〔Chiba Med. 65, 67～74, 1989〕

〔綜説〕 PHARMACOLOGCIAL AND ANTICARCINOGENIC EFFECTS OF *CAPSELLA BURSA-PASTORIS* EXTRACT

Keiko KURODA*

Received November 19, 1988. Accepted January 5, 1989.

SUMMARY

Capsella bursa-pastoris herb has been eaten and used medicinally in China and Japan for many centuries and some chemical and pharmacological studies on the herb extract were made in the early period of this century. Our studies showed that the herb extract had some other pharmacological activities including oxytocic, antiulcer and anti-inflammatory actions. Thereafter, the herb extract was found to inhibit the solid growth of Ehrlich tumor in mice. Fumaric acid was isolated and identified as the active component responsible for the antitumor action and also for the antiulcer action. When fumaric acid was given to the tumor-bearing mice in combination with mitomycin C, an efficient but highly toxic antitumor antibiotic, it was found that fumaric acid reduced selectively the side effects of mitomycin C. Fumaric acid was then shown to exhibit an inhibitory effect on the chemical carcinogenesis i.e. the induction of stomach and lung cancers by a nitrofuran in mice, and that of hepatomas by 3-methyl-4′-dimethylaminoazobenzene or thioacetamide in rats. These activities of fumaric acid were associated with its enhancing effect on the DNA synthesis of the pertinent tissues to overcome the damages by these toxic agents.

Key words: *Capsella bursa-pastoris*, Fumaric acid, Anticarcinogenic effect, Antitumor activity, DNA synthesis.

1. Introduction

In an old Chinese book written in the 6 th century, it is recorded that people had a custom of eating seven spring herbs with rice gruel on January 7th. An old Japanese book, "ENGISHIKI", written in the 10 th century says the same things. This practice has been believed to help stomachs from exhaustion after the New Year Feasts, and still followed by many Japanese people today. Figs. 1 and 2 show the seven herbs.

1. *Oenanthe stolonifera*, Umbelliferae,
2. *Capsella bursa-pastoris*, Cruciferae,
3. *Gnaphalium multiceps*, Compositae,
4. *Stellaria media*, Caryophylaceae,
5. *Lapasna apogonoides*, *Compositae*,
6. *Raphanus sativus*, Cruciferae,
7. *Arabis flagellosa*, Cruciferae.

Capsella bursa-pastoris, commonly called "shepherd's purse", was used as a folk medicine for hemostasis, diuresis, and antipyresis (Fig. 3)[1]. Few studies were made chemically and pharmacologically in the early period of this century[2-5].

2. Pharmacological activities of *Capsella bursa-pastoris* extract

At the beginning of our studies, we examined the pharmacological activities of *Capsella bursa-*

* Research Center for Pathogenic Fungi and Microbial Toxicoses, Division of Mechanism of Fungal Infections, Chiba University, Chiba 280
黒田啓子：*Capsella bursa-pastoris* 抽出物の薬理作用並びに抗腫瘍効果について
千葉大学真核微生物研究センター，感染機構分野
（昭和63年11月19日受付，昭和64年1月5日受理）

