

Novel Application of *Shochu* Distillery By-products to Prophylaxis against Mammary Carcinogenesis Induced by 7,12-Dimethylbenz[*a*]anthracene in Rats

Taku SASAKI,^{1,2} Makoto ABE,¹ Shin'ichi NAKAYAMA,¹ Kazuyuki MORIYAMA,³ Hidetaka TAHARA,³ and Toshichika TAKITA^{1,†}

¹Department of Nutritional Science, Faculty of Applied Biological Science, Tokyo University of Agriculture, Setagaya Ward, Tokyo 156-8502, Japan

²Department of Medicine, Tokai University School of Medicine, Isehara City, Kanagawa 259-1193, Japan

³Kirishima Shuzou Co., Ltd., Miyazaki 885-8588, Japan

Received May 2, 2005; Accepted June 23, 2005

The effect of the heat-dried product of *Shochu* distillery by-products (HSDB) derived from sweet potato on mammary carcinogenesis in rats was investigated. HSDB was fed at 2.5% or 5% of the total feed weight. Dietary HSDB at the 5% level suppressed the incidence and number of tumors, and delayed the latency of mammary tumor development relative to the control diet. Experiments were conducted to determine the relative polarity of the anticarcinogenic constituent(s). The number of tumors per tumor-bearing rat was lower in the diet group fed with an ethyl acetate extract of HSDB than in the control group. The tumor incidence evaluated at both palpation and autopsy was slightly lower in the group fed with a methanol extract than in the control group. These results suggest that HSDB contained at least two constituents of differing polarity that counteracted mammary carcinogenesis.

Key words: carcinogenesis; chemoprevention; mammary cancer; environmental pollution; *Shochu*

Most industrialized countries are facing significant problems as they attempt to minimize environmental pollution. The Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter, 1972 (The London Convention 1972) has been adopted as the international standard to prevent marine pollution by the International Maritime Organization. This convention was reinforced by the 1996 Protocol. In Japan, it had been common practice for distillery by-products of *Shochu* (SDB), a Japanese traditional spirit, to be dumped at sea as sewage sludge, but this treatment was phased out in light of the Protocol by the end of

2000. The current main alternatives to dumping are use in agriculture and deposition on land. However, due to new restrictions on land use that favor conservation and an imbalance in the demand for fertilizer, it is clear that these alternatives are also limited. Therefore, the development of new uses for SDB has become an environmental and economic priority.

Sweet potato, *Ipomoea batatas*, is one of the main sources for *Shochu* which generates an abundance of SDB. Both the sheer quantity and seasonal variance in amount have resulted in insufficient attention being paid to the development of alternative uses for sweet potato SDB. Previous studies have established that this plant contains ingredients that are active against certain diseases.^{1–8)} While many reports have concentrated on the natural pigment, anthocyanin, as the active factor in sweet potato,^{1–4)} recent studies have suggested that elements of white-skinned sweet potato, which lacks anthocyanin, have both an antidiabetic effect *in vivo* and antitumor activity *in vitro*.^{5–8)}

The incidence of diseases associated with certain dietary choices is currently increasing in many industrialized countries. Mammary cancer is one such disease whose development is closely correlated with the diet.^{9,10)} At present, a study of those compounds with antitumor activity derived from vegetables and other plants for use as potential therapeutic chemopreventives is a major focus of anticancer research.^{11,12)} We examined in the present study whether the heat-dried product of SDB (HSDB) derived from anthocyanin-free sweet potato was able to prevent 7,12-dimethylbenz[*a*]anthracene (DMBA)-induced mammary cancer in female rats. The intent of the study is to illustrate a novel use for HSDB as a dietary anticarcinogen.

