats were sacrificed by cervical dislocation under anesthesia with lethal doses of diethyl ether.

The protocol for the postinitiation stage is shown in Fig. 2. The rats were divided into six groups. Two weeks after the second AOM injection, rats were administered bitter melon extracts (1.0 or 0.1 g/kg body weight) by intragastric gavage for 12 weeks. Animals were carefully observed daily and weighed weekly. The large intestines of sacrificed rats were removed, expanded with 10% formalin in phosphate buffered saline solution (pH 7.4) on ice for 15 min, and then cut open longitudinally along the main axis. The intestines were cut into three portions : the first was the rectum to 2 cm from the anus, and the remaining colon was divided into two parts called the proximal and distal colon. Then, each segment was placed between two pieces of filter paper, fixed in 10% buffered formalin for 24 hr, and stained with 0.2% methylene blue in saline. ACF was examined under a microscope at a magnification of ×40 according to the procedure of Bird (28).

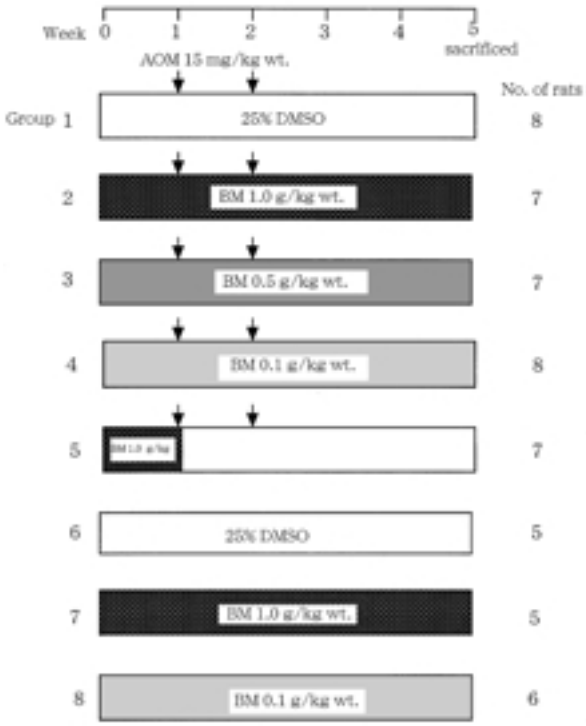

Fig. 1. Experimental protocol for the initiation stage.

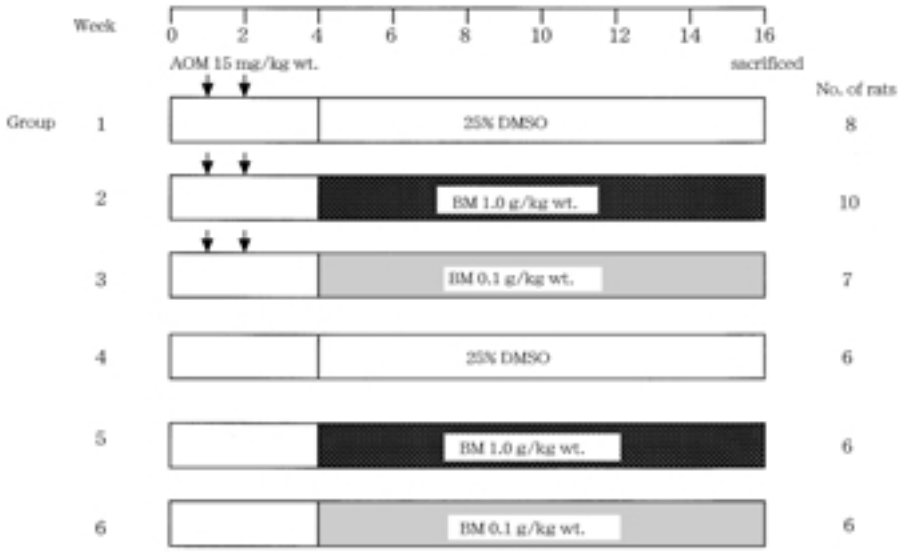

Fig. 2. Experimental protocol for the postinitiation stage.