Fig. 1. Seven herbs

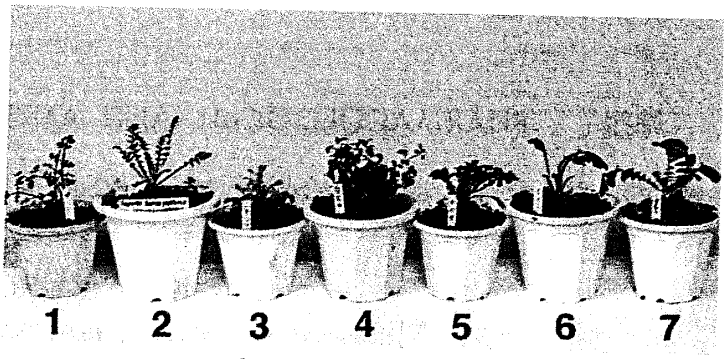


Fig. 2. Seven herbs

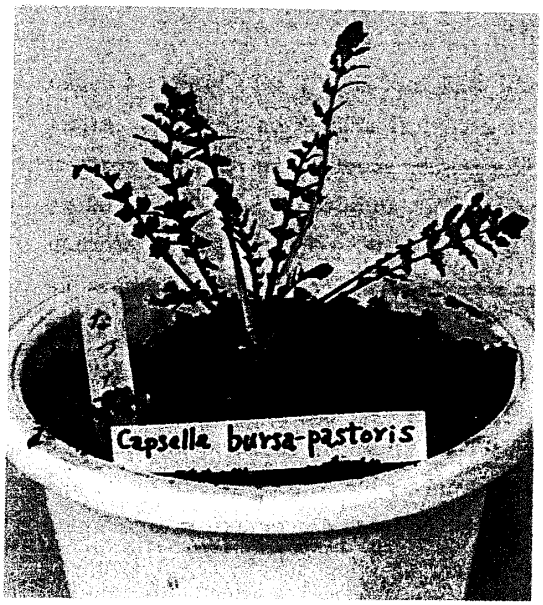


Fig. 3. *Capsella bursa-pastoris*

pastoris herb. The active components of this herb were extracted with 70% ethanol, purified by passing through the columns of charcoal, K_1, and Sephadex G25, K2, and fractionated by absolute-ethanol extraction, K_3 and K_4, and paper chromatography, K_3' (Fig. 4). The isolated materials showed various kinds of pharmacological activities: they decreased blood pressure, increased the coronary blood flow in the heart as did the cardiotonic agents, and contracted small intestine and uterus. These activities advanced with the progress of refinement, and K_3', which consisted of polpeptides, showed the most potent oxytocic activity[6,7]. A strong diuretic activity was exhibited by the absolute-ethanol soluble fraction, K_4, but not in the insoluble fraction, K_3. The herb extract also exhibited anti-inflammatory and antiulcer actions. Our studies have provided some pharmacological basis to the clinical usage of the herb extract[8,9].

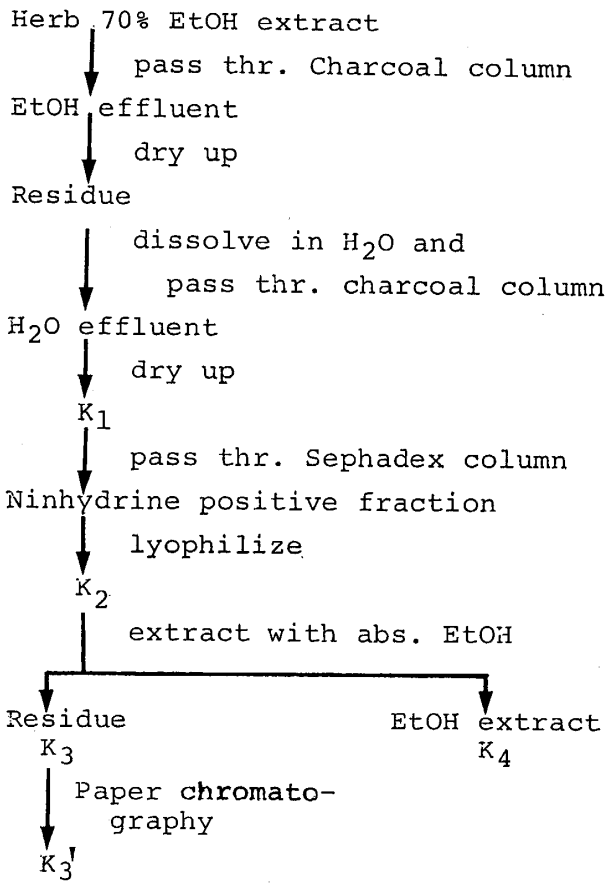


Fig. 4. Fractionation of *CBP* extract for pharmacological activities

3. Inhibitory effect of *Capsella bursa-pastoris* extract on growth of Ehrlich solid tumor

Then, we made experiments to examine whether the herb extract exhibited any anti-tumor activity or not, and found that it inhibited the solid growth of Ehrlich tumor in mice[10]. Accordingly, we tried to isolate and identify the active component, using the anti-tumor activity as a guide. In short, the active component could be extracted from the acidified solution of the herb into ether, and an active substance was isolated in the crystalline form (Fig.

