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Inhibitory Effect of Kale Juice on the Growth and DNA Incorporation of Human Cancer Cells

Seon-Mi Lee and Kun-Young Park†

Dept. of Food Science and Nutrition, and Pusan Cancer Research Center, Pusan National University, Pusan 609-735, Korea

Abstract

The inhibitory effects of kale juice on the growth and DNA incorporation of human cancer cells, using HT-29 colon cancer cells, MG-63 osteosarcoma cells, AGS gastric adenocarcinoma cells and K-562 leukemia cells, were studied. The growth of human cancer cells were inhibited in the presence of kale juice(10, 20 and 40μl/ml) and the effects were the juice concentration- and incubation time-dependent up to 6 days. When 20μl/ml of kale juice was added to the media of HT-29, MG-63, AGS and K-562 cancer cells, the cell growth after 6 or 4 days of incubation was retarded by 83~95% of control group. Morphological changes of HT-29 colon cancer cells were studied under inverted microscope. As the concentration of kale juice increased up to 20μl/ml, degree of cell aggregation was decreased. Moreover, the DNA incorporation of AGS gastric adenocarcinoma cells and MG-63 osteosarcoma cells which were labeled with [^{3}H] thymidine was significantly reduced after 2 days of incubation at 37°C with kale juice. Therefore, we concluded that kale juice strongly decreased the growth of various human cancer cells.

Key words: kale juice, human cancer cells, DNA incorporation

INTRODUCTION

In the recent years, many efforts to seek for naturally nontoxic antimutagenic and anticancer compounds have been continued through various anticancer medicines and foods. Related to food, these compounds have been continuously discovered from vegetables, fruits, grains and seaweeds, etc.(1-4). By several researches performed until now, it is reported that daily consumption of green-yellow vegetables remarkably reduced the risk of many types of cancers, such as lung, larynx, oral, pharynx, esophagus, stomach, liver and bladder cancer, etc. Thus, it is believed that green-yellow vegetables are one of the prominent foods for the anticancer activities and health(5). Especially, *Brassica* vegetables, like cabbage, broccoli and cauliflower, are known to have the stronger anticancer effects than other green-yellow vegetables(6-9). Our laboratory has made progress in researches about antimutagenic and anticancer effects from green-yellow vegetables and *Brassica* vegetables, which can be taken daily and do not have any harmful activities(10,11).

Kale(*Brassica oleracea* var. *acephala*), one of *Brassica* vegetables, is originated from Asia Minor as a type of cabbage(12), and has various kinds such as tree kale, marrow kale, bush kale, portuguese kale, kitchen kale and chosun kale, etc. Kale, broadly used as materials of vegetable juices and kale-wrapped rice in Korea, contains high levels of vitamins and minerals, especially vitamin C(146mg/100g), vitamin U(54mg/100g), β-carotene (70.3μg/g), calcium(181mg/100g), phosphorous(69mg/100g), iron(3.4mg/100g) and potassium(380mg/100g), etc. And kale is also known to contain anticancer compounds such as chlorophyll, flavonoids, benzyl isothiocyanate and phenethyl isothiocyanate(13-18). It is found that kale contains 35.5% of dietary fiber by enzymatic gravimetric method and 32.5% of dietary fiber by urea enzymatic dialysis method as a dry basis(19), and it is reported that these soluble and insoluble dietary fiber greatly reduced the mutagenicity induced by aflatoxin B_1(AFB_1) and 3,2'-dimethyl-4-amino-biphenyl (DMAB), etc.(20,21). Choi et al.(22) reported the juice of kale reduced the mutagenicity induced by AFB_1 under *Drosophila* system *in vivo*. Chung et al.(23) mentioned the juice of kale reduced the phospholipid and cholesterol concentration in hypercholesterolemic rats.

†Corresponding author

Thus, the protective effects of kale on cancer is expected to attribute to complex actions of various compounds, like flavonoid, chlorophyll, dietary fiber, vitamin C, β-carotene, benzyl isothiocyanate and phenethyl isothiocyanate, present in kale.

Recently, the first screening method for anticancer compounds which have an anticancer effect tends to perform with *in vitro* test on the panel of cancer cell lines obtained from representative cancers in human (24-28). As the methods measuring the toxicity of this cancer cell, there are relatively the diverse methods, such as Clonogenic assay calculating the colony numbers formed by the survival cells after culturing the cells with sample, tritiated thymidine uptake assay measuring the utilization degree of nucleic acid of cancer cells(29), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide(MTT) assay measuring the enzyme activity of survival cancer cells(30), and sulforhodamine (SRB) assay measuring the protein amount within cells.