6) DNA adduct formation

The experimental procedure for detecting DNA adduct formation is shown in Fig. 3. Rats were divided into eight groups and received AOM as described above. Animals were sacrificed 12 hr after the second AOM injection and then the livers and colons were removed. The colon was slit open longitudinally and all the waste contents were removed by washing with saline. Then, the colon was laid flat on a glass plate, and the colonic mucosa was scraped with a glass slide. The livers, colonic mucosa and colonic muscular layers were kept at -80°C until DNA adduct analysis.

7) High performance liquid chromatography (HPLC) of O^6-meG and N^7-meG

DNA adduct formation was assayed by the method of Netto *et al*. (29). The DNA was isolated from the liver and colon by phenol extraction (30) followed by incubation with RNase A, RNase T_1, and Proteinase K for purification. The purified DNA was dissolved in 10 mM sodium cacodylate (pH 7.0) at a concentration of 5 mg/ml. The DNA solution was hydrolyzed by neutral thermal hydrolysis at 100°C for 30 min to release N^7-meG (31). The partially apurinic DNA was precipitated and hydrolyzed by acid hydrolysis at 70°C for 30 min. Neutral thermal and acid hydrolysates were analyzed for the specific adducts by HPLC (Shimadzu LC-5A) with a Chemcosorb 7-SCX cation exchange column (4.6×250 mm, Chemco Co., Osaka) and 200 mM ammonium formate (pH 3.0) as a mobile phase at a flow rate of 1.0 ml/min. Elution of the fluorescent bases was monitored using a Shimadzu fluorescence detector with an excitation wavelength of 280 nm and an emission wavelength of 365 nm.

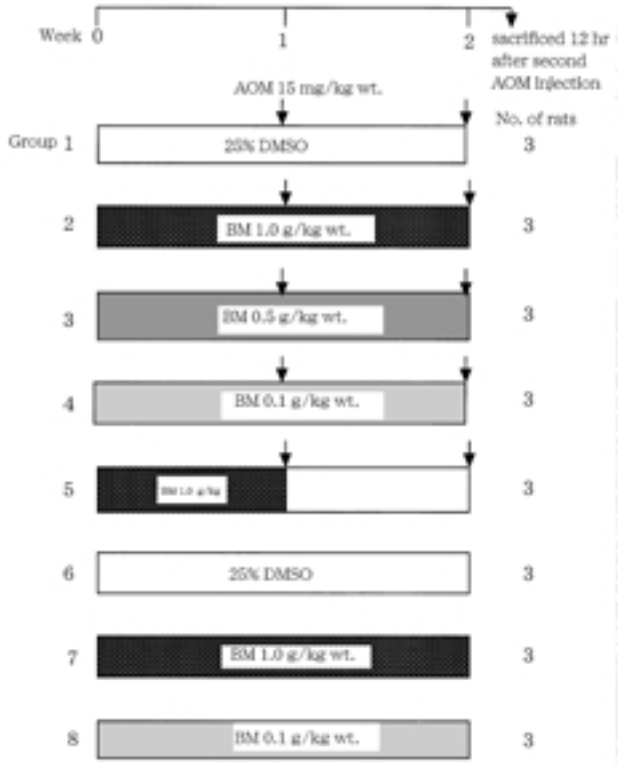

Fig. 3. Experimental protocol for DNA adduct formation.

Statistical Analysis

The data were analyzed by one way analysis of variance.

RESULTS

1) Antimutagenicity of bitter melon

Bitter melon extract was nonmutagenic in the *Salmonella typhimurium* mutation assay using strains TA98 and TA100 in the presence and absence of S-9 mix (data not shown). The antimutagenic activity of bitter melon extract against various known mutagens is shown in Table 1. Bitter melon extract inhibited the mutagenicity of IQ, MeIQ, MeIQx, PhIP, Glu-P-2 and AFB_1.

2) Effect of bitter melon extract on AOM-induced ACF formation

The mean body weight of rats in each group during the initiation stage is shown in Fig. 4. The average

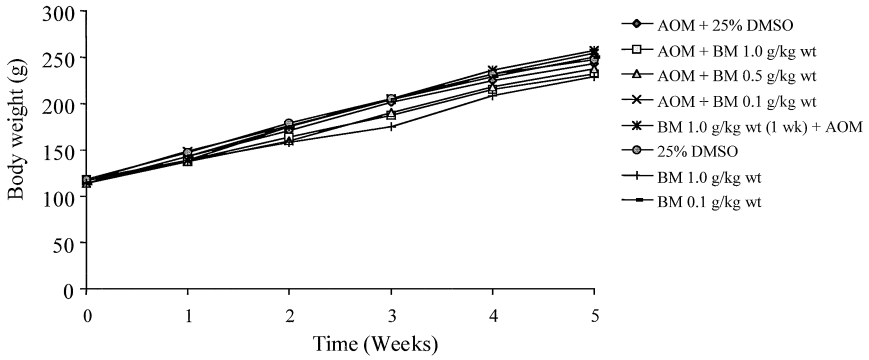

Fig. 4. Mean body weight of F344 rats in each group during the initiation stage.