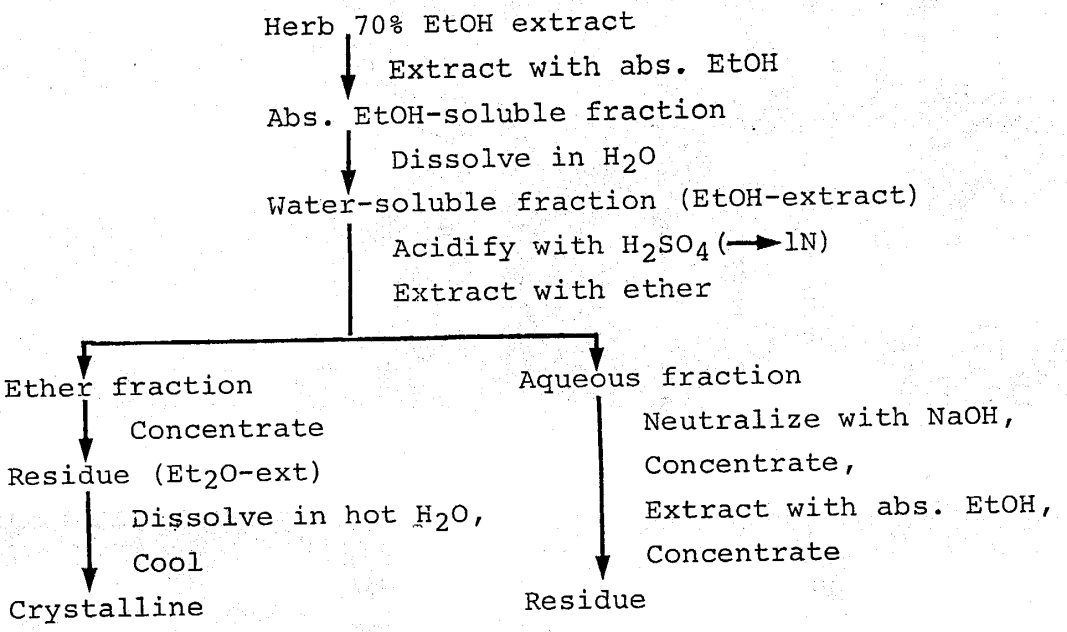


Fig. 5. Fractionation of *CBP* extract for antitumor activity

Table 1. Anti-tumor Activities of the Fractions

Fraction	Control	Residue	EtOH-ext	Et_2O-ext	Crystalline
Dose (mg/kg)[a]	—	80	80	80	40
Tumor wt(g)	2.2±0.6	2.4±0.5	1.5±0.5[b]	1.0±0.4[b]	0.7±0.4[c]

[a] Given i.p. daily for 14 days. Each value represents the mean±S.D.
[b] $P<0.02$, [c] $P<0.01$, relative to Control.

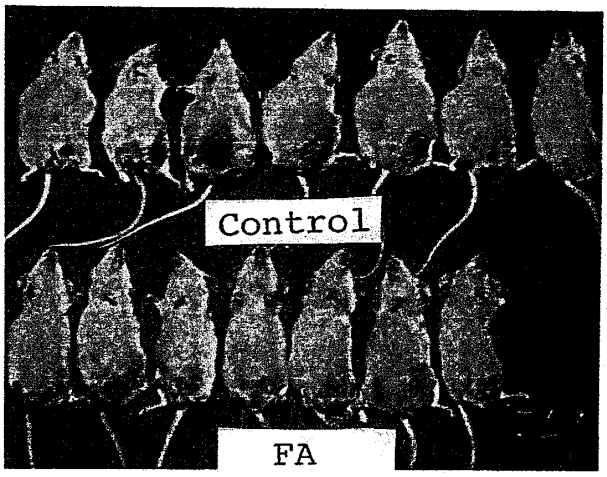
Table 2. Physical and Chemical Properties of the Crystalline Substance

Monoclinic prismatic needles; mp 285-286°.
IR (KBr)/cm; 1660, 1425, 1320, 1275, 1234, 1010, 900.
NMR in dimethylsulofoxide-d_6 δ ppm from tetramethylsilane; 6.65 (2H, singlet).
Mass spectrum m/e; 116 (M^+).
Elementary analysis;

$C_4H_4O_4$

Calculated: C 41.39, H 3.47.
Found; C 41.66, H 3.52.

5). Table 1 shows the anti-tumor activities of the fractions. The anti-tumor assay was made on ICR strain mice which were inoculated with 2 millions of Ehrlich tumor cells into the subcutaneous tissue of the left inguinal region. Each fraction of the herb extract was given to mice intraperitoneally for 14 days at the dose given in the Table 1. Then, the animals were killed and their tumor lumps were weighed. The anti-tumor activity was noted in the ether soluble fraction and also in the crystalline substance isolated from this fraction. The physical and chemical properties of the crystalline substance, including Melting Point, Infrared (IR), Nuclear Magnetic Resonance(NMR)- and Mass- spectra, and elementary analysis, completely coincided with those of authentic fumaric acid (Table 2). Figs. 6 and 7 show the tumor nodules isolated from the subcutaneous tissue of mice. In contrast to saline-control, fumaric acid effectively inhibited the growth of tumor nodules. Fumaric acid was also responsible for the antiulcer action of the herb extract (Fig. 8)[11].