In this study, kale that showed the highly anticancerous and antimutagenic effect among various *Brassica* vegetables was selected from previous experiments on *Brassica* vegetables. The anticancerous effects of kale juice on cancer cells were studied in terms of cell growth, morphological changes and DNA incorporation using various human cancer cells.

MATERIALS AND METHODS

Preparation of sample

Kale was purchased from a local market in the city of Pusan, Korea. After the sample was selected, washed and blended by a juicer, the portion of kale juice was obtained by refrigerated centrifugation(4°C) at 9,000 rpm for 30min. The supernatant was sterilized through milipore filter(0.45μm) before adding to the experimental system(31).

Materials

Dulbeco's modified Eagle's medium(DMEM), fetal calf serum(FCS), 0.05% trypsin-0.02% EDTA, and 100 units/ml penicillin-streptomycin were purchased from GIBCO Co.(Gaithersburg, MD, USA). CO_2 incubator (Sanyo, model MCO96, Japan) was used for cell culture. Inverted microscope(Olympus, model PM-10AK, Japan) was used to see the morphological changes of cancer cells. The liquid scintillation counter(Beckman LS250) was used for DNA incorporation experiments.

Cell culture

AGS human gastric adenocarcinoma cell, HT-29 human colon cancer cell, MG-63 human osteosarcoma cell and K-562 human leukemia cell were obtained from Korea Cell Bank(Medical School, Seoul National University). AGS, HT-29(32) and MG-63 cancer cells(33) were cultured routinely at 37°C in DMEM, supplemented with 100 units/ml of penicillin-streptomycin and 10% FCS in 5% CO_2 incubator. Media were changed twice or three times every week. After six or seven days, cultured cancer cells were washed with PBS. Cells were harvested after trypsin-0.02% EDTA treatment followed by centrifugation. Media were added to integrated cells and it was evenly dispersed with pipette. Five ml of medium containing cancer cells were transfered to the cell culture flask, and it was cultured for further experiment. For a long term storage, the harvested cells in DMEM containing 10% FCS and 10% dimethylsulfoxide were stored in the liquid nitrogen. K-562 leukemia cells, suspension cell, were cultured routinely at 37°C in DMEM, supplemented with 100 units/ml of penicillin-streptomycin and 10% FCS. The cell was maintained same procedure described as the above(24,27, 28,30).

Cell growth experiments

Adherent cell AGS, HT-29 and MG-63 cancer cells, were plated in 24-well plates and cultured for 24 hours in 10% FCS supplemented DMEM at a plating density of 20,000 cells/ml. After cancer cells were attached to plate, they were cultured in 5% CO_2 incubator at 37°C, changing culture medium with kale juice every other day. After 6 days, the cells were treated with 0.05% trypsin-0.02% EDTA, inhibitory effects of kale juice to that of control on the growth of cancer cells were observed by counting the cell numbers. The suspensioned K-562 leukemia cells, were also seeded in 24-well plate with the density of 20,000 cells/ml, and the kale juice at desired concentrations were added directly in medium. After 4 days, the inhibitory effect on the growth of K-562 cancer cells to that of control was observed(Fig. 1)(33-36). In order to see the morphological change of the cancer cells by kale juice, cancer cells were cultured for 24hr in 10% FCS supplemented DMEM at plating density of 20,000 cells/ml. After cancer cells were attached

