THE *IN VIVO* EFFECT OF A *BRASSICA OLERACEA VAR. CAPITATA* EXTRACT ON EHRLICH ASCITES TUMORS OF *MUS MUSCULUS* BALB/C MICE

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SUMMARY

An extract of *Brassica oleracea var. capitata* juice was prepared using petroleum ether, ether, ethanol and an Al_2O_3 column. The healing and tumor protecting effects of this extract were tested on Ehrlich ascites (EA) solid tumors of *Mus musculus* BALB/C mice. Complete disappearance of the tumors was observed in 54.5% of the animals in the experimental group (n=22) which received 20 mg/day of the extract i.p. for 28 days. Regression of the tumors (27%), fixation of tumor size (4%) and an increase in tumor size (18%) were also recorded. Neither tumor size fixation nor regression was recorded in the control group which received physiological serum (0.5 ml/day). The healing effect was found to be related to the starting tumor size. The healed animals in the experimental group were followed for 6-7 months and no tumor recurrence was recorded. The protective effect of this extract on tumor formation was also tested. Experimental animals (n=35) received 20 mg/day of the extract i.p. for 20 days. Physiological serum was administered to a control group (n=30). Transplantation of solid tumors was performed on the 20^{th} day and extract administration was discontinued. Transplantation success was recorded 20 days after transplantation. In the experimental group, only three out of 35 mice showed tumor development, whereas in the control group the number was 23 out of 35 mice. It was also observed that the extract prevented the development of liquid EA tumors. This extract was also found to be nontoxic. *Brassica oleracea var. capitata* had a healing effect as well as a protective effect on EA solid tumors of mice. These results are in agreement with our previous results obtained from a liquid *Brassica oleracea var. acephala* juice extract.