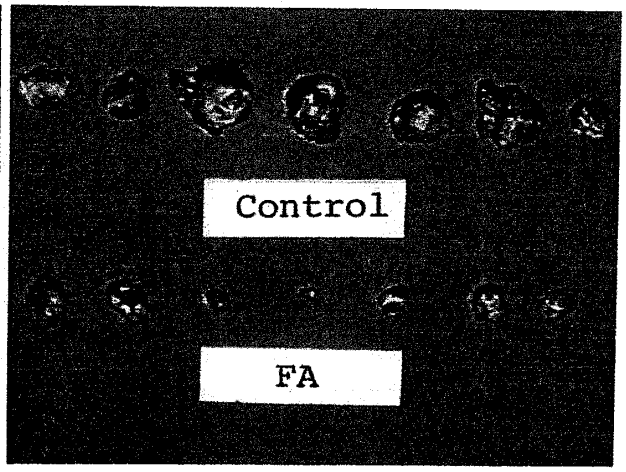


Fig. 6, 7. Inhibitory effect of fumaric acid on the solid growth of Ehrlich tumor in mice

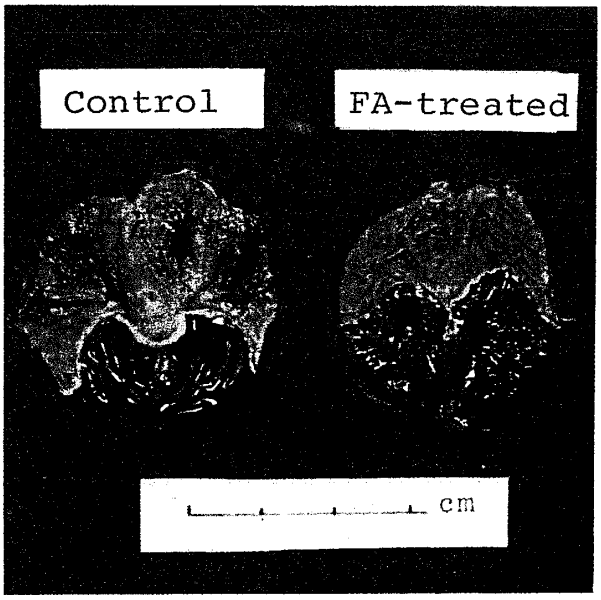


Fig. 8. Antiulcer action of fumaric acid

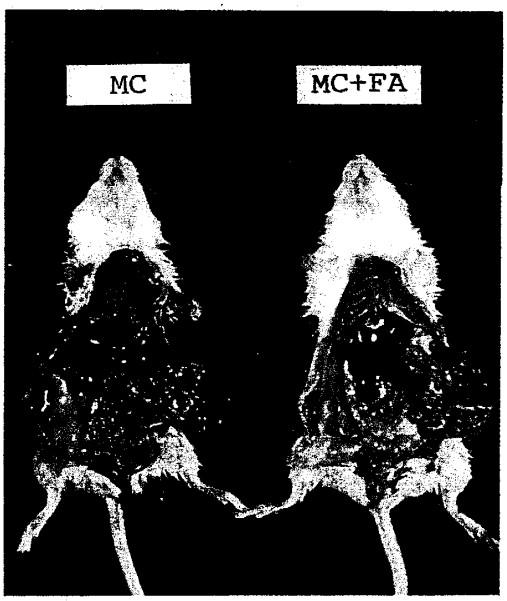


Fig. 9. Inhibitory effect of fumaric acid on the occurrence of toxic symptoms in mice given mitomycin C

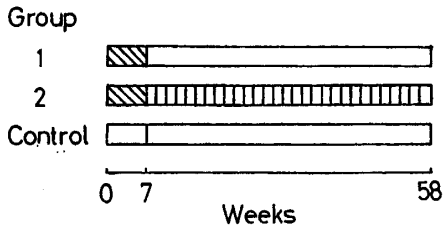


Fig. 10. Experimental methods. ▨ =given a diet containing 0.06% 3-methyl-DAB; ▥ =given a diet containing 1% fumaric acid and drinking water containing 0.025% fumaric acid; □ =given a basal diet.

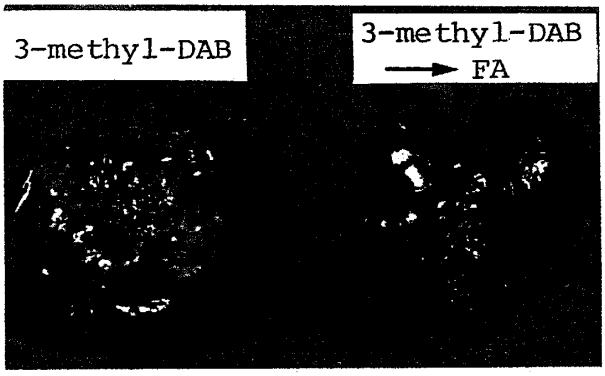


Fig. 11. 3-Methyl-DAB-induced hepatoma and the inhibitory effect of fumaric acid on the induction of hepatoma