† To whom correspondence should be addressed. Tel: +81-3-5477-2443; Fax: +81-3-5477-2626; E-mail: takita@nodai.ac.jp

Abbreviations: Et-Ac, ethyl acetate-soluble fraction of HSDB; DMBA, 7,12-dimethylbenz[*a*]anthracene; HSDB, heat-dried product of *Shochu* distillery by-products; Me, methanol-soluble fraction of HSDB; SDB, *Shochu* distillery by-products

Materials and Methods

Experiment 1. Five-week-old female Sprague-Dawley rats (CLEA Japan, Tokyo, Japan) were housed in individual apartment cages in an air-conditioned room ($22 \pm 1^\circ\text{C}$ and $50 \pm 10\%$ humidity) under a 12-hr alternating light-dark cycle. The animals were fed on a MF-2 rat diet (Oriental Yeast, Tokyo, Japan) and water *ad libitum* for 1 week as adaptation to the experimental conditions. Fifty-one rats were subsequently divided into three dietary groups. The average body weight was similar in each group. HSDB was derived from *Ipomoea batatas* L. cv. Koganesengan (Kirishima Shuzou Co., Miyazaki, Japan), a cultivar that lacks anthocyanin. The composition of HSDB was analyzed by Japan Food Research Laboratories (Tokyo, Japan). The proportions of ingredients in all test diets were adjusted equally (Table 1). The total dietary concentrations of BSD were (w/w) 0% in the control group, 2.5% in the low group, and 5% in the high group. The semipurified diets were stored at -30°C . All animals were fed with the

appropriate diet for 11 weeks at 17:00 h; the remaining food was removed at 9:00 h the following morning to avoid the ingestion of lipid peroxides. A single dose of 50 mg/kg of body weight of DMBA (Sigma Chemical Co., St. Louis, MO, U.S.A.) dissolved in 1 ml of corn oil was administered orally to each rat on day 49 after birth. The rats were palpated daily to monitor tumor development. At the termination of the treatment period, the rats were killed under anesthesia with sodium pentobarbital (Dainippon Pharmaceutical Co., Osaka, Japan). All experimental procedures involving the animals were performed according to the guidelines for animal experimentation at the Tokyo University of Agriculture.

Experiment 2. HSDB was suspended overnight in ethyl acetate and then filtered. The residue was re-extracted with ethyl acetate as already described. The solutions obtained from the two extractions were mixed and evaporated at 50°C . The residue after evaporation was used as the ethyl acetate-soluble fraction of HSDB (Et-Ac).

Table 1. Diets Used in Experiment 1

Ingredient	Group		
	Control	Low	High
	<i>g/100 g</i>		
Casein	20.0	19.45	18.89
DL-methionine	0.3	0.3	0.3
Sucrose	45.0	44.22	43.45
Corn starch	15.0	15.0	15.0
Mineral mix (AIN-76 TM)	3.5	3.39	3.28
Vitamin mix (AIN-76 TM)	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
Cellulose	5.0	4.06	3.12
Corn oil	10.0	9.88	9.76
HSDB	—	2.5	5.0

HSDB, heat-dried product of *Shochu* distillery by-products. *Shochu* is derived from *Ipomoea batatas* L. cv. Koganesengan.

Table 2. Body Weight Gain and Organ Weights for the DMBA-Treated Rats in Experiment 1

	Group		
	Control	Low	High
Body wt. gain (g)	130 ± 6 (100)	141 ± 6 (108)	129 ± 5 (99.2)
Liver (g/100 g of body wt.)	3.03 ± 0.09 (100)	2.96 ± 0.07 (97.8)	2.85 ± 0.06 (93.9)
Spleen (mg/100 g of body wt.)	229 ± 23 (100)	244 ± 44 (107)	191 ± 6 (83.5)
Kidney (mg/100 g of body wt.)	606 ± 11 (100)	616 ± 24 (102)	606 ± 17 (100)
Lung (mg/100 g of body wt.)	451 ± 17 (100)	472 ± 24 (105)	426 ± 18 (94.5)

Each value is the mean ± SEM. Values in parentheses are percentages of means relative to those of the control group fed on a diet without HSDB.