Table 1. Effect of bitter melon extract on mutagenicity of heterocyclic amines and known mutagens

Mutagen	Dose (μg/plate)	Strain	S-9	His⁺ revertant colonies/plate (% mutagenicity)						
				Concentration of BM (mg/plate)						
				0	0.31	0.63	1.25	2.5	5.0	10.0
IQ	0.004	TA98	+	795	550	675	636	661	559	562
				(100)	(69)	(85)	(80)	(83)	(70)	(71)
MeIQ	0.0025	TA98	+	3116	2131	1932	1930	1879	1927	1685
				(100)	(68)	(62)	(62)	(60)	(62)	(54)
MeIQx	0.01	TA98	+	1376	806	1048	960	993	1081	899
				(100)	(59)	(76)	(70)	(72)	(79)	(65)
PhIP	1.0	TA98	+	1114	771	662	848	782	739	589
				(100)	(69)	(59)	(76)	(70)	(66)	(53)
Trp-P-1	0.05	TA98	+	1453	1658	1622	1698	1914	2073	2301
				(100)	(114)	(112)	(117)	(132)	(143)	(158)
Trp-P-2	0.1	TA98	+	1101	1290	1280	1347	1822	1938	1347
				(100)	(117)	(116)	(122)	(165)	(176)	(122)
Glu-P-1	0.05	TA98	+	2399	1828	1852	2173	2644	2293	2209
				(100)	(76)	(77)	(91)	(110)	(96)	(92)
Glu-P-2	4.0	TA98	+	1673	1330	1241	1390	1344	1093	995
				(100)	(79)	(74)	(83)	(80)	(65)	(59)
AαC	5.0	TA98	+	1538	1927	1635	1845	1861	1865	1565
				(100)	(125)	(106)	(120)	(121)	(121)	(102)
MeAαC	5.0	TA98	+	548	807	718	940	948	1313	1773
				(100)	(147)	(131)	(172)	(172)	(240)	(324)
AFB_1	0.1	TA98	+	1169	627	509	428	478	447	317
				(100)	(54)	(44)	(27)	(41)	(38)	(27)
B(*a*)P	2.5	TA100	+	292	225	270	206	222	266	311
				(100)	(77)	(92)	(71)	(76)	(91)	(107)
AOM	4.0*	YG7108	+	869	681	580	626	670	693	648
				(100)	(78)	(67)	(72)	(77)	(80)	(75)

*mg/plate

body weight of rats fed 1 g of bitter melon extract per kg body weight for one week before the first AOM injection (group 5) was slightly but significantly higher than that of rats given AOM alone at the day of sacrifice ($p<0.05$).

The effect of bitter melon on AOM-induced ACF formation during the initiation stage is shown in Table 2. No abnormality was seen with the naked eye in any group. ACF formation was observed in rats treated with AOM alone and AOM with bitter melon extracts (groups 1-5). No ACF were induced in rats not treated with AOM (groups 6-8). The mean number of colonic ACF of rats in group 1 (AOM alone) was the highest. The average numbers of colonic ACF were significantly decreased by feeding bitter melon extract at a dose of 0.5 g/kg body weight (group 3, $p<0.05$) and by pretreatment for one week before the first AOM injection (group 5, $p<0.005$). The tendency of the decrease in ACF was especially true in the total number of ACF, which was significantly decreased by feeding bitter melon extracts (groups 2-5). However, the observed effect of bitter melon was not dose-dependent.

The mean body weight of rats in the postinitiation stage is shown in Fig. 5. The average weight of rats not injected with AOM was significantly higher than in those treated with AOM alone ($p<0.05$).

