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Brief communication (Original)

Anticlastogenic potential of Thai vegetable, Siamese cassia, using mouse erythrocyte micronucleus assay

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Background: Some Thai vegetables may enhance the activities of some phase II enzymes and decrease activities of phase I enzymes. Thus, they may possess cancer chemo-preventive potentials.
Objective: Determine the anti-clastogenic activity of Thai vegetable, Siamese cassia (SC), against an indirect-acting clastogens, cyclophosphamide (CYP), and 7, 12-dimethylbenz(a)anthracene (DMBA).
Methods: Male mice were fed either with semi-purified diet, containing 2% or 4% of ground lyophilized SC leaves, for two weeks prior to administration of clastogens. The anti-clastogenicity of SC leaves using the *in vivo* erythrocyte micronucleus assay in mice was performed. Blood samples were collected and counted for reticulocytes with and without a micronucleus using the fluorescent microscope.
Results: Feeding SC leaves at 2% or 4% in the diets reduced the number of micronucleated peripheral reticulocytes (MNRETs) induced by both CYP and DMBA. However, the effect was statistically significant only at 4% in CYP-induced mice.
Conclusion: Siamese cassia leaves possess anti-clastogenic activity against clastogens in mice, particularly in a high dose.

***Keywords*:** Anticlastogenic, micronucleus, MNRETs, Siamese cassia

The risk of some cancers may be reduced by the consumption of various kinds of vegetables and fruits [1]. Many vegetables and fruits contain various kinds of chemicals possessing chemo-preventive potentials. In several of them, the chemoprevention of cancers is under clinical trials [2, 3].

Chemo-preventive agents may function by a variety of mechanisms. These mechanisms are directed at all major stages of carcinogenesis [4], induction of phase II detoxification enzymes, the inhibition of phase I activating enzymes, and the inhibition of the mutagenicity/clastogenicity of chemical carcinogens [5].

Some Thai vegetables, such as neem flowers as well as sesbania flowers, Thai and Chinese bitter gourd fruits, leaves of sweet basil, Siamese cassia, ivy gourd, and Indian mulberry, possess antimutagenic activity towards *Salmonella typhimurium* [6, 7]. According to Kusamran et al. [8] and Tepsuwan et al. [9], some of them could increase the activity of some phase II enzymes while decreasing the activity of some phase I enzymes. In addition, these vegetables could inhibit some chemically induced carcinogenesis [7, 10, 11].

Siamese cassia (*Cassia siamea* Britt, SC) is one of Thai native vegetable whose young leaves and flowers are used as common Thai dish and have some medicinal properties. It has been reported that the ethanol extract of SC leaves contained phenolic compounds, a chemopreventive agents [12]. Previously, we reported that SC leaves could inhibit 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary gland carcinogenesis in rats [10].

The *in vivo* rodent micronucleus assay in bone marrow cells has been accepted for evaluation of the clastogenicity of chemical compounds. This technique is very useful as a short-term assay for evaluating the anti-clastogenicity as well as chemo-preventive

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potential of compounds [13-15]. MacGregor et al. [16] utilized mouse peripheral blood instead of bone marrow cells in the micronucleus assay. Recently, Hayashi et al. [17] used acridine orange as supravital staining of blood cells. These modifications offer many advantages to conventional bone marrow assay and widely used to evaluate chemical clastogenicity. In this study, we determined the anticlastogenicity of Siamese cassia leaves by applying the mouse erythrocyte micronucleus assay.

Materials and methods

Cyclophosphamide (CYP) was purchased from ASTA Medica AG (Frankfurt am Main, Germany). Acridine orange (AO) was obtained from E. Merk (Germany), DMBA and all vitamins used for the preparation of vitamin mixture were obtained from Sigma Chemicals Co (St. Louis, USA). Chemicals used for the preparation of salt mixture were obtained from Fluka Chemicals Co (Buchs, Switzerland). Casein (EM HV milk protein) was the product of D.M.V. Co (Veghel, The Netherland).

SC was purchased from the local markets in Bangkok. It was washed with tap and distilled water, only leaves portion were chopped into small pieces and lyophilized. Freeze-dried samples were blended to powder and kept at -20°C until use.