Table 3. Mammary Tumors for the DMBA-Treated Rats in Experiment 1

	Group		
	Control	Low	High
Tumor incidence at necropsy (%)	76.5 (100)	68.8 (89.9)	62.5 (81.7)
Latent period (days)	52 ± 2.7 (100)	55 ± 2.7 (107)	59 ± 1.7* (114)
Total no. of tumors	53 (100)	69 (130)	34 (64.2)
No. of tumors/tumor-bearing rat	4.08 ± 1.04 (100)	6.27 ± 1.33 (154)	3.40 ± 0.67 (83.4)
Total tumor wt./group (g)	40.1 (100)	52.6 (131)	17.8 (44.4)
Tumor wt./tumor-bearing rat (g)	3.09 ± 1.48 (100)	4.78 ± 1.84 (155)	1.78 ± 0.40 (57.6)
Wt. of 1 primary tumor (g)	0.757 ± 0.204 (100)	0.772 ± 0.174 (102)	0.523 ± 0.106 (69.1)

Each value is the mean ± SEM. Values in parentheses are percentages of means relative to those of the control group fed on a diet without HSDB.

*The symbol denotes a significant difference ($P < 0.05$) as compared with the value for the control group (Mann-Whitney *U*-test).

The residue obtained from the second extraction using ethyl acetate was left in a fume hood in order to remove the solvent by evaporation. This residue was then extracted with methanol to obtain the methanol-soluble fraction of HSDB (Me). The extraction process was performed as described for Et-Ac.

The Et-Ac and Me extracts are regarded as lipid and carbohydrate, respectively. The composition of the diet for experiment 2 is shown in Table 4. The total dietary concentrations of HSDB-derived material were (w/w) 0% for group I, 0.29% Et-Ac for group II, and 0.71% Me for group III. Both the strain and age of the rats were the same as those used in experiment 1. The animals were administered the control or treatment diet for 9 weeks at 17:00 h, and the remaining food was removed at 9:00 h the following morning. The rats were exposed to DMBA according to the protocol described for experiment 1.

Table 4. Diets Used in Experiment 2

Ingredient	Group		
	I	II	III
	<i>g/100 g</i>		
Casein	20.0	20.0	20.0
DL-methionine	0.3	0.3	0.3
Sucrose	45.0	45.0	44.29
Corn starch	15.0	15.0	15.0
Mineral Mix (AIN-76™)	3.5	3.5	3.5
Vitamin Mix (AIN-76™)	1.0	1.0	1.0
Choline bitartrate	0.2	0.2	0.2
Cellulose	5.0	5.0	5.0
Corn oil	10.0	9.71	10.0
Et-Ac	—	0.29	—
Me	—	—	0.71

Et-Ac and Me are the ethyl acetate- and methanol-soluble fraction of HSDB, respectively.
The extraction process is described in the Materials and Methods section.

Statistical analysis. Each value is expressed as the mean \pm SEM. Treatment differences relative to values for the control group were analyzed by the Mann-Whitney *U* test with Statview software (Cricket Software, Philadelphia, PA, U.S.A.). Probability values of less than 0.05 are considered significant. A regression analysis was also conducted with the Statview software.

Results and Discussion

This study was designed to examine the contribution of HSDB to chemically-induced mammary cancer. No treatment-related changes in clinical signs or body weight gain were observed in either experiment (Tables 2 and 5). All tissue weights, with the exception of the spleen weight, were unaffected by feeding with HSDB or fractions thereof. In both experiments, the spleen weight of the rats fed on the control diet tended to be higher than the spleen weight in the HSDB-treated groups. This splenomegaly was associated with the development of tumor weight: the spleen weight was

Table 5. Body Weight Gain and Organ Weights for the DMBA-Treated Rats in Experiment 2

	Group		
	I	II	III
Body wt. gain (g)	216 \pm 5 (100)	217 \pm 5 (100)	216 \pm 5 (100)
Liver (g/100 g of body wt.)	3.30 \pm 0.13 (100)	3.13 \pm 0.07 (94.8)	3.12 \pm 0.06 (94.5)
Spleen (mg/100 g of body wt.)	235 \pm 41 (100)	188 \pm 6 (80.0)	182 \pm 7 (77.4)
Kidney (mg/100 g of body wt.)	624 \pm 13 (100)	596 \pm 13 (95.5)	597 \pm 13 (95.7)
Lung (mg/100 g of body wt.)	371 \pm 14 (100)	360 \pm 10 (97.0)	371 \pm 10 (100)

Values are means \pm SEM. Values in parentheses represent percentages of mean values v.s those of Group I fed the control diet.