KEY WORDS

Brassica oleracea var. capitata, cancer, cabbage

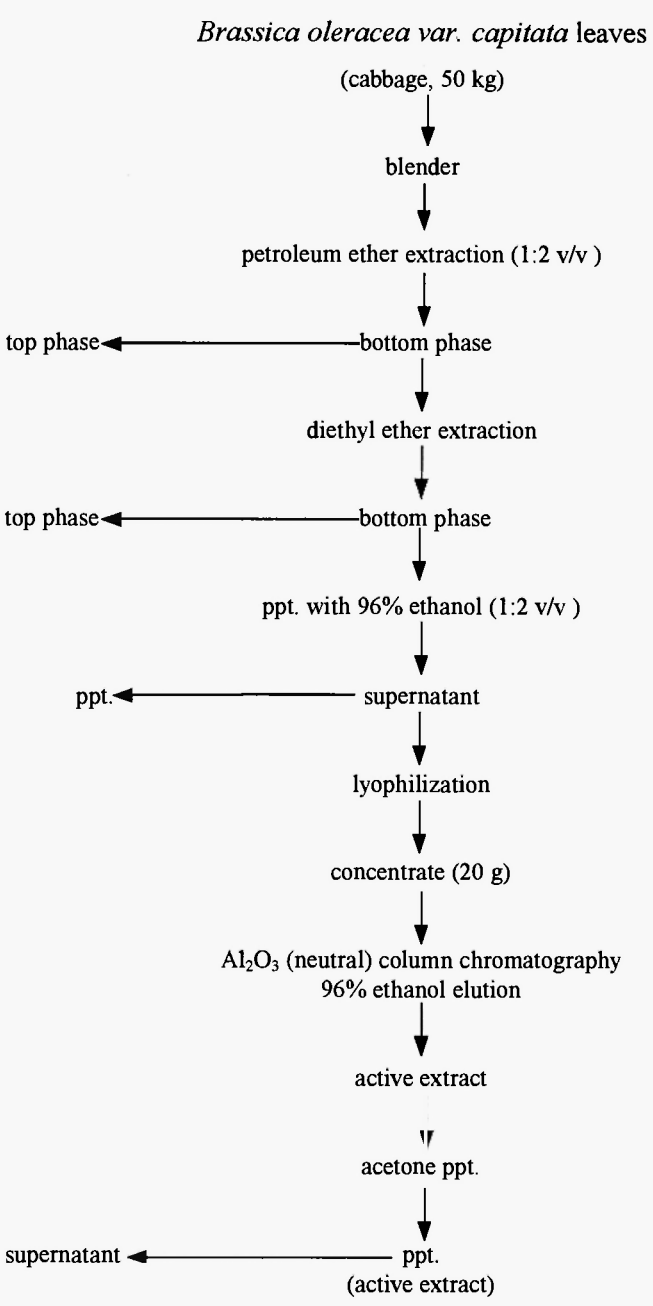
INTRODUCTION

In recent years there have been a number of studies reporting that Cruciferae plants affect carcinogenesis. There is a correlation between consumption of these plants and decrease in the incidence of cancer in man /1-3/. Several compounds isolated from these plants have been reported to inhibit carcinogenesis in animals /4-5/. A cytolytic effect of an alkaloid compound obtained from the leaves of *Brassica oleracea var. capitata* and *var. acephala* has been demonstrated by Gürkan *et al.* /6-8/. An anticoagulant effect of cruciferous plants has also been reported /9/ together with antitumoral, antibacterial /8/ and goitrogenic effects /10,11/.

The aim of the present study was to investigate the biological activities of the fractions of *Brassica oleracea var. capitata* isolated by column chromatography using Al_2O_3 following petroleum ether and diethyl ether extraction /12,13/.

METHODOLOGY

The following isolation procedure of the biologically active extract from *Brassica oleracea var. capitata* was employed. The following flow chart describes schematically the experimental method used:



RESULTS AND DISCUSSION

In the present study, an alcoholic Al_2O_3 column extract was obtained from *Brassica oleracea var. capitata* juice. The healing and tumor protecting effects of this extract were tested on Ehrlich ascites (EA) solid tumors of *Mus musculus* BALB/C mice. Complete disappearance of the tumors was observed in 54.5% of the animals. Regression of the tumors was seen in 27%, fixation of the tumor size in 4%, and increase in tumor size in 18% were recorded in the experimental group (n=22) which received 20 mg/day of the extract i.p. for 28 days. Neither tumor size fixation nor regression was recorded in any of the animals in the control group which received physiological serum (0.5 ml/day) (n=15). The healing effect was found to be related to the starting tumor size (Table 1). The healed animals in the experimental group were followed for 6-7 months and no tumor recurrence was recorded.

The protective effect of this extract on tumor formation was also tested. Experimental animals (n=35) received 20 mg/day of the extract i.p. for 20 days. Physiological serum (0.5 ml/day) was administered to the control group (n=30). Solid tumor transplantation was performed on the 20^{th} day and extract administration was discontinued. Transplantation success was recorded 20 days after transplantation. In the experimental group only three out of 35 mice had tumor development whereas in the control group this ratio was 23 out of 35 mice. It was also observed that the extract prevented the development of liquid EA tumors. This extract was also found to be nontoxic.

Brassica oleracea var. capitata has a healing effect as well as a protective effect on EA solid tumors of mice. These results are in accordance with previous results obtained with liquid *Brassica oleracea var. acephala* juice extract /13,14/.

Several biological activities of an extract obtained from *Brassica oleracea var. capitata* and its *in vivo* effects on some biochemical parameters have been investigated /15-17/. It was previously reported that the contents of cruciferous plants such as cabbage, Brussels sprouts, cauliflower, etc. affect carcinogenesis /1,3/. Epidemiological studies have established a relationship between the consumption of these vegetables and the incidence of cancer in humans /1-3/. It has been claimed that some substances isolated from these plants inhibit carcinogenesis in animals /4,5/.