Animals, diets, and experimental procedure

Male ICR mice, five weeks old, were obtained from the National Laboratory Animal Center, Mahidol University, Nakhon Pathom, Thailand. Animals were maintained at the Laboratory Animal Facility of the National Cancer Institute according to the Institutional Care Guidelines by the Animal Ethics Committee of the institute. All animals were housed in shoes box stainless steel cages in an air-conditioned room at 23±2°C and relative humidity 50±20% with 12 hour light/dark cycle. For each experiment, animals were acclimatized for five to seven days by giving a modified AIN-76 semi-purified diet [18, 19] before starting the experiment.

Clastogenicity testing

After acclimation for five to seven days with basal AIN-76 diet, male mice were divided into three groups (8-10 mice each), as shown in **Figure 1.** Group 1 was control group fed with basal diet. Groups 2 and 3 were experimental groups given ground freeze-dried SC leaves at 2% and 4%, respectively, by mixing with AIN-76 diet, for two weeks. Both control and experimental groups were pair-fed as previously described [7] and water *ad libitum*. The experimental design is summarized in **Figure 1**. At two weeks after feeding SC leaves diets, blood samples were collected and dropped on AO-coated slides, which are described later.

Anticlastogenicity testing

Experiment **A**: CYP was intra-peritoneally injected into mice that have been used previously for clastogenicity test at 50 mg/kg BW just after the blood sample was collected. Then blood sample was collected at 24 and 48 hours after CYP injection (**Figure 1**) and analyzed for AO-stained reticulocytes under the fluorescent microscope.

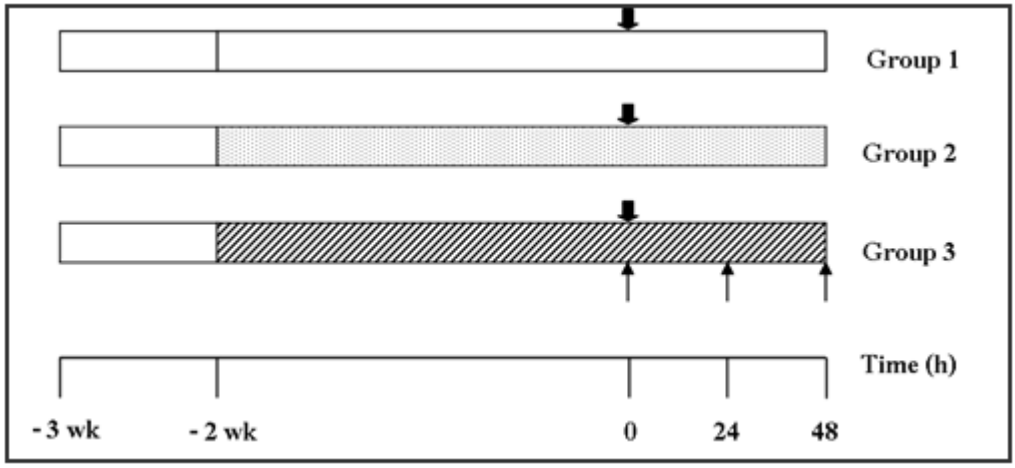

Figure 1. Schematic diagram of experimental design. Black arrows indicate clastogen administration and arrows indicate blood samples collection.

Experiment **B**: The rest of the experimental group for clastogenicity study was administered with DMBA at 40 mg/kg BW (in corn oil) by gavage. Blood samples were collected at 24 and 48 hours after clastogen administration (**Figure 1**) and analyzed for reticulocytes as in experiment **A**.

Preparation of AO-coated glass slides and analysis of MNRETs

AO-coated glass slides were prepared following Hayashi et al. protocol [20]. Five to seven microliters of mouse peripheral blood was placed on the center of an AO-coated glass slides and immediately covered with 22 x 40 mm cover slip. After a few hours, the blood cells were ready for counting micronucleated peripheral reticulocytes (MNRETs) under the fluorescent microscope with a blue excitation and a yellow barrier filter. The frequencies of MNRETs were recorded based on the observation of all 1000 reticulocytes per mouse. Reticulocytes for counting of micronuclei were restricted to types I, II, and III as classified by Vander et al. [21].

Statistical analysis

The significant difference in the frequencies of MNRETs between the experimental and control groups was analyzed using Kruskal-Wallis H and nonparametric Mann-Whitney U tests at $p < 0.05$.

Results

Body weight and food consumption were recorded daily during the experiment. It was found that there were no significant differences between the control and experimental groups (data not shown). **Figure 2** shows the frequency of MNRETs in mice treated with CYP (experiment **A**) or DMBA (experiment **B**).