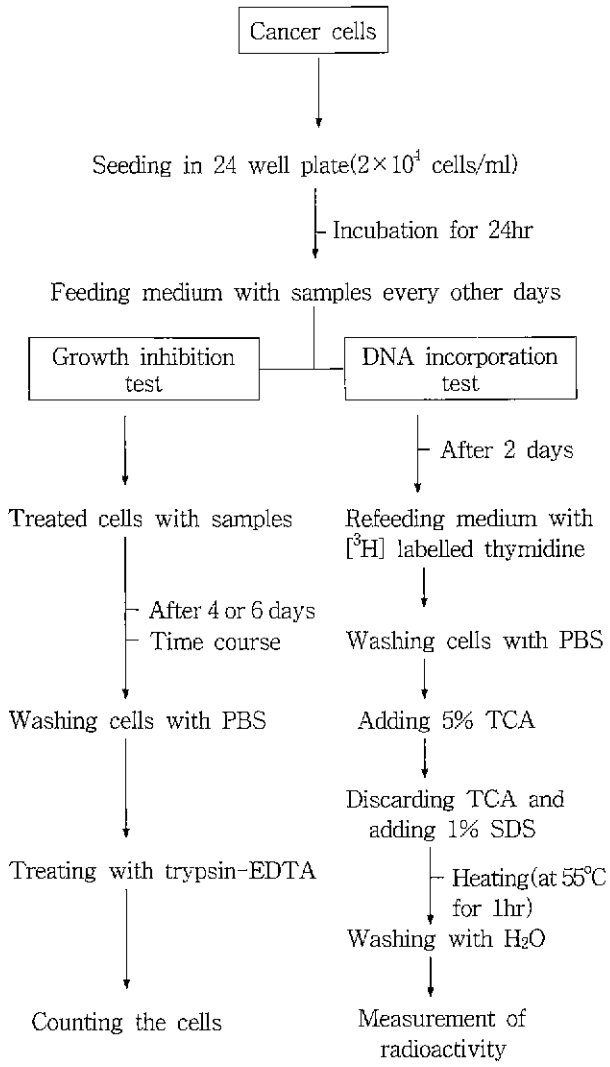

Fig. 1. A schematic diagram for the tests of growth inhibition and DNA incorporation in cancer cells.

to the plate, they were cultured with medium containing kale juice in 5% CO_2 incubator at 37°C. The shape of cancer cell was observed under the inverted microscope(Olympus Co., Japan)(36).

DNA incorporation

AGS and MG-63 cancer cells were plated in 24-well plates and cultured for 24hr in 10% FCS supplemented DMEM at a plating density of 20,000 cells/ml. After cells were attached to the plate, cultured medium was replaced with new medium containing 10% FCS and kale juice. They were cultured in 5% CO_2 incubator at 37°C. After 48hr, medium was replaced with the indicated medium labeled with 3μCi/ml of [^{3}H] thymidine. After 2hr of incubation, the indicated medium was discarded and the solid component was washed twice with PBS. Cells were kept refrigerated at 4°C with 1ml of 5% cold TCA. After 1hr, TCA was removed and 250μl of 1% SDS was added. The cells were heated to separate from 24-well plate for 1hr at 55°C. After cells were transfered into scintillation vial, they were washed twice with 125 μl of H_2O. The radioactivity was measured with Beckman LS250 scintillation counter after adding 3.5ml of scintillation cocktail(29)(Fig. 1).

Statistical analysis

Student's *t*-test was used for statistical analysis, reflecting data obtained from control and sample groups.

RESULTS AND DISCUSSION

The inhibitory effects of kale juice on the growth of various human cancer cells of AGS gastric adenocarcinoma cells, HT-29 colon cancer cells, MG-63 osteosarcoma cells and K-562 leukemia cells were studied (Fig. 2). When the kale juice was added to the medium

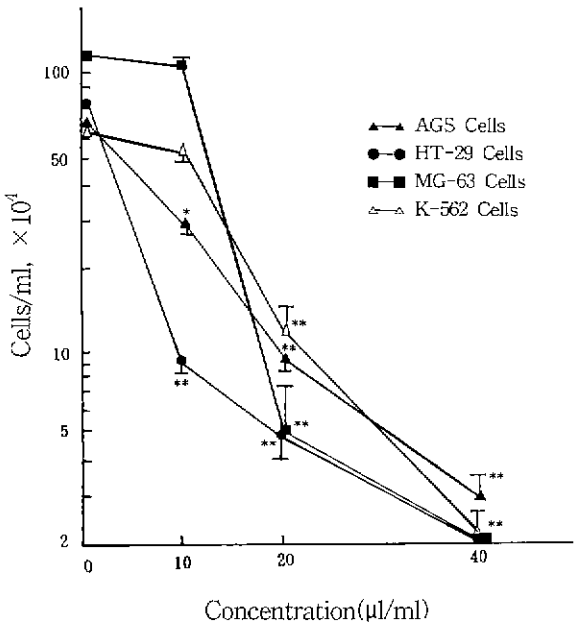

Fig. 2. Inhibitory effect of the filtered fresh juice of kale on the growth of AGS gastric adenocarcinoma cells, HT-29 colon cancer cells, MG-63 osteosarcoma cells, and K-562 leukemia cells.
(Counted after 6 days of incubation: AGS, HT-29 and MG-63. Counted after 4 days of incubation: K-562 cells)
*Significantly different from the control at the $p<0.05$ level.
**Significantly different from the control at the $p<0.01$ level.