TABLE 1

The effect of cabbage extract on solid Ehrlich ascites tumors

Mouse No.	Tumor Size (cc) Beginning	Tumor Size(cc) Day 28
CONTROL GROUP		
1	0.30x0.40x0.23	1.30x1.30x1.20
2	0.30x0.50x0.50	1:42x1.50x1.30
3	0.40x0.50x0.32	1.60x1.43x0.80
4	0.50x0.50x0.50	1.52x1.30x1.30
5	0.50x0.80x0.72	1.71x2.10x1.30
6	0.52x0.41x0.32	1.32x1.21x1.00
7	0.60x0.50x0.60	1.20x1.21x1.30
8	0.62x0.50x0.30	1.42x1.20x0.90
9	0.63x0.60x0.50	1.35x1.80x0.90
10	0.70x0.61x0.40	1.36x1.40x0.90
11	0.80x0.80x0.62	1.80x2.25x1.00
12	0.81x0.70x0.53	1.80x2.15x1.60
13	0.90x1.20x0.80	2.32x2.53x1.40
14	1.00x0.90x0.50	2.53x1.80x0.90
15	1.00x1.00x0.62	2.60x2.15x1.30
EXPERIMENTAL GROUP		
1	0.30x0.30x0.30	0
2	0.30x0.32x0.40	0
3	0.50x0.50x0.60	0
4	0.60x0.40x0.32	0
5	0.62x0.51x0.42	0
6	0.72x0.70x0.50	0
7	0.72x0.60x0.52	0
8	0.73x0.42x0.70	0
9	0.80x0.70x0.62	0
10	0.82x0.80x0.43	0
11	0.82x0.91x0.40	0
12	0.90x0.72x0.52	0
13	0.74x0.72x0.80	0.30x0.35x0.42
14	0.80x0.82x0.91	0.53x0.42x0.60
15	0.80x0.70x0.91	0.34x0.30x0.52
16	0.90x0.90x0.60	0.45x0.42x0.30
17	1.00x1.00x0.72	0.73x0.70x0.40
18	1.00x1.40x1.30	1.12x0.90x0.70
19	1.40x1.00x0.92	1.42x1.30x1.00
20	1.50x1.50x1.22	1.51x1.70x1.10
21	1.60x1.50x1.52	3.00x1.85x1.75
22	1.62x1.51x1.07	2.90x1.80x1.75

It was previously established that the juice of the leaves of *Brassica oleracea var. capitata* inhibited mitosis secondary to morphological changes in the embryoblasts of rats /8/. A Dragendorff-positive, possibly alkaloid substance obtained from this liquid caused the death of the cells of tumors and embryos /7/ and inhibited the mitosis of seeds of *Plasedus vulgaris* /6/.

It has been reported that feeding cabbage to aflatoxin B_1-induced cancerous mice reduced markers of tumor activity and also inhibited hepatic DNA-aflatoxin B_1 binding /18/. It was shown that *Brassica oleracea var. acephala* had an antitumor effect. This was determined by following the reproduction of erythroleukemic K562 and BALB/C normal spleen cells in tissue culture /13,14/. It was observed that the extract did not have a lethal effect against BALB/C spleen cells at the concentrations used, but it did have a tumoricidal effect against K562 cells at a concentration of 2.5-5.0 μg extract/ml and above /12,13/. It was also shown that this extract did not have any stimulatory effect against NK (natural killer) or LAK (lymphokine-activated killer) systems, which are important mechanisms in "immune surveillance" /13,14/.

Scientific studies concerning other biological activities of cruciferous plants have been published. It has been reported that cabbage juice increases gastric secretion /19/, decreases the level of blood sugar /20/, and that it contains a goitrogenic agent /10,11/. In another study, it was found that an extract from black cabbage did not affect the level of blood sugar in rabbits /21/.

Recently, it was shown that eating cooked Brussels sprouts did not affect T_3 or T_4 plasma levels in humans /22/. It has been demonstrated that in animals fed with cruciferous plants or with substances obtained from these plants, there was an increase in mixed function oxidases in the liver /23/; glutathione reductase also increased /19,24/; hepatic DNA destruction and lipid peroxidation were inhibited /25/, and cytochrome C-reductase was also inhibited /18/.