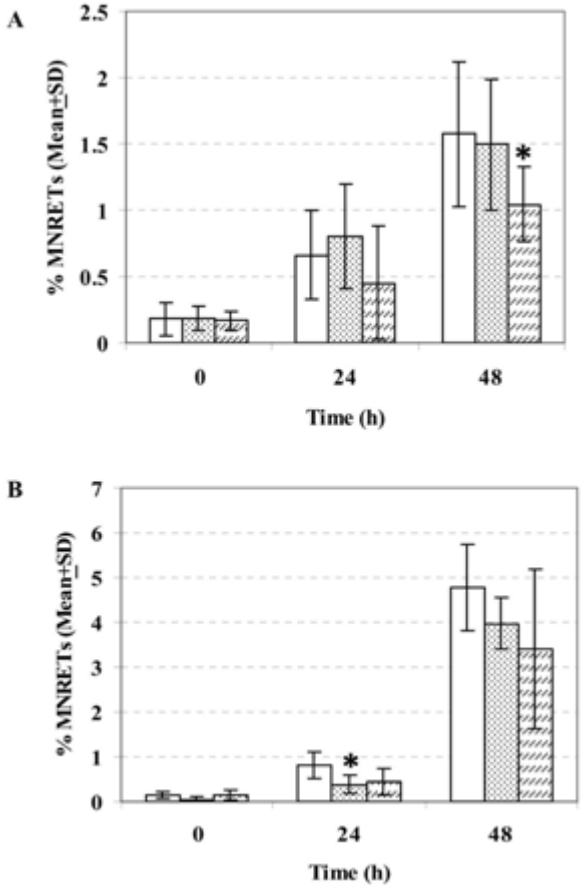

Figure 2. Mean frequencies of MNRETs in mice after administration of clastogens, CYP (**A**) and DMBA (**B**). The number of MNRETs in the control group received AIN-76 (basal) diet (open bar) and the experimental groups (dot bar and hatched bar) received ground freeze-dried SC leaves at 2% and 4%, respectively, in the diets.

In experiment **A**, the number of MNRETs slightly increased at 24 hours and showed peak frequency at 48 hours of both control and experimental groups. The percent inhibition was calculated to compare the inhibitory effect of SC leaves against clastogens induced MNRETs at 48 hours after treatment. SC leaves at 2% in the diet caused a slight reduction in MNRETs only at 48 hours. However, SC leaves at 4% in the diet resulted in a reduction in MNRETs at both time point but statistically significant ($p=0.02$) at 48 hours with 34% inhibition.

In experiment **B**, the pattern of MNRETs formation was similar to CYP induction. Feeding of SC leaves at either 2% or 4% in the diets resulted in a reduction in MNRETs but significant difference was achieved ($p=0.036$) only in the low dose group at 24 hours.

Discussion

Since SC leaves has bitter taste, it was mixed in the diets in a lower concentration, only at 2% and 4%. In the present experiment, SC leaves had no effect to the growth rate of the mice. In addition, it had no effect on the spontaneous formation of MNRETs, indicating that SC leaves had no clastogenic effect but possesses anti-clastogenic activities.

The present result demonstrated that SC leaves, at both low and high doses, reduced micronucleus formation induced by CYP and DMBA. Notably, its reduction was statistically significant in CYP only, compared with the control group. Our results correlated well with the previous report by Tepsuwan et al. [10] evaluating the activities of chemical carcinogen metabolizing enzymes and on mammary gland carcinogenesis in rats. However, we must note that our inhibitory effect was not high due to a low volume of SC leaves in the diets because mice did not eat the diets with high percent SC leaves.

In conclusion, the Thai vegetable, Siamese cassia leaves, contained some anti-clastogens, indicating that they might have chemo-preventive potential against genotoxicants. The inhibitory effect of this vegetable correlated well with the capacity to enhance phase II enzyme activity with decrease in phase I enzyme activity. Micronucleus assay may be useful as screening method for detecting chemo-preventive agents.