of AGS gastric adenocarcinoma cells in the concentration of 10, 20 and 40μl/ml, 58.0%, 87.0% and 95.9% of growth inhibition to that of control was observed, respectively ($p<0.05$). In the case of HT-29 cells, 88.3% and 98.7% of cell growth were inhibited in the presence of 10μl/ml and 40μl/ml of kale juice, respectively($p<0.01$). And also same effect was observed from MG-63 osteosarcoma cells, 95.4% of growth inhibition was observed from 20μl/ml of kale juice, and 99.0% inhibition was occured when 40μl/ml of kale juice was added($p<0.01$). In the case of K-562 leukemia cells, there was no inhibitory effect of cell growth in 10μl/ml of kale juice. But, when the kale juice concentrations increased up to 20 and 40μl/ml, 82.6% and 96.8% of inhibitory effects were observed, respectively($p<0.01$). As the concentrations of kale juice was increased, the growth of human cancer cells of AGS gastric adenocarcinoma cells, HT-29 colon cancer cells, MG-63 osteosarcoma cells and K-562 leukemia cells were more retarded as concentration dependent.

Fig. 3 shows the inhibitory effect of kale juice on the growth of AGS gastric adenocarcinoma cell according to the incubation time. When 40μl/ml of kale juice was added to cultured cell medium for 2, 4 and 6 days of incubation, 89.7%, 91.7% and 97.7% of inhibitory effect were found, repectively. When 20μl/ml of kale juice was added, 69.0%, 79.8% and 79.7% of inhibitory effects were shown for 2, 4 and 6 days, respectively. Therefore, it was observed that adding 20μl/ml to sample was less effective than adding 40μl/ml to sample. Lawson et al. (6) reported that the consumption of *Brassica* vegetables was in inverse relation to the generation of colon, stomach, breast, and prostate cancer, they explained that indole compound in *Brassica* vegetables might decrease the gastric cancer which is induced by 3,4-benzo[a] pyrene(Bp) and the breast cancer that is induced by 7,12-dimethyl-benz[a]anthracene(DMBA). National Research Council(37) in USA recommended to consume more *Brassica* vegetables as a means of decreasing the growth of human cancer, based on results of animal experiments and epidemiological researches.

The inhibitory effect of fresh kale juice on the growth of HT-29 colon cancer cells was also carried out according to the incubation time(Fig. 4). When kale juices were added to cultured medium in the concentration of 40μl/ml for 2, 4 and 6 days of incubation, 28.6%, 60.8% and 88.3% of inhibitory effect were found, respectively.

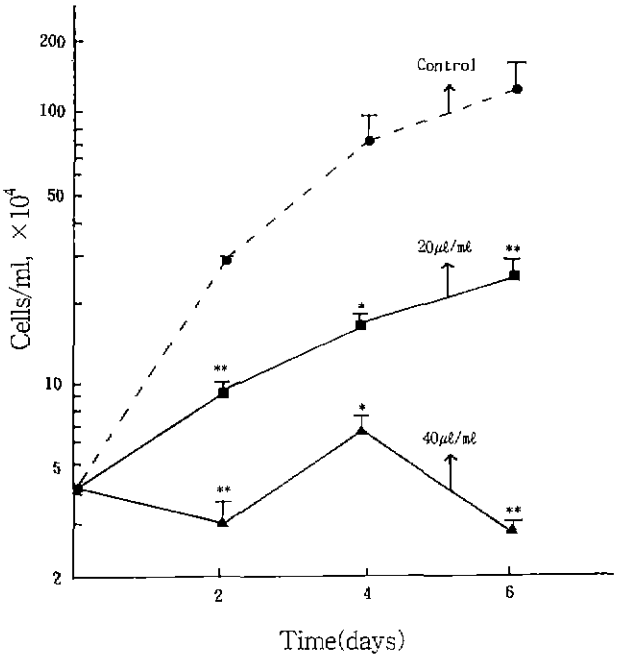

Fig. 3. Inhibitory effect of the filtered fresh juice of kale (μl/ml) during the growth of AGS gastric adenocarcinoma cells.