Table 3. Effects of Mitomycin C and Fumaric Acid on Liver and Kidney as to Contents of Nuclei Acids and Incorporation of Either [^{3}H]Thymidine into DNA or [^{14}C]Orotic Acid into RNA[a]

Treatment[b]	DNA		RNA	
	Content (mg/g tissue)	Incorporation of [^{3}H] thymidine (dpm×10^{-3}/mg DNA)	Content (mg/g tissue)	Incorporation of [^{14}C] orotic acid (dpm×10^{-4}/g tissue)
Liver				
Control	2.35 ± 0.17	5.04 ± 1.53	8.40 ± 1.09	3.09 ± 0.31
MC	3.52 ± 0.15[d]	2.46 ± 0.43[d]	7.90 ± 0.21	3.20 ± 0.13
MC+FA	2.88 ± 0.52[c]	11.7 ± 3.3[d]	8.00 ± 0.70	3.37 ± 0.45
Kidney				
Control	3.70 ± 0.11	3.13 ± 0.18	4.90 ± 0.25	40.0 ± 4.2
MC	4.42 ± 0.19[d]	2.39 ± 0.16[d]	4.98 ± 0.09	50.7 ± 5.9[d]
MC+FA	3.89 ± 0.39	6.58 ± 2.54[d]	4.89 ± 0.13	46.7 ± 5.9

[a] Mice were divided into three groups, and each group of animals were treated with two i.p. injections on the first and third days. On the fifth day the contents of tissue nucleic acids and the incorporation of either [^{3}H]thymidine into DNA or [^{14}C]orotic acid into RNA were determined. Each value represents the mean±S.D. of six determinations.

[b] Control, givinen 0.2 ml of 0.9% NaCl; MC-treated, given 4 mg/kg of mitomycin C; MC+FA-treated, given 4 mg/kg of mitomycin C and 40 mg/kg of fumaric acid.

[c] $P<0.05$, relative to Control.

[d] $P<0.01$, relative to Control.

4. Reduction by fumaric acid of side effects of mitomycin C

An attempt was made to examine the combination effect of fumaric acid with mitomycin C, a potent but highly toxic anti-tumor antibiotic[12]. Then it was found that the toxic symptoms in ICR mice given mitomycin C were reduced by the concurrent administration of fumaric acid (Fig. 9). Fumaric acid did not reduce the antitumor activity of mitomycin C against either the solid or the ascitic form of the Ehrlich tumor. Fumaric acid reduced the lethal and hematologic toxicities of mitomycin C, and studies on the nucleic acids of animal tissues indicated that mitomycin C inhibited selectively DNA synthesis of liver and kidney, whereas fumaric acid exerted an enhancing effect, antagonistic to mitomycin C, on DNA synthesis of these tissues (Table 3).

5. Anticarcinogenic activity of fumaric acid

The third activity of fumaric acid against cancer is an anticarcinogenic action. This activity of fumaric acid was shown by the following three experiments.

(1) A study was carried out to examine the effect of fumaric acid on the induction of hepatomas in the rats that had been given a hepatocarcinogen, 3-methyl-4'-(dimethylamino) azobenzene (3-methyl-DAB)[13]. Animals used were male Donryu strain rats and the basal diet fed to the animals was a semisynthetic diet, CE-2. Rats, 2 months of age, were fed a diet containing 0.06% 3-methyl-DAB until they consumed 0.5 g of the carcinogen, which required about 50 days. Then the animals were divided into 2 groups. The rats of Group 1 were followed to be given the basal diet and ordinary drinking water for 51 weeks. Group 2 rats were given a diet containing 1% fumaric acid and the drinking water containing 0.025% fumaric acid for the same period. Control rats were maintained on the basal diet and ordinary drinking water throughout (Fig. 10). All 15 animals of the untreated control group showed no neoplastic changes in their livers. Hepatic tumors developed in 11 of 13 animals of Group 1 fed solely 3-methyl-DAB. In the rats of Group 2 given fumaric acid after ingestion of 3-methyl-DAB, most livers were macroscopically almost normal, and their surfaces were generally smooth (Fig. 11). Only 2 cases of hepatocellular