The mean numbers of ACF and aberrant crypts/focus (AC/ACF) in the colon and rectum are shown in Table 3. The total number of ACF in rats fed 1 g of bitter melon extract per kg body weight was significantly lower than that of rats in the control group ($p<0.05$). The bitter melon extract significantly inhibited ACF formation with four or more crypts per focus (≧4C/F), which is predictive of tumor incidence. This effect was dose-dependent.

3) Effect of bitter melon extract on AOM-induced DNA adduct formation

The effect of bitter melon on DNA adduct formation is shown in Table 4. The bitter melon extract dose-dependently decreased the average of O^6-meG in colonic mucosa and it significantly decreased O^6-meG ($p<0.05$) and N^7-meG formation ($p<0.05$) in the colonic muscular layer at a dose of 0.5 g/kg body weight (group 2). Pretreatment with bitter melon extract at a concentration of 1 g/kg body weight before the first AOM injection (group 5) also significantly de-

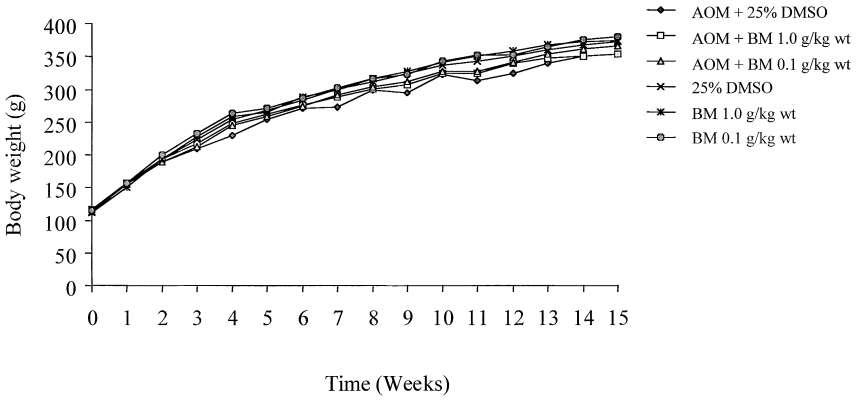

Fig. 5. Mean body weight of F344 rats in each group during the postinitiation stage.

Table 2. Inhibitory effect of bitter melon extract on the formation of AOM-induced aberrant crypt foci during the initiation stage

Group	Treatment	No	Colon		Rectum		Total	
			ACF	AC/ACF	ACF	AC/ACF	ACF	AC/ACF
1	AOM + 25% DMSO	8	162.8±47.2[a]	2.02±0.10	33.9±16.6	1.81±0.20	196.6±46.1	1.95±0.07
2	AOM + BM 1.0 g/kg	7	125.4±35.8	2.02±0.14	29.0±9.45	1.89±0.29	154.4±37.1[b]	1.98±0.18
3	AOM + BM 0.5 g/kg	7	109.3±39.7[b]	2.09±0.07	31.9±13.4	2.08±0.28[b]	141.1±28.0[c]	2.09±0.12
4	AOM + BM 0.1 g/kg	8	129.3±18.6	2.16±0.13[b]	31.0±9.07	1.85±0.19	160.3±17.3[b]	2.05±0.10
5	BM 1.0 g/kg (1 wk) + AOM	7	99.6±31.5[c]	2.16±0.20[b]	36.6±18.4	1.99±0.27	136.1±40.4[c]	2.11±0.21[b]
6	25% DMSO	5	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]
7	BM 1.0 g/kg	5	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]
8	BM 0.1 g/kg	6	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]

a) Mean±SD.
b) Significantly different from the AOM-treated control group at $p<0.05$, c) $p<0.005$, and d) $p<0.0005$.

Table 3. Effect of bitter melon extract on the formation of AOM-induced aberrant crypt foci during the postinitiation stage

Group	Treatment	No	Colon			Rectum			Total		
			ACF	AC/ACF	≧4 C/F	ACF	AC/ACF	≧4 C/F	ACF	AC/ACF	≧4 C/F
1	AOM + 25% DMSO	8	235.4±35.6[a]	3.34±0.11	92.4±14.8	44.1±6.8	3.07±0.20	13.4±2.0	279.5±33.4	3.25±0.13	105.8±14.7
2	AOM + BM 1.0 g/kg	10	194.0±15.9	2.83±0.13[c]	55.4±6.4[c]	34.4±4.6	2.42±0.07[c]	6.6±1.4[c]	228.3±15.5[b]	2.69±0.11[d]	62.0±6.7[d]
3	AOM + BM 0.1 g/kg	7	199.3±16.1	3.06±0.12	64.0±5.0[b]	31.3±3.7[b]	2.59±0.26[b]	5.9±1.8[c]	230.6±16.4	2.90±0.16[b]	69.9±6.3[c]
4	25% DMSO	6	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]
5	BM 1.0 g/kg	6	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]
6	BM 0.1 g/kg	6	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]	0±0[d]

a) Mean±SD.
b) Significantly different from the AOM-treated control group at $p<0.05$, c) $p<0.005$, and d) $p<0.0005$.