REFERENCES

1. Graham S. Results of case-control studies of diet and cancer in Buffalo, New York. Cancer Res 1983; 43 (Suppl): 2409.
2. Graham S, Dayal H, Swanson M, Mittelman A, Wilkinson G. Diet in the epidemiology of cancer of the colon and rectum. J Natl Cancer Inst 1978; 61: 709-712.
3. National Research Council. Diet, Nutrition and Cancer. Washington, DC: National Academy Press, 1982; 11.
4. Boyd JN, Babish JG, Stoewsand GS. Modification by beet and cabbage of aflatoxin B_1 induced rat plasma α-fetoprotein elevation, hepatic tumorogenesis and mutagenicity of urine. Food Chem Toxicol 1982; 20: 47-50.
5. Stoewsand DS, Babish JB, Wimberly HC. Inhibition of hepatic toxicities from polybrominated biphenyls and aflotoxin B_1 in rats fed cauliflower. J Environ Pathol Toxicol 1978; 2: 399-404.
6. Gürkan E, Köksal G. The cytotoxic effect of two *Brassica* species. Fitoterapia 1988; LIX: 47-48.
7. Gürkan E, Küçükcezzar R. *Brassica oleracea var. capitata*'nın (Beyaz Lahana) sitotoksik etkisi ve bu etkinin biyolojik önemi. İstanbul: ÇNAEM-R-209, 1982; 1-16.
8. Şengün A, Çevikbaş A, Özalpan A, Gürkan E. *Brassica oleracea*'nın doku kültüründe yetiştirilen hücreler ve bakteriler üzerine tesiri. IV. Bilim Kongresi Ankara 5-8 Kasım 1973; 1-4.
9. Carter TH, Everson BA, Ratnof OD. Cabbage seed protease inhibitor: a slow tight binding inhibitor of trypsin with activity toward trombin, activated stuart factor (factor X a), activated Hageman factor (factor XII a) and plasmin. Blood 1990; 75: 108-115.
10. Heany RK, Fenwick GR. Chemical and biological properties of indole glucosinolates (glucobrassicins): a review. Food Chem Toxicol 1988; 26: 59 70.
11. Nugon-Baudon L, Rabot S, Szylit O, Raiboud P. Glucosinolate toxicity in growing rats: interactions with the hepatic detoxification system. Xenobiotica 1990; 20: 223-230.
12. Baytop T. Türkiye'de bitkilerle tedavi. Istanbul Üniversitesi yayını, İstanbul 1984; 40.
13. Yurtsever E, Yardımcı T, Çevikbaş A, Ugur Ş, Gürkan E, Savaş B, Akoğlu T, Ulution ON. Kara lahana ekstesinin antitumoral, antiagregan, profibronolitik ve antitromolitik etkileri. Bitkisel Ilaç Hammeddeleri Toplantısı, Bildiriler 16-19 Mayıs 1991. Ed Başer KHC. 1992; 115-124.
14. Yardımcı T, Yurtsever E, Gürkan E, Çevikbaş A, Savaş B, Kavalalı E, Bayraktar E, Akoglu T. The antitumoral effect of an extract isolated from *Brassica oleracea var. acephala.* Int Pharmacy J 1991; Suppl F.I.P. Washington: abst #4, P-MP-029.
15. Yurtsever E, Tamer L, Yarman A, Isbir T, Yardımcı T. The effect of extract of *Brassica oleracea var. capitana* on lipid peroxidation in *Mus musculus*

BALB/C mice. First International Meeting on Pharmacy and Pharmaceutical Sciences, Istanbul, September, 1994.
16. Yaman A, Yurtsever E, Özsavcı M, Yardımcı T. The effect of *Brassica oleracea var. capitana* extract on leucocyte count and hematocrit values in rats. Second Meeting of the Balkan Clinical Laboratory Federation, Istanbul, September, 1994.
17. Yurtsever E, Yardımcı T. The in vivo effect of *Brassica oleracea var. capitana* extract (EC) on rat blood cell glutathione and hemoglobin levels of BALB/C mice with and without Ehlich ascites tumors. International Meeting on Pharmacy and Pharmaceutical Sciences, Istanbul, September, 1998.
18. Whitty JP, Bjeldanes LF. The effect of dietary cabbage on xenobiotic-metabolizing enzymes and the binding of aflotoxin B_1 to hepatic DNA in rats. Food Chem Toxicol 1987; 25: 581-587.
19. Bykoff KM. Influence of cabbage juice ingested with different kinds of food upon the secretary function of the gastric glands. Physiol Abstr 1922; 8. 366-370.
20. Dubin HE, Corbit HB. Hypoglycemia producing substances. US Patent Dec 1928; 22: 845.
21. Kuşçu İ, Can A. Action of two drugs from folk medicine on blood sugar level. Acta Pharm Turcica 1984; 26: 46-48.
22. McMillan M, Spinks EA, Fenwick GR. Preliminary observations on the effect of dietary Brussels sprouts on thyroid function. Hum Toxicol 1986; 5: 15-19.
23. Wattenburg L. Inhibition of chemical carcinogens by minor dietary components. In: Arnott MS, Van Eys J, Wang YM, eds. Molecular Interaction and Cancer. New York: Raven Press, 1982; 43-56.
24. Bogards I, Van Ommen B, Falke HE, Williams MI, Van Blanderen PI. Glutathione S-transferase subunit induction patterns of Brussels sprouts isothiocyanate and goitrin in rat liver and small intestinal mucosa: a new approach for the identification of inducing xenobiotics. Food Chem Toxicol 1990; 28: 81-88.
25. Stohs SJ, Lawson TA, Anderson L, Bueding E. Effect of oltipraz, BHA, ADT and cabbage on glutathione metabolism, DNA and lipid peroxidation in old mice. Mech Aging Dev 1986; 37: 137-145.