A 90-day oral toxicity study of purple corn color, a natural food colorant, in F344 rats

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Abstract

A subchronic oral toxicity study of purple corn color (PCC), a natural food colorant, was performed with groups of 10 male and 10 female F344 rats fed the agent at dietary levels of 0%, 0.5%, 1.5% and 5.0% for 90 days. No mortalities occurred during the treatment period. No treatment-related changes in the body weight, food and water consumption, ophthalmology, hematology, organ weight data and histopathology were observed. Regarding general conditions and gross pathology, staining of fur and black feces were noted in rats of the 1.5% and 5.0% diet groups. Moreover, brown urine and black material in the stomach, small and large intestine were evident in rats receiving 5.0%. These changes were considered due to the anthocyanin content. On clinical chemistry analysis, total cholesterol, phospholipid and triglyceride were significantly lowered in both sexes of the 5.0% group, but these were not considered to be toxicologically significant.

Thus, the No-observed-adverse-effect-level (NOAEL) was judged to be 5.0% in diet for both sexes (male: 3542 mg/kg/day, female: 3849 mg/kg/day) for PCC under the present experimental conditions.
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1. Introduction

Purple corn color (Maize morado color, PCC), is extracted from the cobs and kernel of *Zea mays* LINNE, and it has been consumed in Peru of the South America from Incan. In Japan, it is widely used in beverages, jellies, and candies since 1970s. The number of toxicological studies of anthocyanines have been limited, and the acceptable daily intake (ADI) was earlier determined at 0–2.5 mg/kg/day based on a short-term toxicity study of grape-skin extract only (JECFA, 1982).

Recently, Tsuda et al. (2003) demonstrated dietary PCC to significantly suppress the development of obesity and ameliorate the hyperglycemia induced by high fat diet feeding in mice. Moreover, it has been reported that the PCC may have some hope for colorectal cancer prevention (Hagiwara et al., 2001). These results provide a biochemical and nutritional basis for the use of PCC as a new dietary ingredient in dietary supplement.

The present 90-day toxicity study was therefore conducted to determine any toxic effects of PCC and estimate

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the possible toxicological risk to human with administration in the diet to both sexes of F344 rats.

2. Materials and methods

2.1. Test chemicals

PCC (containing 26.4% of anthocyanins, 57.7% of polyphenol and glucose and 10% of citric acid) was manufactured commercially by San-Ei Gen F.F.I., Inc. lot no. 020508; storage condition, at 2–4 °C and protected from light (in a refrigerator). The chemical formula is provided in Fig. 1.

2.2. Diet preparation and analysis

The test compound was incorporated at the required levels into irradiated (6.0 kGy) powder diet MF (Oriental Yeast Co., Ltd., Tokyo, Japan). In this study the stability of PCC in prepared diets was analyzed, and it was confirmed to be stable for 5 weeks at room temperature. Therefore, diet preparation was performed at intervals of 3–4 weeks. Analysis of homogeneity and content in the prepared diets confirmed the homogeneity to be satisfactory, and the contents of each diet analyzed were within the acceptable ranges.

2.3. Animals and their husbandry

Four-week-old F344/DuCrj rats were obtained from Charles River Japan, Inc. (Kanagawa, Japan) and allowed a 5 days quarantine and acclimation period, during which body weight and health conditions were monitored, and an ophthalmologic examination was performed for all animals. After confirmation of normal health status they were used for the studies at the age of 5 weeks. The animals were administered PCC from the age of 6 weeks.

The animals were housed in transparent polypropylene cages on hardwood chip bedding (Beta chip, Northeastern Products Co., NY, USA) in an environment-controlled room. Constant conditions of temperature (19–23 °C), relative humidity (50–62%), and ventilation (more than 15 times/h) were maintained, and the room was artificially illuminated for 12 h (7:00–19:00) each day. All experimental procedures were performed in accordance with Standards Relating to the Care and Management of Experimental Animals, Notification No. 6, March 27, 1980, Amendment, December 21, 1999 of the Prime Minister's Office, Guideline for Animal Experimentation, Japanese Association for Laboratory Animal Science and Law for the Humane Treatment and Management of Animals, Law No. 105, October 1, 1973.
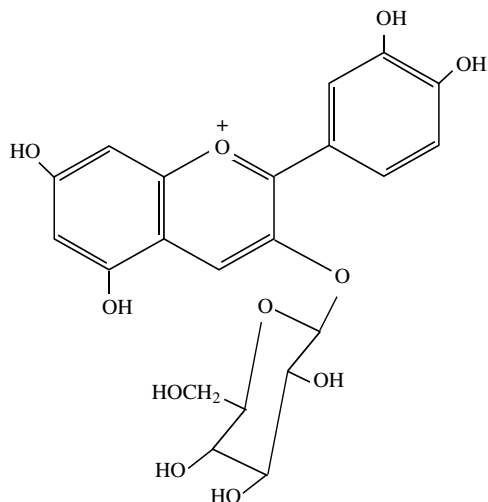