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References

1. Block G, Patterson B., Subar A. Fruits, vegetables and cancer prevention: a review of the epidemiologic evidence. Nutr Cancer. 1992; 18:1-29.
2. Wattenberg LW. Chemoprevention of cancer. Cancer Res. 1985; 45:1-8.
3. Kelloff GJ, Boone CW, Crowell JA, Steele VE, Lubet R, Sigman CC. Chemopreventive drug development: Perspectives and progress. Cancer Epidemiol Biomarkers Prev. 1994; 3:85-98.
4. Wattenberg LW. Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. J Natl Cancer Inst. 1997; 58:395-8.
5. Talalay P. The role of enzyme induction in protection against carcinogenesis. In: Wattenberg LW, Lipkin M, Boone CW, Kelloff GJ, editors. Cancer Chemoprevention. Boca Raton: CRC; 1992. p. 469-78.
6. Rojanapo W, Tepsuwan A. Antimutagenic and mutagenic potentials of Chinese radish. Environ Health Perspect Suppl. 1993; 101:247-52.
7. Kusamran WR, Tepsuwan A, Kupradinun P. Antimutagenic and anticarcinogenic potentials of some Thai vegetables. Mutat Res. 1998; 402:247-58.
8. Kusamran WR, Ratanavila A, Tepsuwan A. Effects of neem flowers, Thai and Chinese bitter gourd fruits and sweet basil leaves on hepatic mono-oxygenases and glutathione-*S*-transferase activities and *in vitro* metabolic activation of chemical carcinogen in rats. Fd Chem Toxicol. 1998; 36:475-84.
9. Tepsuwan A, Kusamran WR. Effect of the leaves of Siamese Cassia, Indian mulberry and Asiatic pennywort on the metabolizing enzymes of chemical carcinogens in rat liver. Bull Dept Med Serv. 1997; 22: 425-37.
10. Tepsuwan A, Kupradinun P, Kusamran WR. Effect of Siamese cassia leaves on the activities of chemical carcinogen metabolizing enzymes and on mammary gland carcinogenesis in the rat. Mutat Res. 1999; 428: 368-73.
11. Tepsuwan, A, Kupradinun, P. and Kusamran, WR. Chemopreventive potential of neem flowers on

carcinogen-induced rat mammary and liver carcinogenesis. Asian Pac. J. Cancer Prev. 2002; 3: 231-8.

12. Nakanishi K, Sasaki S, Kiang AK, Goh J, Kakisawa H, Ohashi M, et al. Phytochemical survey of Malaysian plants preliminary chemical and pharmacological screening. Chem Pharm Bull. 1965; 13:882-90.
13. Heo MY, Jae LH, Jung SS, Au WW. Anticlastogenic effects of galangin against mitomycin C-induced micronuclei in reticulocytes of mice. Mutat Res.1996; 360:37-41.
14. Yamagishi M, Osakabe N, Natsume M, Adachi T, Takizawa T, Kumom H, Osawa T. Anticlastogenic activity of cacao: inhibitory effect of cacao liquor polyphenols against mitomycin C-induced DNA damage. Fd Chem Toxicol. 2001; 39:1279-83.
15. Hwang KM, Jung KO, Song CH, Park KY. Increased antimutagenic and anticlastogenic effects of *Doenjang* (Korean fermented soybean paste) prepared with bamboo salt. J Med Food. 2008; 11:717-22.
16. MacGregor JT, Wehr CM, Henika PR, Shelby MD. The in vivo erythrocyte micronucleus test: measurement at steady state increases assay efficiency and permits integration with toxicity studies. Fund Appl Toxicol. 1980; 14:513-22.
17. Hayashi M, Sofuni T, Ishidate M Jr. An application of acridine orange fluorescent staining to the micronucleus test. Mutat Res. 1983; 120:241-7.
18. Bieri LG, Stoewsand GS, Briggs GM, Phillips RW, Woodard JC, Knapka JJ. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. J Nutr. 1997; 107:1340-8.
19. Reeves PG, Nielsen FH, Fahay Jr GC. AIN-93 purified diet for laboratory rodents: Final report of the American Institute of Nutrition Ad Hoc Writing Committee on the reformulation of the AIN-76 rodent diet. J Nutr. 1993; 123:1939-51.
20. Hayashi M, Morita T, Kodama Y, Sofuni T, Ishidate Jr M. The micronucleus assay with mouse peripheral blood reticulocyte using acridine orange-coated slides. Mutat Res. 1990; 245:245-9.
21. Vander BJ, Harris AC, Ellis RS. Reticulocyte counts by means of fluorescence microscopy. J Lab Clin Med. 1963; 62:132-40.