Table 6. Mammary Tumors for the DMBA-Treated Rats in Experiment 2

	Group		
	I	II	III
Tumor incidence at necropsy (%)	66.7 (100)	77.8 (117)	61.1 (91.7)
Latent period (days)	43 \pm 2.9 (100)	51 \pm 1.7 (118)	50 \pm 2.5 (116)
Total no. of tumors	64 (100)	44 (68.8)	47 (73.4)
No. of tumors/tumor-bearing rat	5.33 \pm 0.90 (100)	3.14 \pm 0.77* (58.9)	4.27 \pm 0.89 (80.1)
Total tumor wt./group (g)	65.4 (100)	15.2 (23.2)	23.5 (35.9)
Tumor wt./tumor-bearing rat (g)	5.45 \pm 2.46 (100)	1.08 \pm 0.43 (19.9)	2.13 \pm 0.80 (39.2)
Wt. of 1 primary tumor (g)	1.02 \pm 0.43 (100)	0.345 \pm 0.087 (33.8)	0.499 \pm 0.138 (48.9)

Each value is the mean \pm SEM. Values in parentheses are percentages of means relative to those of the group I fed on the control diet.

*The symbol denotes a significant difference ($P < 0.05$) as compared with the value for the control group (Mann-Whitney *U*-test).

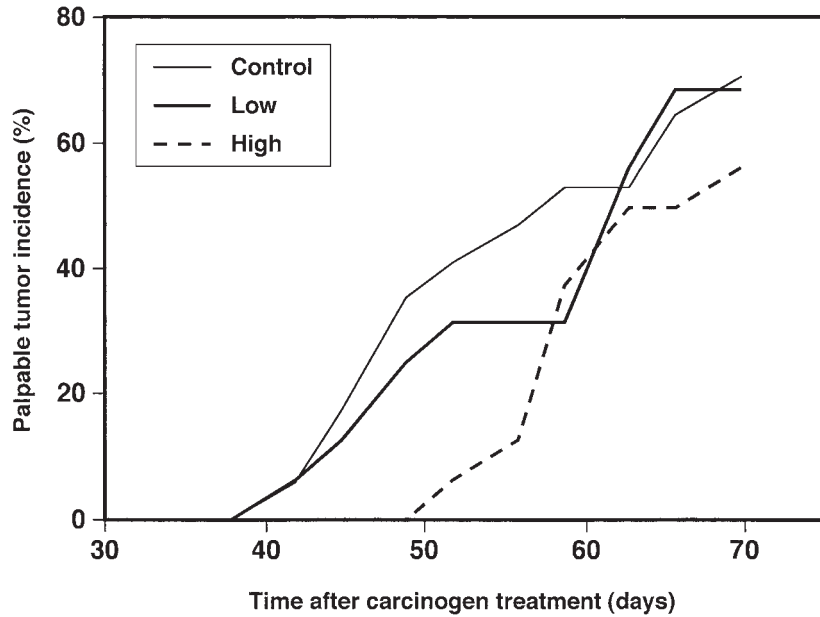


Fig. 1. Cumulative Palpable Tumor Incidence in the DMBA-Treated Rats in Experiment 1.

Thin solid line, the control group; thick solid line, the low group; thick dashed line, the high group. The final tumor incidences diagnosed by palpation were 70.6% for the control group, 68.8% for the low group, and 56.3% for the high group. The values for the low group and the high group as a percentage of the control were 97.4% and 79.7%, respectively.