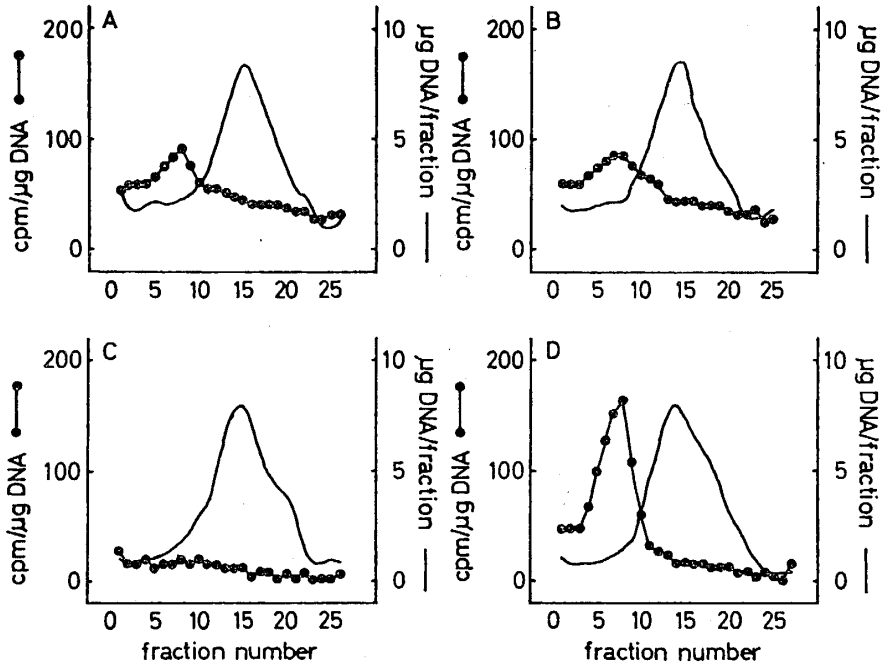


Fig. 12. Effect of an i. v. injection of mitomycin C and fumaric acid on the distribution of the radioactivity of hepatocyte DNA in NaI isopycnic gradients. (A) Hepatocytes from a rat given 8ml/kg of 0.9% NaCl solution. (B) Hepatocytes from a rat given 40 mg/kg of fumaric acid. (C) Hepatocytes from a rat given 0.5mg/kg of mitomycin C. (D) Hepatocytes from a rat given both mitomycin C and fumaric acid. At 24hr after the injection, hepatocytes were isolated from rat liver and exposed in culture to [^{3}H]dThd and BrdUrd. The DNA of the hepatocytes was semipurified and distributed in NaI isopycnic gradients. Sedimentation was from right to left. The quantity of DNA in each fraction was determined fluorometrically and the radioactivity in DNA was determined in a liquid scintillation counter. Curves are representative of 3 separate experiments.

carcinomas developed in this group. The liver on the left was found in a rat of Group 1. The neoplasm is greyish white, soft, and lobulated. The tumor nodule with central umbilication occupies a large part of the median lobe. The liver on the right is a representative example of a normal looking liver from a rat of Group 2.

(2) Another inhibitory effect of fumaric acid on hepatocarcinogenesis was examined in rats fed thioacetaminde (TAA)[14]. A group of male Donryu rats were fed TAA at a level of 0.035% in the diet for 40 weeks and then fed a basal diet for 40 weeks. Hepatic carcinomas developed in 9 of 41 animals of this group fed TAA alone. The effect of fumaric acid on the carcinogenesis was examined in 2 groups fed both TAA and fumaric acid; one group of rats were fed fumaric acid at 1% in a basal diet after ingestion of TAA, and another group of rats were fed TAA plus a supplement of 1% fumaric acid in the diet. The inhibitory effect of fumaric acid on TAA carcinogenesis was so marked that no hepatic carcinomas were found in both groups fed fumaric acid in combination with TAA.

(3) The inhibitory effect of fumaric acid on carcinogenesis by potassium 1-methyl-7-[2-(5-nitro-2-furyl) vinyl] -4-oxo-1, 4-dihydro-1, 8-naphthyridine-3-carboxylate (NFN), a strong stomach and lung carcinogen, was examined histologically with male ICR/JCL mice[15]. NFN was fed to mice at a dose level of 0.012% in the diet for 14 weeks. These mice were then divided into 2 groups. One group was given a basal diet, and the other group was given a diet containing 1% fumaric acid in the subsequent 39 weeks. In the group of 30 mice fed NFN alone, squamatous cell carcinomas were found in the stomachs of 7 mice, multiple papillomas in the stomachs of 13 mice, and multiple and large

