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Effect of Dietary Phenols on Body Tissue Oxidative State and Cancer Prevention

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식이내 페놀류들이 생체조직의 산화상태와 항암작용에 미치는 영향

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요 약

본 연구에서는 phenol이 암예방에 어떻게 영향을 미치는가를 규명하고자 시도하였으며 phenol이 TBARS에 미치는 영향, TBRAS와 암과의 상관관계 규명에 초점을 맞추었다. 식이 phenol이 조직산화와 종양 발생(tumor onset)에 미치는 영향을 측정하기 위하여 영양적으로 우수한 amino acid-based diet와 transgenic mouse model을 이용한 protocol을 사용하였다. Mice는 human lymphotropic virus (type-1) transactivator (tax1) gene을 carry하며 동시에 종양이 외부로 나타난다. 25마리의 transgenic mice를 대조군, 2, 4, 8 mmol catechin/kg diet 군 및 wine solid 군으로 구분하였으며, 대조군은 catechin과 wine solid를 전혀 주지 않았으며, wine solid 군은 red wine 750 ml/kg을 주었다. Mice는 매일 관찰하여 맨 처음 종양이 발현하는 날짜를 기록하였다. Catechin과 wine solid를 섭취한 mice에서 종양이 발현하는 시기가 대조군보다 유의적으로 낮았으며 더욱이 4 mmol catechin diet 군과 8 mmol catechin diet 군에서는 실험기간동안 각각 1마리에서 종양이 발견되지 않았다. Catechin과 wine solid를 섭취한 mice의 뇌조직과 비장의 TBARS 수준은 대조군 mice의 동일한 조직과 비교하였을 때 유의적으로 낮았다. 또한 조직의 TBARS 수준은 종양 발생과 유의적으로 상관관계가 있었다. 본 연구의 결과는 phenol의 종류에 상관없이 식이 phenol에 조직의 항산화(산화억제)를 통해 암예방(cancer prevention)에 영향을 미친다는 것을 제시해 준다.

INTRODUCTION

Dietary intake of antioxidant nutrients is an important and practical approach to protect against tissue oxidative damage.

Flavonoids and phenols are some of the most characteristic constituents of higher plants : they are very widely distributed therein and may occur in quite large amounts in the normal diet. Catechin is a plant phenolic flavonoid that has shown to be non-toxic[1] and have hepatoprotective effects[2]. It has previously been reported that catechin acts as a free radical scavenger and antioxidant and prevents lipid peroxidation and LDL oxidation[3]. Catechin also has demonstrated inhibitory effects on chemically induced carcinogenesis or mutagenesis[4,5,6] and inhibitory properties for nitrosation, aromoatic amines, and benzo(a)pyrene[7]. Catechin has also been reported to alter the DNA binding of 2-acetylaminofluorene in rat hepatocytes[8].

Red wine is desirable food source of polyphenols for experimental purposes because it contains many diverse phenols in high concentrations[9,10]. Wine phenolic compounds have been reported to reduce coronory heart disease by inhibiting the oxidation of LDL[11]. Incorporating wine solids into a diet[12,13] has been used to examine the protective effect of wine phenolic compounds against diseases. Grapes contain significant amounts of phenols and flavonoids which are retained in wine[9]. The phenol concentrations in the red table wine are catechin (1.22 mmol/L wine), epicatechin (0.34 mmol/L wine) malvidin-3-glucoside (0.1016 mmol/L wine), gallic acid (0.054 mol/L wine), quercetin (0.035 mmol/L wine) and myr-

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ecetin (0.031 mmol/L wine). Wine has a simple food matrix making it convenient to incoporate into highly purified test diets for experimental animal studies. Transgenic mice models are superior to chemically induced cancer models for evaluating the post-initiation cancer prevention activity of natural foods and food components. The transgenic mice used in this study carry the human T-lymphotropic virus type-1 (HTLV-1) transactivator(tax1) gene[14] and develop nerve sheath tumors similar to human neurofibromatosis. Time of tumor onset, tumor incidence, and tissue involvement are well characterized[15,16].