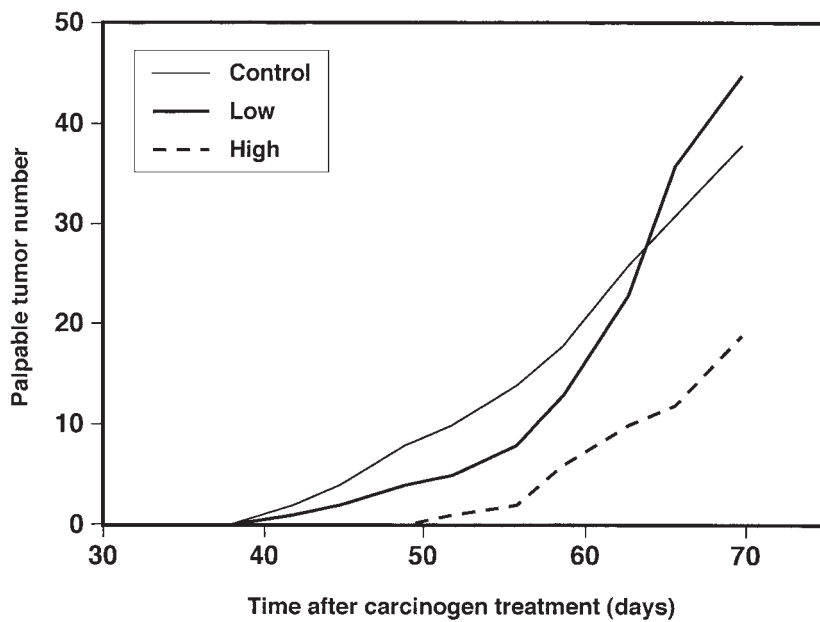


Fig. 2. Cumulative Palpable Tumor Number in the DMBA-Treated Rats in Experiment 1.

Thin solid line, the control group; thick solid line, the low group; thick dashed line, the high group. The final tumor numbers diagnosed by palpation were 38 for the control group, 45 for the low group, and 19 for the high group. The values for the low group and the high group as a percentage of the control were 118% and 50%, respectively.

positively correlated with an increase in the weight of mammary tumors per tumor-bearing rat (experiment 1, $r = 0.584$; experiment 2, $r = 0.913$; data from both experiments; $r = 0.753$). Although further studies not involving the administration of a carcinogen are needed to assess the relationship between HSDB-treatment and splenomegaly, we have previously reported that the

groups with larger DMBA-induced mammary tumor weight were prone to splenomegaly.¹³⁾

The palpatory assessment in experiment 1 indicated a suppression of the incidence and number of tumors in the high group (Figs. 1 and 2). These observations were consistent with the autopsy results (Table 3). The latent period for the high group was significantly delayed

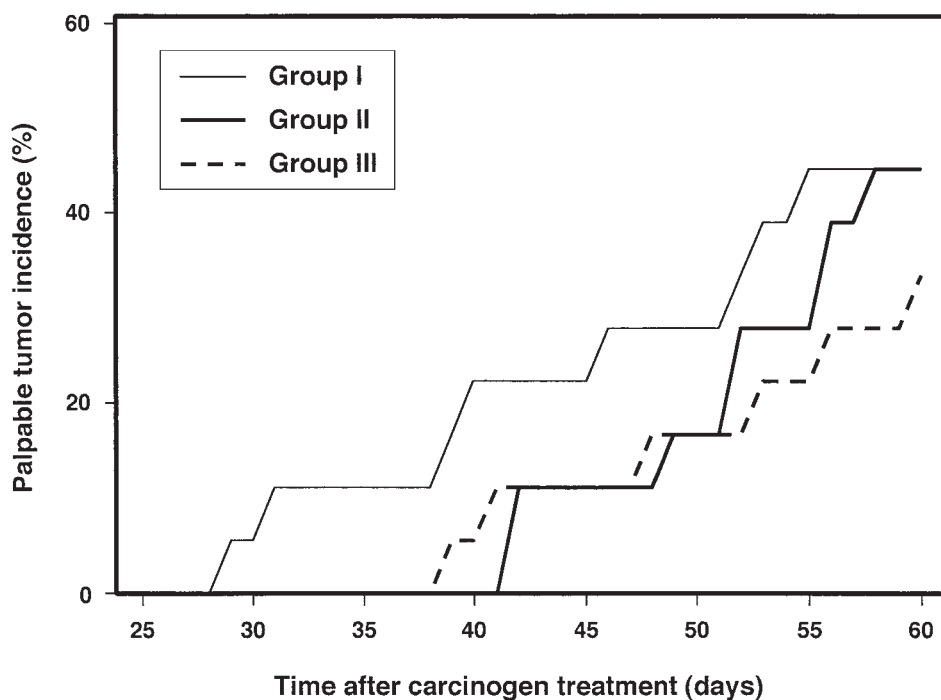


Fig. 3. Cumulative Palpable Tumor Incidence in the DMBA-Treated Rats in Experiment 2.