*Significantly different from the control at the $p<0.05$ level.

**Significantly different from the control at the $p<0.01$ level.

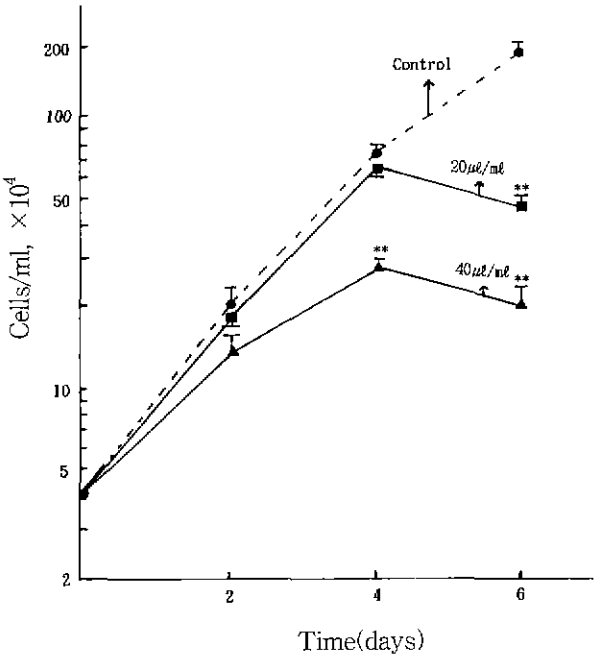

Fig. 4. Inhibitory effect of the filtered fresh juice of kale (μl/ml) during the growth of HT-29 colon cancer cells.

**Significantly different from the control at the $p<0.01$ level.

When 20μl/ml of kale juice was added, the inhibitory effect on the cell growth was not significantly changed after 4 days of incubation, but those effect was significant after 6 days of incubation. We observed the inhibitory effect of kale juice on the growth of HT-29 colon cancer cells is less effective than that of AGS gastric adenocarcinoma cells. Lee et al.(20) showed the soluble dietary fiber(SDF) of kale significantly decreased the mutagenicities mediated by AFB_1, DMAB, 2-amino-3, 4-dimethyl-imidazo[4,5-f]quinoline(MeIQ) and 3-amino-1-methyl-5H-pyrido[4,3-b]indole(Trp-p-2), and also reported strong inhibitory effect on the growth of human AZ-521 gastric adenocarcinoma cells.

The pictures shown in Fig. 5 are the photomicrographs of HT-29 colon cancer cells incubated for 6 days with or without the filtered juice of kale by inverted microscope. The more kale juice was added to the medium, the less aggregation of cell mass was observed, compared to the control group.

The inhibitory effects of DNA incorporation within cells labeling [^{3}H] thymidine after 2 days of incubation with kale juice were examined by liquid scintilation counter, using AGS gastric adenocarcinoma cells and MG-63 osteosarcoma cells(Fig. 6). Adding 10μl/ml of kale juice to AGS gastric adenocarcinoma cells showed significant inhibitory effect on DNA incorporation. When the concentration of kale juice was increased to 20μl/ml and 40μl/ml, 89.6% and 98.2% of inhibitory effects were appeared, respectively($p<0.01$). Also, adding kale juice up to 20μl/ml in MG-63 osteosarcoma cells had few inhibitory effects of DNA incorporation, but when 40μl/ml of kale juice was added, 94.7% of inhibition on DNA incorporation was observed. Therefore, DNA incorporation in AGS gastric adenocarcinoma cells was much more reduced than that in MG-63 osteosarcoma cells.