Many reports demonstrate the inhibitory effects of phenols against tumorigenesis and oxidation, but no clear conclusions have been yet identified.

We observed the effects of dietary phenols on body tissue oxidative state and the relationship between TBARS and cancer prevention to see the effects of phenols on cancer prevention and also we observed the effects of catechin and wine phenolic compounds to see the results of different kinds of phenols.

MATERIALS AND METHODS

1. Reagent and wine

(+)-Catechin, 2-thiobarbituric acid, butylated hydroxytoluene, β-nicotinamide adenine dinucleotied phosphate, *n*-butanol were purchased from Sigma Chemical Co. (St. Louis, Mo., USA). Ammonium sulfate, sodium chloride, methanol, hexane, acetone were obtained from Fisher Scientific (Fair Lawn, NJ).

Nitrogen was bubbled through the wine (Sonoma Valley, California Zinfandel, 1990 harvest, purchased locally) for ~2 hours at 20℃ to remove some of the ethanol. The wine was poured into stainless steel trays to a depth of ~1 cm and lyophilized (Model 50SRC, Virtis Co., Gardiner, NY). The viscous residue was scraped from the trays, dissolved in ~15 mL H_2O for each 750 mL original wine, transferred to an amber bottle, purged with argon and stored at −20℃ until it was incorporated into diet. A 500 g quantity of diet (Table 1) was placed in a food processor (Model DLC - 7P, Cuisinart, Greenwich, CT) and 7.5 mL of the wine solids solution was slowly added while blending. Wine solid was added to the diet to yield a total phenolic content of approximately 0.1%.

A typical adult human diet would contain a total phenolic content of 0.17%, assuming a daily intake of 1 g phenolics/day[17] in ~600 g total food in USA. Therefore the concentration of phenolic compounds in the diet as well as the intake of phenols by the mice, per unit metabolic size, was approximately equivalent to that of typical human diet. Small amounts (500 g) of the supplemented diet were prepared at a time to ensure that it was fresh when presented to the mice. The diets were stored at 3℃ until fed to the mice.

2. Transgenic mice

Mice carrying the human T-lymphotropic virus type-1 (HTLV-1) transactivator(tax1) gene in their germline under control of its own long terminal repeat (LTR), the transcriptional regulatory region of the virus, were previously described[14]. This strain was originally derived via micro injection of the LTR-tax1 gene construct into fertilized eggs from super ovulated CD1 females crossed with C57BL/6×DBA2 F1 males. Mouse pups were genotyped at ~10 days of age[14] to give 25 transgenic mice for experiments.

3. Treatment protocols

At weaning, twenty-five male transgenic mice were systematically assigned into 5 groups of five mice each, control group, 2 mmol catechin/kg diet, 4 mmol catechin/kg diet, 8 mmol catechin/kg diet groups and wine solid diet group. Mice in control group were without catechin, mice in wine solid group received red wine 750 mL/kg diet. The mice were housed in individual stainless steel wire bottom cages in a room with a 12-h light: 12-h dark cycle, a temperature of 20~23℃ and a relative humidity of 50%. Mice were

Table 1. Compositon of the expermental diet

Ingedients	Control group	2	4	8	Red wine solids
Dextrin(g)	403.23	403.23	403.23	403.23	403.23
Sucrose	201.12	201.12	201.12	201.12	201.12
Cellulose(Solka Folc 40)	50	50	50	50	50
Corn oil(with 0.15%BHT)	100	100	100	100	100
Amino acid mixture[1]					
Vitamin mix[2]	2.38	2.38	2.38	2.38	2.38
Mineral mix[3]					
Catechin(mmol /kg)	–	2	4	8	
Red wine solid(ml /kg)	–	–	–	–	750