Thin solid line, group I; thick solid line, group II; thick dashed line, group III. The final tumor incidences diagnosed by palpation were 44.4% for group I, 44.4% for group II, and 33.3% for group III. The values for group II and group III as a percentage of the control were 100% and 75.0%, respectively.

compared to that for the control group. The 5% HSDB diet markedly decreased the total tumor weight per group compared to the control diet. Both the tumor weight per tumor-bearing rat and the weight of one primary tumor tended to be lower in the high group. In the low group, although the incidence of tumors was lower than that in the control group, both the number and weight of tumors were elevated. Although the underlying reason for this requires clarification of the dose-response relationship between HSDB and tumor growth, the findings for the high group revealed that HSDB contained an active component against chemically-induced mammary carcinogenesis *in vivo*. Thus, the current study suggests the possibility of using HSDB as a source of chemopreventive factors beneficial for human health.

Additional experiments were undertaken to identify the factor(s) in HSDB that counteracts cancers *in vivo*. In experiment 2, the number of tumors per tumor-bearing rat was significantly lower in group II than in group I (Table 6). In light of this result, it is likely that an apolar constituent had a pronounced effect on the growth of tumor cells. This hypothesis is consistent with a study reporting that certain components extracted by ethyl acetate from several plants exerted an antiproliferative effect on melanoma cells.¹⁴⁾

The anticarcinogenic compound(s) was not identified in the present study. It is possible that some constituents produced by the Maillard reaction, during the fermentation and/or heating of SDB, accounted for the prophyl-

lactic effect. Oxidative stress correlates very well with carcinogenesis, and the effectiveness of antioxidants has been evaluated.^{15,16)} Rabah *et al.*⁵⁾ have reported that an aqueous extract of the sweet potato cultivar, Koganesengan, the same cultivar from which the HSDB used in the present study was derived, had antioxidative activity which was intensified by baking. However, Ye *et al.*¹⁷⁾ have reported that the number of amino groups in sweet potato SDB was very low, which would tend to minimize the possibility of significant involvement of Maillard reaction products in the inhibitory behavior of HSDB observed in the current study.

Experimental evidence is available that shows that fruits and vegetables contain certain anticancer compounds.^{11,12)} Polyphenols are representative of compounds which protect cells during both the initiation and promotion of carcinogenesis.¹²⁾ Recent studies have shown that sweet potato SDB contained high levels of polyphenols, and have identified caffeic acid as the dominant polyphenol component.^{17,18)} Caffeic acid exhibited many physiological activities against cancer *in vitro*, including radical-scavenging activity,^{19,20)} anti-mutagenesis,²¹⁾ and the inhibition of both metastasis and angiogenesis.²²⁾ In addition to these reports, it is worth noting that caffeic acid can inhibit the growth of mammary tumors *in vitro*.²³⁾ Thus, the current results obtained for the dietary administration of HSDB may be at least partly due to caffeic acid.

In experiment 2, the total numbers of tumors observed were lower at both palpation and autopsy in