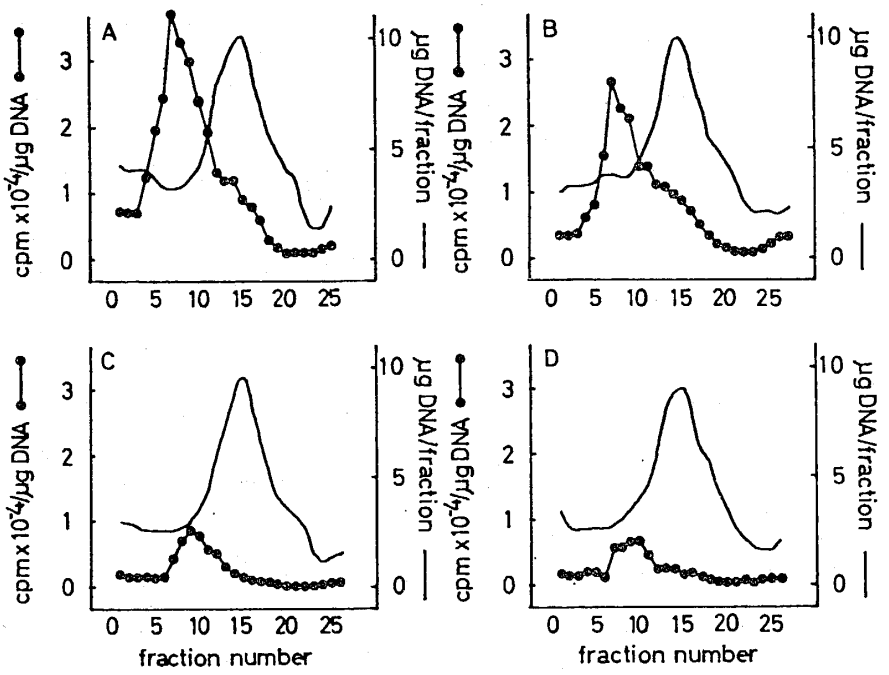


Fig. 13. Effect of an i.v. injection of mitomycin C and fumaric acid on the distribution of the radioactivity of 3-methyl-DAB-induced hepatoma cell DNA in NaI isopycnic gradients. (A) Hepatoma cells from a rat given 8 ml/kg of 0.9% NaCl solution. (B) Hepatoma cells from a rat given 40 mg/kg fumaric acid. (C) Hepatoma cells from a rat given 0.5 mg/kg of mitomycin C. (D) Hepatoma cells from a rat given both mitomycin C and fumaric acid. Rats were inoculated with 3-methyl-DAB-induced transplantable AH136B hepatoma cells in the abdomen and 8 days after given an i. v. injection as described above. At 24 hr after the injection, hepatoma cells were harvested from the abdominal ascites and exposed in culture to [^{3}H]dThd and BrdUrd. Analysis of the density gradients was done as described in Fig. 12. Curves are representative of 3 separate experiments.

papillary adenocarcinomas in the lungs of 27 animals. The administration of fumaric acid suppressed the NFN-induced stomach and lung carcinogenesis. In the group of 32 mice fed NFN and fumaric acid, no stomach tumors developed except one early-stage of squamatous cell carcinoma. In the lungs, only a small focus of mild atypical hyperplasia and a few early-stage adenocarcinomas were noted in 7 and 11 animals, respectively.

6. Concluding remarks

I would like to conclude our study by mentioning the mode of action of fumaric acid for its anticarcinogenic activity[16]. In our study fumaric acid was given to rats after intake of a carcinogen. Therefore, fumaric acid inhibited the development of hepatomas not so much affecting the metabolism of the carcinogen but more affecting the promotion of carcinogenesis. Fumaric acid has some kinds of activities supposedly relevant to the present finding. First, as mentioned previously, this acid inhibited the solid growth of subcutaneously transplanted Ehrlich tumor in mice[10]. Second, it reduces the growth and viability of Ehrlich, MH134, L1210 mouse tumor cells[17] as well as 3-methyl-DAB-induced rat hepatoma cells in culture. Third, it reduces the toxic symptoms in mice and rats after the injection of mitomycin C, a toxic antitumor antibiotic, or aflatoxin B_1, a hepatotoxin produced by a fungus, *Asperguillus flavus*[12,16].

Fig. 12 shows the DNA synthesis of rat hepatocytes. The activity of DNA synthesis was determined by incubating the hepatocytes with [^{3}H]-labeled thymidine and 5-bromodeoxyuridine in culture, sedimenting the DNA isolated from hepatocytes in NaI isopycnic gradient, and measuring the

radioactivity in the fractionated DNA. The semi-conservative DNA synthesis is shown as the radioactivity sedimenting more rapidly than the peak of DNA detected fluorometrically (Fig. 12A). The injection of mitomycin C at the dose of 0.5mg/kg at 24 hr before sacrifice reduced the activity of hepatocytes' DNA synthesis (Fig. 12C), while the concurrent dosing of 40mg/kg of fumaric acid enhanced the activity and prevented it from being reduced by mitomycin C (Fig. 12D). A similar finding was noted with aflatoxin B_1[16]. The injection of mitomycin C also inhibited the DNA synthesis of 3-methyl-DAB-induced transplanted hepatoma cells that were growdng in the abdominal ascites of rats (Fig. 13C). However, in contrast to the case of hepatocytes, the concurrent dosing of fumaric acid did not enhance the DNA synthesis of the cancer cells. The present selective effect of fumaric acid with regard to the enhancement of DNA synthesis seems to indicate the mode of action of fumaric acid for anticarcinogenic activity, namely, fumaric acid might enhance the DNA synthesis of liver tissue to ameliorate the deleterious alterations brought about by 3-methyl-DAB and also to encourage the normal liver cells to compete with the precancerous and dormant cancer cells for the development in the liver tissue.