1) Amino acid mixture (g /kg diet) : L-Alanine 3.50g, L-Arginine(free base) 11.20g, L-Asparagine · H_2O 6.82g, L-Aspartic acid 35.0g, L-Cystine 3.50g, L-Glutamic acid 35.00g, Glycine 35.00g, L-Histidine(free base) 3.30g, L-Isoleucine 8.20g, L-Leucine 11.10g L-Lysine · HCl 17.99g, L-Methionine 8.20g, L-Phenylalanine 11.60g, L-Proline 3.50g, L-Serine 3.50g, L-Threonine 8.20g, L-Tryptophan 1.74g, L-Tyrosine 1.74g, L-Valine 8.20g, Succinylsulfathiazole 10.00g

2) Vitamin mix(mg /kg diet) : Thiamin · HCl 6.0mg, Riboflavin 7.0mg, Pyridoxine · HCl 7.0mg, Niacin 30.0mg, D-Calcium pantothenate 16.0mg, Biotin 0.20mg, Vit. B_{12}(0.1% titration) 50.0mg, Vit. A palmitate 250,000U /g 16.0mg, Alpha Tocopherol 250U /g 200mg, Vit. D_3 400,000U /g 2.5mg, Menadione $NaSO_3$ 48.0mg, Choline chloride 2,000mg, Folic acid 5.00mg

3) Mineral mix (mg /kg diet) : $CaCO_3$ 14,645mg, $CaHPO_4 \cdot 2H_2O$ 215mg, KH_2PO_4 17,155mg, NaCl 12,370mg, $MgSO_4 \cdot 7H_2O$ 4,990mg, $Fe(C_6H_5O_7) \cdot 6H_2O$ 623mg, $CuSO_4$ 78mg, $MnSO_4 \cdot H_2O$ 78mg, $ZnCl_2$ 181.5mg, KI 0.25mg, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 1.25mg, $Na_2SeO_3 \cdot 5H_2O$ 0.75mg, $CrK(SO_4)_2 \cdot 12H_2O$ 19.25mg, NaF 2.25mg, CH_3CO_2Na 8,080mg

fed and weighed daily and had free access to the diet shown in Table 1. The use and care of mice was approved by the IACAUC(Institutional Animal Care and Use Committee) at the University of California at Davis.

Mice were examined daily for the appearance of a first tumor developing externally on the snouts ears, feet or tails. The age of the mouse on the day that the first tumor appeared was the age of tumor onset on the mouse. At the age from 126 to 150 days, the blood was collected in a sterile, 3.5 mL tube cotaining 0.06 mL of 7.5% K_3-EDTA solution. Plasma was seperarated following centrifugation (1,000×g, 5min, 40℃) and immediately frozen on dry ice. Liver, brain, kidney, muscle and spleen were removed, weighed, and immediately frozen on dry ice. The tissues were stored at −80℃ until analysis.

4. Tissue homogenates preparation

Tissues were weighed and 100 μL of ice cold 0.2% BHT in ethanol was added to each sample. Ice cold 1% NaCl was added and the mixture was homogenized for approximately 30s (Polytron Homogenizer, Brinkman Instruments.) to yield a 20% homogenate (w/w). Two aliquots a 10 μL and a 100 μL were removed for protein and thiobarbituric acid(TBA) assay respectively. The TBA aliquot was immediately frozen on dry ice and stored at −80℃ overnight before analysis.

5. Thiobarbituric acid assay

The TBA assay was performed using a modification of the method of Uchiyama previously[18].

A 200~300 μL aliquot of 20% tissue homogenate was mixed with 3ml of 1% phosphoric acid and 1 mL of 0.6% TBA. The mixture was stoppered, heated in a boiling water bath for 45min, cooled on ice and extracted with 4mL of *n*-butanol. Absorbance of the organic layer at 520 and 534 nm was measured versus a reagent blank. Standards, ranging in concentration from 0.08 to 8 nmol MDA, were prepared using MA sodium salt. A standard curve was obtained using the difference in absorbance at 534 and 520 nm and sample TBA reactive substance (TBARS) concentrations calculated correspondingly.

6. Protein analysis

The Coomassi Blue dye-binding assay was used to detemine tissue protein concentrations. A 100 μL aliquot of appropriately diluted tissue homegenate was added to the dye reagent, mixed, and absorbance at 594 nm was measured versus a reagent blank. A standard curve using bovine serum albumin was used to calculate protein concentration.