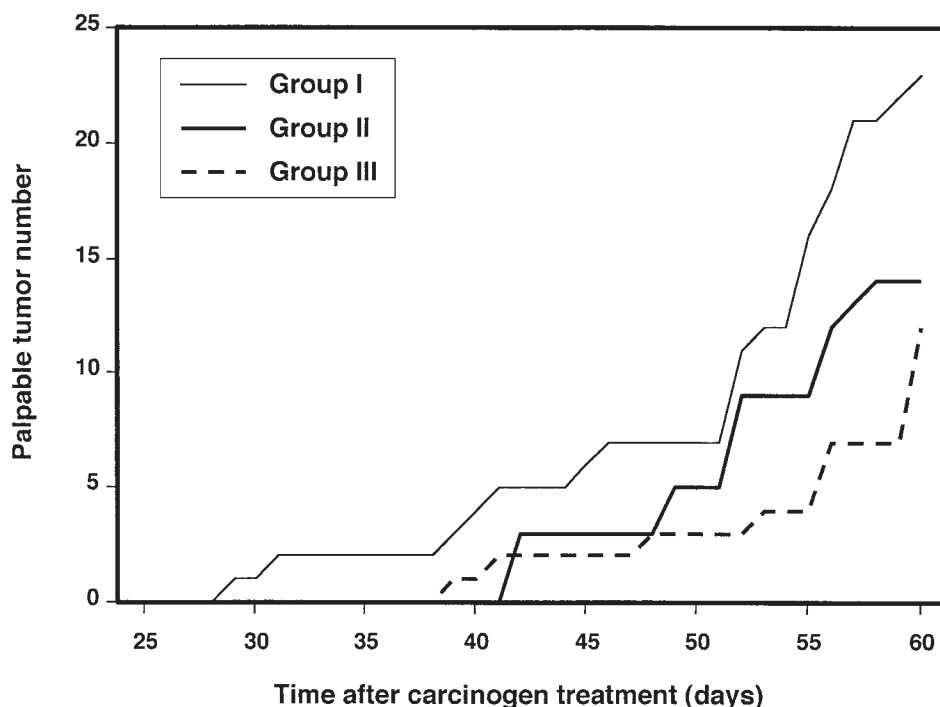


Fig. 4. Cumulative Palpable Tumor Number in the DMBA-Treated Rats in Experiment 2.

Thin solid line, group I; thick solid line, group II; thick dashed line, group III. The final tumor numbers diagnosed by palpation were 23 for group I, 14 for group II, and 12 for group III. The values for group II and group III as a percentage of the control were 60.9% and 52.2%, respectively.

Groups II and III (Fig. 4, Table 6). All the indices for tumor weight tended to improve with feeding of either diet as compared to the control diet. Taken together, these results suggest that HSDB contained multiple anticarcinogenic constituents with different polarity. The anticancer activity observed by feeding the diets containing HSDB fractions was lower than that obtained with the 5% HSDB diet (unpublished data), so the contribution of HSDB to the inhibition of mammary carcinogenesis may have been due to the synergistic effect of more than two components.

In conclusion, this investigation has shown that HSDB had the ability to inhibit mammary carcinogenesis *in vivo*. Although further study is needed to elucidate the mechanisms involved in these physiological activities, the current findings are of value for the development of novel alternative uses of sweet potato SDB.

References

- Hagiwara, A., Yoshino, H., Ichihara, T., Kawabe, M., Tamano, S., Aoki, H., Koda, T., Nakamura, M., Imaida, K., Ito, N., and Shirai, T., Prevention by natural food anthocyanins, purple sweet potato color and red cabbage color of 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP)-associated colorectal carcinogenesis in rats initiated with 1,2-dimethylhydrazine. *J. Toxicol. Sci.*, **27**, 57–68 (2002).
- Konczak-Islam, I., Yoshimoto, M., Hou, D. X., Terahara, N., and Yamakawa, O., Potential chemopreventive properties of anthocyanin-rich aqueous extracts from *in vitro* produced tissue of sweetpotato (*Ipomoea batatas* L.). *J. Agric. Food Chem.*, **51**, 5916–5922 (2003).
- Yoshimoto, M., Okuno, S., Yoshinaga, M., Yamakawa, O., Yamaguchi, M., and Yamada, J., Antimutagenicity of sweetpotato (*Ipomoea batatas*) roots. *Biosci. Biotechnol. Biochem.*, **63**, 537–541 (1999).
- Matsui, T., Ebuchi, S., Kobayashi, M., Fukui, K., Sugita, K., Terahara, N., and Matsumoto, K., Anti-hyperglycemic effect of diacylated anthocyanin derived from *Ipomoea batatas* cultivar Ayamurasaki can be achieved through the α -glucosidase inhibitory action. *J. Agric. Food Chem.*, **50**, 7244–7248 (2002).
- Rabah, I. O., Hou, D. X., Komine, S. I., and Fujii, M., Potential chemopreventive properties of extract from baked sweet potato (*Ipomoea batatas* Lam cv. Koganesengan). *J. Agric. Food Chem.*, **52**, 7152–7157 (2004).
- Ludvik, B., Neuffer, B., and Pacini, G., Efficacy of *Ipomoea batatas* (Caiapo) on diabetes control in type 2 diabetic subjects treated with diet. *Diabetes Care*, **27**, 436–440 (2004).
- Ludvik, B., Waldhäusl, W., Prager, R., Kautzky-Willer, A., and Pacini, G., Mode of action of *Ipomoea batatas* (Caiapo) in type 2 diabetic patients. *Metabolism*, **52**, 875–880 (2003).
- Kusano, S., and Abe, H., Antidiabetic activity of white skinned sweet potato (*Ipomoea batatas* L.) in obese Zucker fatty rats. *Biol. Pharm. Bull.*, **23**, 23–26 (2000).
- Carter, C. A., Milholland, R. J., Shea, W., and Ip, M. M., Effect of the prostaglandin synthetase inhibitor indome-