creased O^6-meG formation in the colonic muscular layer ($p<0.005$), but this treatment significantly increased O^6-meG formation in the liver.

DISCUSSION

The present study demonstrated that bitter melon extract was antimutagenic for AFB_1 and heterocyclic amines including Glu-P-2, IQ, MeIQ, MeIQx and PhIP, which have been identified in cooked meat, fish and chicken (32). The mutagenicity of AOM in YG7108 was weakly inhibited by the addition of the extract. The extract itself was nonmutagenic with strains TA98 and TA100 both with and without metabolic activation. This is consistent with the findings of Basaran *et. al.* (16). Guevara *et al.* (14) reported that induction of micronucleated polychromatic erythrocytes by mitomycin C was inhibited by the acylglucosylsterols contained in bitter melon. The interactions of some components of bitter melon with mutagens might contribute to the decrease in their mutagenicity.

During the initiation stage of AOM-induced ACF

Table 4. Effect of bitter melon extract on the level of DNA adduct formation in the large intestines of AOM-induced F344 rats

Group	Treatment	No	O^6-Methylguanine (μmol/mol guanine)			N^7-Methylguanine (mmol/mol guanine)		
			Mucosa	Muscular layer	Liver	Mucosa	Muscular layer	Liver
1	AOM + 25% DMSO	3	108.2±12.2[a]	91.9±10.8	365.9±58.2	1.88±0.27	1.66±0.29	4.94±0.38
2	AOM + BM 1.0 g/kg	3	76.8[e]	108.6±25.2	375.2±57.1	1.63±0.26	1.40±0.05	4.65±0.09
3	AOM + BM 0.5 g/kg	3	95.3[e]	64.5±3.3[b]	344.7±64.8	1.92±0.16	1.29±0.17[b]	4.62±0.40
4	AOM + BM 0.1 g/kg	3	116.7±30.3	78.5±9.2	340.6±23.1	1.99±0.24	1.45±0.22	4.68±0.80
5	BM 1.0 g/kg (1 wk) + AOM	3	89.7±28.0	58.9±7.2[c]	485.8±15.3[c]	1.67±0.19	1.68±0.04	4.82±0.82
6	BM 1.0 g/kg	3	<1.5±0.15[d]	<1.9±0.0[d]	<2.3±0.2[d]	<0.62±0.06[d]	<0.75±0.01[d]	<0.92±0.07[d]
7	BM 0.5 g/kg	3	<1.5±0.06[d]	<2.0±0.2[d]	<2.1±0.2[d]	<0.60±0.03[d]	<0.81±0.08[d]	<0.83±0.06[d]
8	BM 0.1 g/kg	3	<1.5±0.14[d]	<2.0±0.3[d]	<2.5±0.2[d]	<0.59±0.05[d]	<0.81±0.12[d]	<0.99±0.08[d]

a) Mean±SD. b) Significantly different from the AOM-treated control group at $p<0.05$, c) $p<0.005$, and d) $p<0.0005$.
e) Average of two samples.

formation, feeding bitter melon extract at 0.5 g/kg body weight significantly decreased the number of ACF in rats. The inhibitory effects were consistent with the inhibition of O^6-meG DNA adduct formation in the colonic mucosa and muscular layer. In the present study, we measured the levels of N^7-meG and O^6-meG. N^7-meG is quantitatively the major alkylation product in methylating agent-treated animals (33), but the level and persistence of O^6-meG in the target tissue is more closely correlated with carcinogenicity (34). During DNA replication, O^6-meG mispairs with thymine resulting in a G to A point mutation (33) and this mutation has been implicated in oncogene activation (33, 35). AOM is metabolized to methylazoxymethanol (MAM) by P450 2E1 in the liver (36). Then MAM is transported to the colon via the blood stream (36) or via the bile duct after glucuronidation in the liver (37). Previous studies found that oral feeding of bitter melon reduced the increased expression of cytochrome P450 1A2, 2B1, 2E1, and 3A in streptozotocin-induced diabetic rats (38). Bitter melon might inhibit AOM-induced ACF formation by selectively inhibiting the expression of cytochrome P450 2E1 which is responsible for the metabolic activation of AOM. However, O^6-meG in the liver was not decreased and rather increased in the pretreatment group. Enhanced DNA repair, or other mechanisms may possibly be involved in the inhibition of ACF formation.