7. Statistical analysis

Statistical significance of the mean differences in tissue TBARS and protein concentrations between diet groups were evaluated by ANOVA and Duncan's multiple range test. Linear regressin equation were developed to describe the relation between tissue TBARS(y variable) and tumor onset(x variable)

RESULTS AND DISCUSSION

1. The effects of phenol on cancer

Table 2 shows the weight gain, food intake, tumor onset date during the 24 experimental weeks. Mice fed the three catehin diets and wine solid-supplemented diet grew normally and had similar growth rates to mice fed the nonsupplemented diet.

Mean age of tumor onset was significantly delayed in experimental diet groups(Table 2). Nonsupplemented mice had a first tumor by 45 days of age, while not until 124 days of age did 4 mMol /kg, 8 mMol /kg diet groups of mice exhibit tumors. No tumor appeared in one mouse of each 4 mmol /kg and 8 mmol /kg diet group during the whole experiment period. Also tumor didn't appear until 112 days in wine solid diet group.

Table 2. Tumor onset age, body gain and food intake of mice fed experimental diet

Group	Tumor onset age(days)		Body gain (g /week)	Food intake
Control	46.90±5.09[1]	b[2]	1.80±0.06 NS	10.04±0.41 NS
2 mmole	53.60±4.45	b	1.85±0.13	11.26±0.34
4 mmole	140.0 ±5.95	a	1.90±0.18	11.77±0.72
8 mmole	148.60±1.40	a	1.95±0.10	11.39±0.54
Wine solid	121.80±3.65	a	1.85±0.12	10.44±0.59

1) Values are means + SEM.
2) Values within a colum not followed by the same letter are significantly different at a=0.05 level by Duncan multiple test.

Therefore supplementing an amino acid based diet with catechin and red wine solids delayed tumor onset in transgenic mice, while no difference was found in results between catechin groups and wine solid diet group.

Pingzhang et al.[23] reported that no tumors were found green tea groups in spite of the different doses of catechin. Wang et al.[24] demonstrated that green tea decreased tumor formation. Similar results were found in our study(Fig. 1).

2.The relationship among phenol, TBARS and cancer

The TBA test has been the most commonly employed method for measuring lipid oxidation. TBARS is an index of oxidative damage of tissues. The TBARS concentrations of plasma, liver, heart, brain, kidney, muscle, spleen of mice fed the catechin diets and red wine solid were consistently lower than that observed in organs of mice fed nonsupplemented diet(Table 3).

The antioxidant compoents in wine, green tea have potent antioxidant properties toward oxidation[23,24,25,26]. Pingzhang et al.[23] demonstrated that SOD(supoeroxide dismutase) activity in

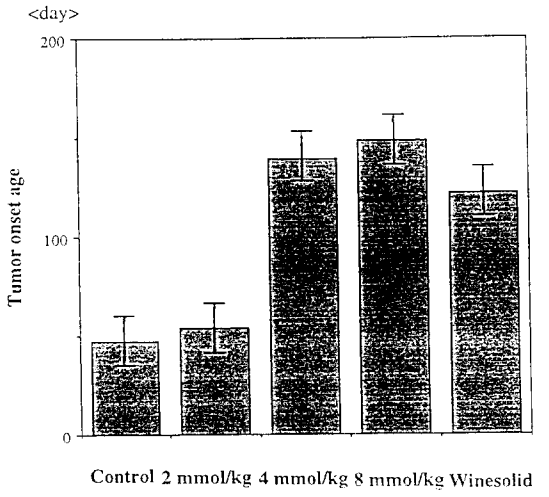


Fig. 1. Tumor onset age of each groups.

mice received green tea polyphenols is much higher than that in mice treated with 1,2-DMH. These results indicated that green tea polyphenol inhibition of tumorigenesis in Kunming mice may be attributed to the antioxidant activities of green tea polyphenols. Ebeler[25] reported that tissue levels of TBARS, MA, acetaldehyde, formaldehyde may have been effected in the regulation of cell growth and the cancer process.