要　旨

古来，中国並びに本邦では，十字花科植物なずなは薬用植物として食されまたは利用されてきたが，その成分や薬理効果についての研究は，今世紀の初め，若干行われたにすぎない。我々の研究から，その抽出物が子宮収縮作用，抗胃潰瘍，抗炎症作用等いくつかの有用な薬理作用を持つことがわかった。次いで抽出物がマウスにおけるエーリッヒ固型癌の成長を抑制することも見出され，抗癌作用や抗胃潰瘍作用の有効成分としてフマール酸を単離，同定した。有効な抗癌性物質マイトマイシンCは副作用も強いがフマール酸と併用投与することにより，副作用のみが選択的に軽減される。フマール酸は，又，化学物質による発癌，例えば，ニトロフランによる胃癌や肺癌の誘発，アゾ色素やチオアセタミドによる肝癌の誘発を抑制する。これらフマール酸の活性は組織DNA合成を促進してこれら毒物による組織損傷を修復することを助けることに基づくものと考えられる。

References

1) Grimme C: Altes und Neues über Capsella bursa-pastoris. Ph Zentralhalle **60** : 237–251, 1919.
2) Boruttau H and Cappenberg H: Beiträge zur Kenntnis der wirksamen Bestandteile des Hirtentäschelkrautes. Arch Pharm **259** : 33–52, 1921.
3) Bombelon E: Acidum bursinicum. Ph Ztg **33** : 151–152, 1888.
4) Gilg E: Kurze vorläufige Mitteilung über die Wirkung der Herbe Bursae pastoris. Angewandte Botanik **4** : 74–77, 1922.
5) Harste W: Wissenschaftlicher Teil. Arch Pharm **266** : 133–151, 1928.
6) Kuroda K and Takagi K: Physiologically active substance in *Capsella bursa-pastoris*. Nature **220** : 707–709, 1968.
7) Kuroda K and Kaku T: Pharmacological and chemical studies on the alcohol extract of *Capsella bursa-pastoris*. Life Sci **8** : 151–155, 1969.
8) Kuroda K and Takagi K: Studies on *Capsella bursa-pastoris*. I. General pharmacology of ethanol extract of the herb. Arch Int Pharmacodyn **178** : 382–391, 1969.
9) Kuroda K and Takagi K: Studies on *Capsella bursa-pastoris*. II. Diuretic, anti-inflammatory and anti-ulcer action of ethanol extracts of the herb. Arch Int Pharmacodyn **178** : 392–399, 1969.
10) Kuroda K, Akao M, Kanisawa M and Miyaki K: Inhibitory effect of *Capsella bursa-pastoris* extract on growth of Ehrlich solid tumor in mice. Cancer Res **36** : 1900–1903, 1976.
11) Kuroda K and Akao M: Inhibitory effect of fumaric acid and dicarboxylic acids on gastric ulceration in rats. Arch Int Pharmacodyn **226** : 324–330, 1977.
12) Kuroda K and Akao M: Reduction by fumaric acid of side effects of mitomycin C. Biochem Pharmacol **29** : 2839–2844, 1980.
13) Kuroda K, Terao K and Akao M: Inhibitory effect of fumaric acid on 3-methyl-4'-(dimethylamino)azobenzene-induced hepatocarcinogenesis in rats. J Natl Cancer Inst **71** : 855–857, 1983.
14) Kuroda K, Terao K and Akao M: Inhibitory effect of fumaric acid on hepatocarcinogenesis by thioacetamide in rats. J Natl Cancer Inst **79** : 1047–1051, 1987.
15) Kuroda K, Kanisawa M and Akao M: Inhibitory effect of fumaric acid on forestomach and lung carcinogenesis by a 5-nitrofuran naphthyridine derivative in mice. J Natl Cancer Inst **69** : 1317–1320, 1982.
16) Kuroda K, Akao M and Terao K: Fumaric acid enhances DNA synthesis of rat hepatocytes by counteracting the toxicities of mitomycin C and aflatoxin B_1. Jpn J Cancer Res (Gann) **77** : 750–758, 1986.
17) Kuroda K and Akao M: Antitumor and anti-intoxication activities of fumaric acid in cultured cells. Jpn J Cancer Res (Gann) **72** : 777–782, 1982.