Bitter melon extract also significantly and dose-dependently inhibited ACF formation during the postinitiation stage. It especially decreased the number of ACF with four or more crypts per focus. Bitter melon inhibits the function of guanylate cyclase, which converts guanosine triphosphate to cyclic guanosine 3', 5'-monophosphate (cGMP) (40). The cGMP plays an important role in cell growth (41-45), and an increase of the cGMP level occurs in some types of tumors including colon adenocarcinoma (46). Therefore, we assumed that the bitter melon extract inhibits the growth of ACF by inhibition of cell proliferation.

Dietary administration of bitter melon extract effectively inhibited AOM-induced ACF formation in the rat colon during both the initiation and postinitiation stages. In addition, bitter melon inhibited the mutagenicity of various known mutagens. Wattenberg (4) discussed chemopreventive agents as blocking agents that prevent carcinogens from reaching or reacting with critical target sites, and as suppressing agents that prevent evolution of the neoplastic process in cells that otherwise would become malignant. Bitter melon extract contains both agents. Although the exact mechanism of the chemopreventive effects of bitter melon is not yet known, bitter melon may be a possible chemopreventive agent against colon carcinogenesis.

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REFERENCES

1. Sugimura T : Multistep carcinogenesis : a 1992

perspective. Science 258 : 603-607, 1992
2. Yokota J, Sugimura T : Multiple steps in carcinogenesis involving alterations of multiple tumor suppressor genes. FASEB J 7 : 920-925, 1993
3. Fearon E R, Vogelstein B : A genetic model for colorectal tumorigenesis. Cell 61 : 759-767, 1990
4. Wattenberg L W : Chemoprevention of cancer by naturally occurring and synthetic compounds. In : Wattenberg L, Lipkin M, Boone C W, Kelloff G J, eds. Cancer Chemoprevention. CRC Press, Boca Raton, 1992, pp.19-39
5. Platel K, Srinivasan K : Plant foods in the management of diabetes mellitus:vegetables as potential hypoglycaemia agents. Nahrung 41 : 68-74, 1997
6. Lee-Huang S, Huang P L, Nara P L, Chen H C, Kung H F, Huang P, Huang H I and Huang P L : MAP30 : a new inhibitor of HIV-1 infection and replication. FEBS Lett 272 : 12-18, 1990
7. Lee-Huang S, Huang P L, Huang P L, Bourinbaiar A S, Chen H C, Kung H F : Inhibition of the integrase of human immunodeficiency virus (HIV) type 1 by anti-HIV plant proteins MAP30 and GAP31. Proc Natl Acad Sci USA 92 : 8818-8822, 1995
8. Bourinbaiar A S, Lee-Huang S : Potentiation of anti-HIV activity of anti-inflammatory drugs, dexamethasone and indomethacin, by MAP30, the antiviral agent from bitter melon. Biochem Biophys Res Commun 208 : 779-785, 1995
9. Bourinbaiar A S, Lee-Huang S : The activity of plant-derived antiretroviral proteins MAP30 and GAP31 against herpes simplex virus *in vitro*. Biochem Biophys Res Commun 219 : 923-929, 1996
10. Jilka C, Strifler B, Fortner G W, Hays E F, Takemoto D J : *In vivo* antitumor activity of the bitter melon (*Momordica charantia*). Cancer Res 43 : 5151-5155, 1983