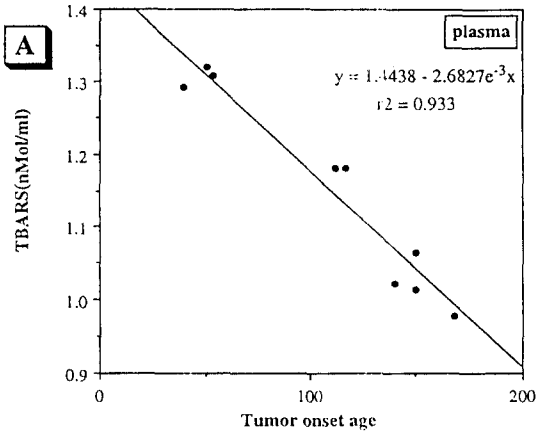
In our results, TBARS levels in mice that received catechin and wine is markedly lower than that in unsupplement mice. TBARS levels in tissues were significantly correlated with tumor onset dates (Fig. 2). These results indicate that dietary polyphenol inhibition of tumor onset may be attributed to the antioxidant activities of catechin and wine polyphenols. Furthermore, catechin and red wine polyphenols are believed to have inhibitory action against tissue oxidation, trapping of ultimate carcinogens. Therefore TBARS may serve as biomarkers for monitoring the tumor.

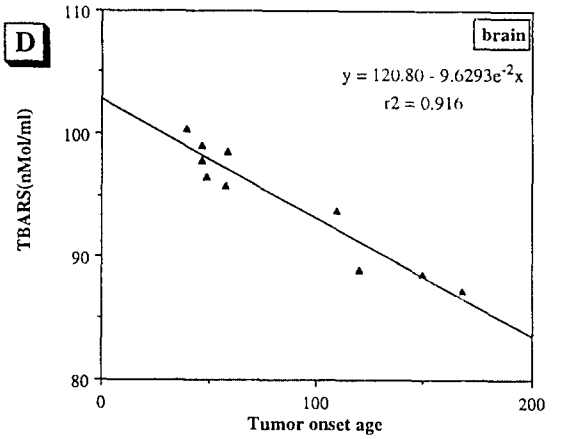
In conclusion, this study might suggest that dietary phenol effects on cancer prevention through tissue antioxidation in spite of different kinds of phenols.

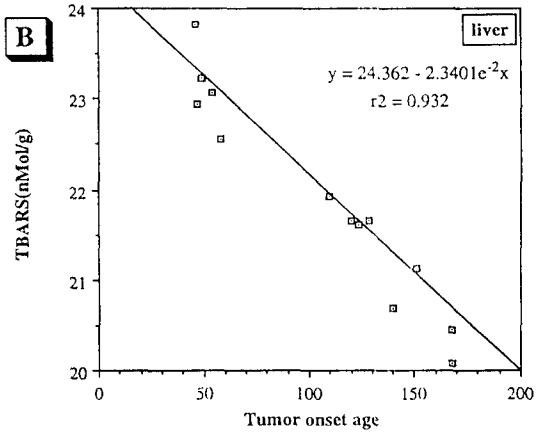
Table 3. TBARS value in tissue of mice treated with five different diets for weeks