- thacin on 7,12-dimethylbenz[a]anthracene-induced mammary tumorigenesis in rats fed different levels of fat. *Cancer Res.*, **43**, 3559–3562 (1983).
- 10) Weisburger, J. H., Role of fat, fiber, nitrate, and food additives in carcinogenesis: a critical evaluation and recommendations. *Nutr. Cancer*, **8**, 47–62 (1986).
 - 11) Wattenberg, L. W., Chemoprevention of cancer. *Cancer Res.*, **45**, 1–8 (1985).
 - 12) Dragsted, L. O., Strube, M., and Larsen, J. C., Cancer-protective factors in fruits and vegetables: biochemical and biological background. *Pharmacol. Toxicol.*, **72** (Suppl. 1), 116–135 (1993).
 - 13) Sasaki, T., Kobayashi, Y., Shimizu, J., Wada, M., In'nami, S., Kanke, Y., and Takita, T., Effects of dietary n-3-to-n-6 polyunsaturated fatty acid ratio on mammary carcinogenesis in rats. *Nutr. Cancer*, **30**, 137–143 (1998).
 - 14) Calliste, C. A., Trouillas, P., Allais, D. P., Simon, A., and Duroux, J. L., Free radical scavenging activities measured by electron spin resonance spectroscopy and B16 cell antiproliferative behaviors of seven plants. *J. Agric. Food Chem.*, **49**, 3321–3327 (2001).
 - 15) Ames, B. N., Dietary carcinogens and anticarcinogens. Oxygen radicals and degenerative diseases. *Science*, **221**, 1256–1264 (1983).
 - 16) Kohen, R., and Nyska, A., Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification. *Toxicol. Pathol.*, **30**, 620–650 (2002).
 - 17) Ye, X. J., Morimura, S., Han, L. S., Shigematsu, T., and Kida, K., *In vitro* evaluation of physiological activity of vinegar produced from barley-, sweet potato-, and rice-*shochu* post-distillation slurry. *Biosci. Biotechnol. Biochem.*, **68**, 551–556 (2004).
 - 18) Yoshimoto, M., Kurata-Azuma, R., Fujii, M., Hou, D. X., Ikeda, K., Yoshidome, T., and Osako, M., Phenolic composition and radical scavenging activity of sweet-potato-derived *Shochu* distillery by-products treated with Koji. *Biosci. Biotechnol. Biochem.*, **68**, 2477–2483 (2004).
 - 19) Ogiwara, T., Satoh, K., Negoro, T., Okayasu, H., Sakagami, H., and Fujisawa, S., Inhibition of NO production by activated macrophages by phenolcarboxylic acid monomers and polymers with radical scavenging activity. *Anticancer Res.*, **23**, 1317–1323 (2003).
 - 20) Kikuzaki, H., Hisamoto, M., Hirose, K., Akiyama, K., and Taniguchi, H., Antioxidant properties of ferulic acid and its related compounds. *J. Agric. Food Chem.*, **50**, 2161–2168 (2002).
 - 21) Yamada, J., and Tomita, Y., Antimutagenic activity of caffeic acid and related compounds. *Biosci. Biotechnol. Biochem.*, **60**, 328–329 (1996).
 - 22) Xu, F., Song, D., and Zhen, Y., Inhibition of tumor metastasis by sodium caffeate and its effect on angiogenesis. *Oncology*, **67**, 88–92 (2004).
 - 23) Hudson, E. A., Dinh, P. A., Kokubun, T., Simmonds, M. S. J., and Gescher, A., Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. *Cancer Epidemiol. Biomark. Prev.*, **9**, 1163–1170 (2000).