11. Ng T B, Liu W K, Sze S F, Yeung H W : Action of α-momorcharin, a ribosome inactivating protein, on cultured tumor cell lines. Gen Pharmacol 25 : 75-77, 1994
12. Lee-Huang S, Huang P L, Chen H C, Huang P L, Bourinbaiar A, Huang H I, Kung H F : Anti-HIV and anti-tumor activities of recombinant MAP30 from bitter melon. Gene 161 : 151-156, 1995
13. Cunnick J E, Sakamoto K, Chapes S K, Fortner G W , Takemoto D J : Induction of tumor cytotoxic immune cells using a protein from the bitter melon (*Momordica charantia*). Cell Immunol 126 : 278-289, 1990
14. Guevara A P, Lim-Sylianco C, Dayrit F, Finch P : Antimutagens from *Momordica charantia*. Mutat Res 230 : 121-126, 1990
15. Rybak S M, Lin J-J, Newton D L, Kung H-F, Monks A, Chen H-C, Huang P L and Lee-Huang S : *In vitro* anti-tumor activity of the plant ribosome inactivating protein MAP30 and GAP31. Int J Oncol 5, 88-94, 1994
16. Basaran A A, Yu T W, Plewa M J, Anderson D : An investigation of some Turkish herbal medicines in *Salmonella typhimurium* and in the COMET assay in human lymphocytes. Teratog Carcinog Mutag 16 : 125-138, 1996
17. Singh A, Singh S P, Bamezai R : *Momordica charantia* (bitter gourd) peel, pulp, seed and whole fruit extract inhibits mouse skin papillomagenesis. Toxicol Lett 16 : 37-46, 1998
18. Pretlow T P, O'Riordan M A, Somich G A, Amini S B, Pretlow T G : Aberrant crypts correlate with tumor incidence in F344 rats treated with azoxymethane and phytate. Carcinogenesis 13 : 1509-1512, 1992
19. Pretlow T P, Barrow B J, Ashton W S, O'Riordan M A, Pretlow T G, Jurcisek J A, Stellato T A : Aberrant crypts : putative preneoplastic foci in human colonic mucosa. Cancer Res 51 : 1564-1567, 1991
20. McLellan E, Bird R P : Effect of disulfiram on 1,2-dimethylhydrazine-and azoxymethane-induced aberrant crypt foci. Carcinogenesis 12 : 969-972, 1991
21. Tachino N, Hayashi R, Liew C, Bailey G, Dashwood R : Evidence for *ras* gene mutation in 2-amino -3-methylimidazo[4,5-*f*]quinoline-induced colonic aberrant crypts in the rat. Mol Carcinog 12 : 187-192, 1995
22. Takahashi S, Ogawa K, Ohshima H, Esumi H, Ito N, Sugimura T : Induction of the aberrant crypt foci in the large intestine of F344 rats by oral administration of 2-amino-1-methyl-6-phenylimidazo [4,5-*b*]pyridine. Jpn J Cancer Res 82 : 135-137, 1991
23. Bird R P : Role of aberrant crypt foci in understanding the pathogenesis of colon cancer. Cancer Lett 93 : 55-71,1995
24. Suaeyun R, Kinouchi T, Arimochi H, Vinitketkumnuen U and Ohnishi Y : Inhibitory effects of lemon grass (*Cymbopogon citratus* Stapf) on formation of azoxymethane-induced DNA adducts and aberrant crypt foci in the rat colon. Carcinogenesis 18 : 949-955, 1997
25. Chewonarin T, Kinouchi T, Kataoka K, Arimochi H, Kuwahara T, Vinitketkumnuen U, Ohnishi Y :

Effects of roselle (*Hibiscus sabdariffa* Linn.), a Thai medicinal plant, on the mutagenicity of various known mutagens in *Salmonella typhimurium* and on formation of aberrant crypt foci induced by the colon carcinogens azoxymethane and 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine in F344 rats. Food Chem Toxicol 37 : 591-601, 1999