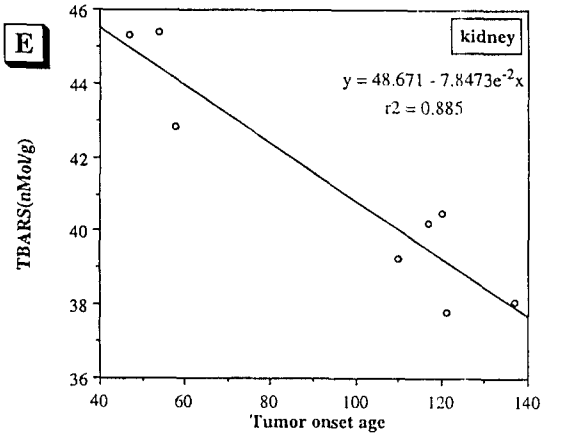
Group	Plasma(nmoles/ml)	Liver(nmoles/g)	Heart(nmoles/g)	Brain(nmoles/g)	Kidney(nmoles/g)	Muscle(nmoles/g)	Spleen(nmoles/g)
Control	1.55±0.12[1] a[2]	23.70±0.64 a	39.32±3.10 a	102.45±2.60 a	42.72±0.98 NS	37.24±4.73 a	87.50±3.84 a
2 mmol	1.45±0.13 a	20.41±1.35 a	34.33±1.28 ab	99.61±1.55 ab	43.74±1.34	27.30±1.40 b	81.58±3.89 ab
4 mmol	1.40±0.17 ab	20.36±0.58 a	32.33±1.18 b	95.84±2.13 ab	43.00±1.62	29.66±0.82 a	73.19±2.51 ab
8 mmol	1.05±0.04 b	19.83±0.58 a	32.86±1.45 b	87.25±3.81 c	41.52±1.54	29.92±2.27 ab	68.90±3.56 c
Wine solid	1.18±0.07 ab	19.42±1.03 b	35.50±1.48 ab	92.03±2.68 bc	40.26±2.05	22.11±2.87 b	71.07±2.78 bc

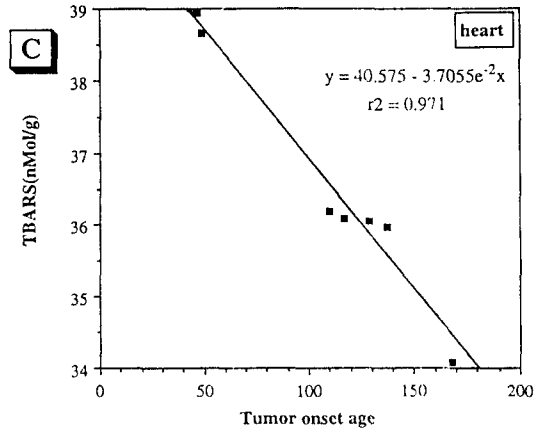
1) Values are means + SEM.
2) Values within a colum not followed by the same letter are significantly different at a=0.05 level by Duncan multiple test.