From the above studies, the fresh kale juice showed

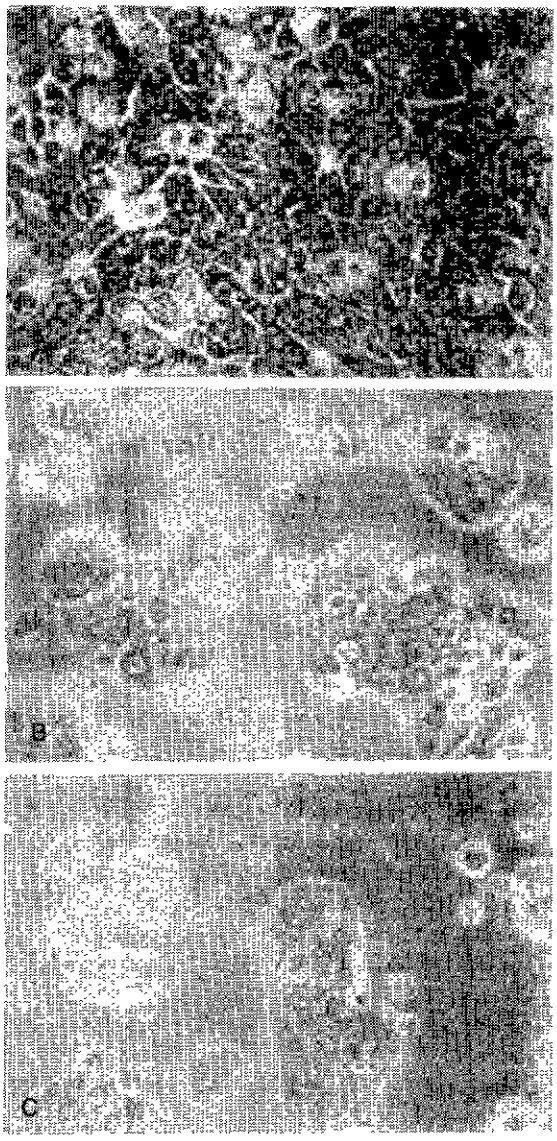

Fig. 5. Photomicrographs of HT-29 colon cancer cells incubated for 6 days with or without the filtered fresh juice of kale(×200).
A: Control
B: Juice(10μl/ml) treated
C: Juice(20μl/ml) treated

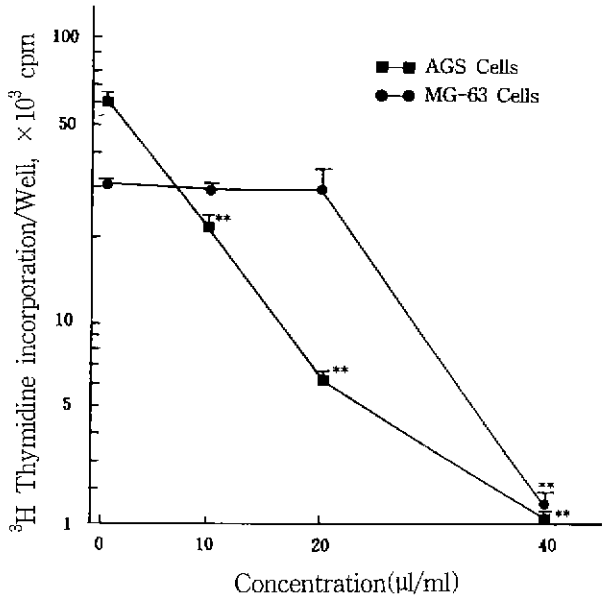

Fig. 6. Inhibitory effect of kale juice on the incorporation of [^{3}H] thymidine in AGS adenocarcinoma cells and MG-63 osteosarcoma cells after 2 days of incubation at 37°C.
**Significantly different from the control at the $p<0.01$ level.

strong inhibition effects on the growth and DNA incorporation of AGS gastric adenocarcinoma cells, HT-29 colon cancer cells, MG-63 osteosarcoma cells and K-562 leukemia cells. These results are in agreement with the epidemiological researches that *Brassica* vegetables exhibit high anticancer effects. Choi et al.(22) reported the fresh juice of kale strongly reduced the mutagenicity induced by aflatoxin B_1 in *Salmonella typhimurium* TA100, and also decreased the genotoxicity induced by AFB_1 in *Drosophila melanogaster* as a result of feeding with kale juice. Kale contains high levels of quercetin(100mg/kg) and kaempferol(211mg/kg) known as anticancer flavonoids(16,17). Wattenberg(18) reported that *Brassica* vegetables such as cabbage, brussels sprout, cauliflower, kale, and turnip contain benzyl isothiocyanate and phenethyl isothiocyanate which are known for inhibiting DMBA, induced mammary tumor formation in female Sprague-Dawley rat. Kale has been identified to contain high levels of vitamin C, β-carotene, various mineral, chlorophyll, dietary fibers, flavonoids, benzyl isothiocyanate and phenethyl isothiocyanate. Thus, the inhibitory effects of kale juice on the growth of cancer cells and DNA incorporation might be due to the activities of these active compounds in kale. We need to study further more the identification of the active compounds, the mechanisms of the anticancer effect, and *in vivo* experiment, in detail.

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