26. Maron D M, Ames B N : Revised methods for the Salmonella mutagenicity test. Mutat Res 113 : 173-215, 1983
27. Yamada M, Sedgwick B, Sofuni T, Nohmi T : Construction and characterization of mutants of *Salmonella typhimurium* deficient in DNA repair of O^6-methylguanine. J Bacteriol 177 : 1511-1519, 1995
28. Bird R P : Observation and quantification of aberrant crypts in the murine colon treated with a colon carcinogen : preliminary findings. Cancer Lett 37 : 147-151, 1987
29. Netto L E S, RamaKrishna N V S, Kolar C, Cavalieri E L, Rogan E G, Lawson T A, Augusto O : Identification of C^8-methylguanine in the hydrolysates of DNA from rats administered 1,2-dimethylhydrazine. Evidence for *in vivo* DNA alkylation by methyl radicals. J Biol Chem 267 : 21524-21527, 1992
30. Gupta R C : Nonrandom binding of the carcinogen *N*-hydroxy-2-acetylaminofluorene to repetitive sequences of rat liver DNA *in vivo*. Proc Natl Acad Sci USA 81 : 6943-6947, 1984
31. Becker R A, Barrows L R , Shank R C : Methylation of liver DNA guanine in hydrazine hepatotoxicity : dose-response and kinetic characteristics of 7-methylguanine and O^6-methylguanine formation and persistence in rats. Carcinogenesis 2 : 1181-1188, 1981
32. Wakabayashi K, Nagao M, Esumi H, Sugimura T : Food-derived mutagens and carcinogens. Cancer. Res 52(Suppl) : 2092s-2098s, 1992
33. Saffhill R, Margison G P, O'Connor P J : Mechanisms of carcinogenesis induced by alkylating agents. Biochim Biophys Acta 823 : 111-145, 1985
34. Margison G P, Kleihues P : Chemical carcinogenesis in the nervous system. Preferential accumulation of O^6-methylguanine in rat brain deoxyribonucleic acid during repetitive administration of *N*-methyl-*N* -nitrosourea. Biochemical J 148 : 521-525, 1975
35. Zalidi N H, Pretlow T P, O'Riordan M A, Dumenco L L, Allay E, Gerson S L : Transgenic expression of human *MGMT* protects against azoxymethane-induced aberrant crypt foci and G to A mutations in the K-*ras* oncogene of mouse colon. Carcinogenesis 16 : 451-456, 1995
36. Sohn O S, Ishizaki H, Yang C S, Fiala E S : Metabolism of azoxymethane, methylazoxymethanol and *N*-nitrosodimethylamine by cytochrome P450IIE1. Carcinogenesis 12 : 127-131, 1991
37. Weisburger J H, Grantham P H, Horton R E, Weisburger E K : Metabolism of the carcinogen *N* -hydroxy-*N*-2-fluorenylacetamide in germ-free rats. Biochem Pharmacol 19 : 151-162, 1970
38. Raza H, Ahmed I, Lakhani M S, Sharma A K, Pallot D, Montague W : Effect of bitter melon (*Momordica charantia*) fruit juice on the hepatic cytochrome P450-dependent monooxygenases and glutathione *S*-transferases in streptozotocin-induced diabetic rats. BiochemPharmacol 52 : 1639-1642, 1996
39. Singh A, Singh S P, Bamezai R : Postnatal efficacy of *Momordica charantia* peel, pulp, seed and whole fruit extract in the detoxication pathway of suckling neonates and lactating mice. Cancer Lett 122 : 121-126, 1998
40. Takemoto D J, Dunford C, Vaughn D, Kramer K J, Smith A, Powell R G : Guanylate cyclase activity in human leukemic and normal lymphocytes. Enzyme inhibition and cytotoxicity of plant extracts. Enzyme 27 : 179-188, 1982
41. Kram R, Tomkins G M : Pleiotypic control by cyclic AMP : interaction with cyclic GMP and possible role of microtubules. Proc Natl Acad Sci USA 70 : 1659-1663, 1973.
42. Rudland P S, Gospodarowicz D, Seifert W : Activation of guanyl cyclase and intracellular cyclic GMP by fibroblast growth factor. Nature 250 : 741-742, 773-774, 1974
43. Seifert W E, Rudland P S : Possible involvement of cyclic GMP in growth control of cultured mouse cells. Nature 248 : 138-140, 1974
44. Watson J, Epstein R, Cohn M : Cyclic nucleotides as intracellular mediators of the expression of antigen-sensitive cells. Nature 246 : 405-409, 1973
45. Lanzani G A, Giannattasio M, Manzocchi L A, Bollini R, Soffientini A N, Macchia V : The influence of cyclic GMP on polypeptide synthesis in a cell-free system derived from wheat embryos. Biochem Biophys Res Commun 58 : 172-177, 1974
46. DeRubertis F R, Chayoth R, Field J B : The content and metabolism of cyclic adenosine 3', 5'-monophosphate and cyclic guanosine 3', 5'-monophosphate in adenocarcinoma of the human colon. J Clin Invest 57 : 641-649, 1976