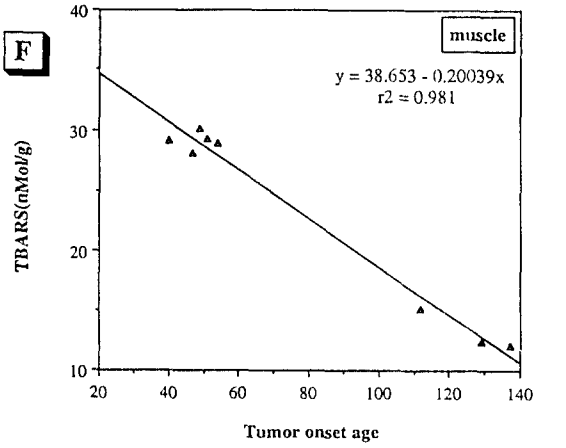

A
plasma
y = 1.4438 - 2.6827e-3x
r2 = 0.933
TBARS(nMol/ml)
Tumor onset age


D
brain
y = 120.80 - 9.6293e-2x
r2 = 0.916
TBARS(nMol/ml)
Tumor onset age


B
liver
y = 24.362 - 2.3401e-2x
r2 = 0.932
TBARS(nMol/g)
Tumor onset age


E
kidney
y = 48.671 - 7.8473e-2x
r2 = 0.885
TBARS(nMol/g)
Tumor onset age


C
heart
y = 40.575 - 3.7055e-2x
r2 = 0.971
TBARS(nMol/g)
Tumor onset age


F
muscle
y = 38.653 - 0.20039x
r2 = 0.981
TBARS(nMol/g)
Tumor onset age

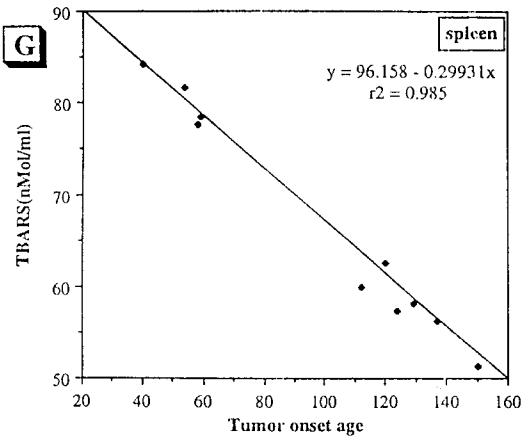


Fig. 2. Correlation between TBARS and tumor onset age.

ABSTRACT

In this study, we tried to figure out how phenol effects on cancer prevention, and for this purpose we focused on phenol effects on TBARS and the relationship between TBARS(thiobarbituric acid-reactive substances) and cancer. A protocol using a nutritionally adequate amino acid-based diet and a transgenic mouse model of neurofibromatosis was used to evaluate the effect of dietary phenols on body tissue oxidation and tumor onset. The mice carry the human T-lymphotropic virus type-1 transactivator (tax1) gene and spontaneously develop externally visible tumors. Twenty-five male transgenic mice were systematically assigned into five groups, control group, 2 mmol, 4 mmol, 8 mmol catechin/kg diet groups and wine solid group. Mice in control group were without catechin, Mice in wine solid group received red wine 750 mL/kg diet. Mice were examined daily, and the age at which a first tumor appeared was recorded. Transgenic mice consuming catechin and wine solid were older when a first tumor appeared. No tumor was found in one mouse of 4 mmol catechin/kg diet and one mouse of 8 mmol catechin diet group. Levels of TBARS in brain and spleen of 8 mmol catechin group and wine solid group were significantly decreased as compared to the same tissue in control group. TBARS levels in tissues were significantly correlated with tumor onset. Results from this study suggest that dietary phenol effects on cancer prevention through tissue antioxidation in spite of different kinds of phenols.

Key words : catechin, wine, TBARS(thiobarbituric acid-reactive substance), transgenic mouse.

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REFERENCES

1. Hennings, G. : Biochemical, pharmacological, and toxicological evaluation of (+) cyanicanol-3 in diseases of the liver. *The Royal Society of Medicine, Internation Congress and Symposium* Series, **47,** 49 (1981).
2. Lapis, K. A., Jeney, A. Divald, G., Vajta, A. Zalatnai and Schaff, Z. : Experimental studies on the effect of hepatoprotective compounds. *Tokai J. Exp. Clin. Med.*, 135 (1986).
3. Mangiapane, H., Thomson, J., Salter, A., Brown, S., Bell, G. D., and White, D. A. : The inhibition of the oxidation of low density lipoprotein by (+)-catechin, a naturally occurring flavonoid, *Biochemical Pharmacology*, **43,** 445 (1992).
4. Nagabhushan, M. and Bhide, S. V. : Antimutagenicity of catechin against environmental mutagens, *Mutagenesis*, **3,** 293 (1988).
5. Steels, C. M., Lalies, M. and Ioannides, C. : Inhibition of the mutagenicity of aromatic amines by the plant flavonoids, (+)-catechin, *Cancer Research*, **45,** 3573 (1985).
6. Kuhnau, J. : The flavonoids. A class of semi-essential food components - their role in human nutrition, *Wld. Rev. Nutr. Diet.* **24,** 117 (1976).
7. Brown, S., Laird, A., Liberman, D. and David, A. W. : The effect of (+)-catechin, a naturally occuring flavonoid on benzopyrene oxidation by cells in culture, *Biochemical Society Transactions*, **21,** 283S (1993).
8. Monteith, D. K. : Catechin inhibition of mutagenesis and alteration of DNA binding of 2-acetylaminofluorene in rat hepatocytes, *Mutation Research*, **240,** 151 (1990).
9. Macheix, J. J., Sapis, J. C., and Fleuriet, A. : Phe-

nolic compounds and polyphenoloxidase in relation to browning in grapes and wines, *Critic. Rev. Food Sci. Nutr.*, **30**, 441 (1991).10. Singleton, V. : Grape and wine phenolics - Background and prospects, *Proc. Symposium University of California-Davis Grape and Wine Centennial*, 215 (1992).

11. Frankel, E. N., Kanner, J., German, J. B., Parks, E. and Kinsella, J. E. : Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine, *The Lancet*, **341**, 454 (1933).
12. Walsem, R. F., and Clifford, A. J. : Folate deficience in rat fed diets dontaining free amino acids or intact proteins, *J. Nutr.*, 118, 1089 (1988).
13. Clifford, A. J., Heid, M. K., Mullr, H. G., and Bills, N. D. : Tissue distribution and prediction of total body folate of rats, *J. Nutr.*, **120**, 1633 (1990).
14. Nerenberg, M., Hinrichs, S. H., Reynolds, R. K., Khoury, G., and Jay, G. : The *tat* gene of human T-lymphotrophic virus type 1 induces mesenchymal tumors in transgenic mice, *Science*, **237**, 1234 (1987).
15. Hinrichs, S. H., Nerenberg, M., Reynolds, R. K., Khoury, G., and Jay, G. : A transgenic mouse model for human neurofibromatosis, *Science* **237**, 1340 (1987).
16. Baird, A. M., Green, J. E., and Hinrichs, S. H. : Animal models of human disease, *Comar. Path. Bull.*, **24**, 3 (1992).
17. Kuhnau, J. : The flavonoids. A class of semi-essential food components - Their role in human nutrition, *Wld. Rev. Nutr. Diet*, **24**, 117 (1976).
18. Uchiyama, M. and Mihara, M. : Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction, *Analytical Biochemistry* **86**, 271 (1978).
19. Francis, A. R., Shetty, T. K. and Bhattacharya, R. K. : Modulatied effect of plant flavonoids on the mutagenicity of N-methyl-N'-nitro-N-nitrosoguanidine, *Carcinogensis*, **10**, 1953 (1989).
20. Stich, H. F., Ohshima, H., Pignatelli, B., Michelon, J. and Bartsch, H. : Inhibitory effect of betel nut extracts on endogenous nitrosation in humans, *Journal of Nation Cancer institute*, **70**, 1047 (1983).
21. Azuine, M. A. and Bhide, S. V. : Adjuvant chemoprevention of experimental cancer - catechin and dietary turmeric in forestomach and oral cancer models, *J. of Ethnopharmacology*, **44**, 211 (1994).
22. Robak, J. and Gryglewski, R. J. : Flavonoids are scavengers of superoxide anions, *Biochemical Pharmacology*, **37**, 837 (1988).
23. Pingzhang, Y., Jinying, Z., Shujun, C., Hara, Y., Qingfan, Z. and Zhengguo Liu : Experimental studies of the inhibitory effects of green tea catechin on mice large intestinal cancer induced by 1,2-dimethylhydrazine, *Cancer letters*, **79**, 33 (1994).
24. Wang, Z. Y., Hung, Z. C. and Bickers, D. R. : Inhibition of chemical and photocarcinogenesis in murine skin by green tea polyphenols, *Proc. Am. Assoc. Cancer Res.*, **31**, 159 (1990).
25. Ebeler S. E., Hinrichs, S. V., Clifford, A. J. and Shibamoto, T. : Volatile carbonyl levels in tissues of transgenic mice with nerve sheath tumors, *J. Chromotography B*, 654 (1994).
26. Chen, H. and Teppel, A. L. : Vitamin E, Selenium, Trolox C, Ascorbic acid palmitate, Acetylcysteine, Coenzyme Q, β-Carotene, canthazanthin, and (+)-catechin protect against oxidative damage to kidney, heart, lung and spleen, *Free Rad. Res.*, **22**, 177 (1995).

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