Fig. 1. Chemical structure of purple corn color.

2.4. Experimental procedure

Groups of 10 male and 10 female rats were given diet containing 0%, 0.5%, 1.5% and 5.0% PCC for 90-days. The animals were observed daily for abnormalities, and individual body weights were recorded weekly. Food and water consumption was measured over a 2-day period before each weighing. A second ophthalmologic examination was performed for five animals in each sex/group during week 13.

Urinalysis of samples collected over a 4-h period (from 9:00 to 13:00) was conducted for all animals in each sex/group during week 13; semi-quantitative estimation (Multi-stix, Bayer-Sankyo, Co. Ltd., Tokyo, Japan) of protein, glucose, ketones, bilirubin, occult blood and urobilinogen was included. Specific gravity values were measured using a reflectance meter (Atago Co., Ltd., Tokyo, Japan). The levels of urinary electrolytes [sodium (NA), potassium (K) and chlorine (CL)] were determined using a Hitachi Biochemical Automatic Analyzer 7070 (Hitachi Ltd., Tokyo, Japan). Urine volumes were measured by weighing. Appearance of urine was examined macroscopically, and urinary sediments stained with Labostain (Muto Pure Chemicals Co., Ltd., Tokyo, Japan) were assessed microscopically. In addition, fresh urine samples were obtained, and urinary pH values measured using a pH meter (Horiba Ltd., Kyoto, Japan).

At the end of week 13, all rats were deprived of food, but not water, overnight and then blood samples were collected via the abdominal aorta under ether anaesthesia to sacrifice the animals by exanguination. Hematological estimations were carried out using an automatic analyzer (Sysmex Model F-820; Sysmex Co., Ltd., Hyogo, Japan) for erythrocyte counts (RBCs), total leukocyte counts (WBCs), hemoglobin concentration (HB), hematoclit value (HT), platelet counts (PLTs), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Coagulation times were measured using a DRIHEMATO system COAG1 (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for prothrombin time (PT) and activated partial thromboplastin time (APTT). Differential counts of leukocytes and reticulocytes were made by microscopy of specimens stained after Wright and Brecher, respectively.

Clinical chemistry determinations were performed with a Hitachi-Biochemical Automatic Analyzer 7070 (Hitachi Ltd., Tokyo, Japan). Parameters included aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), total bilirubin (T-BIL), blood urea nitrogen (BUN), creatinine (CRE), glucose (GLU), total cholesterol (T-CHO), phospholipid (PL), triglyceride (TG), total protein (TP), albumin (ALB), albumin/globulin ratio (A/G), inorganic phosphate (IP), calcium (CA), magnesium (MG), sodium (NA), potassium (K) and chlorine (CL).

Gross pathological examinations were made at autopsy, and any findings recorded. At necropsy, the following organs from each rat were weighed and organ to body weight ratios determined: heart, spleen, thymus, adrenals, pituitary, thyroids (including parathyroid), lungs, salivary glands, liver, kidneys, testes, seminal vesicles, prostate, ovaries (including oviducts), uterus and brain. Samples of these organs and of the aorta, lymph nodes, nasal cavity (turbinates), trachea, tongue, esophagus, stomach, small intestine, large intestine, pancreas, urinary bladder, epididymides, vagina, spinal cord, sciatic nerve, eye, Harderian gland, skin, mammary gland, skeletal muscle, bone and bone marrow, Zymbal's gland, and any gross lesions were fixed in 10% buffered formalin. A full histopathological examination was performed on haematoxylin–eosin-stained tissue sections of the organs and tissues listed above for the control and highest dose group rats. Microscopic examination was also carried out for specimens when gross abnormalities were apparent in the middle and low dose group rats.

2.5. Statistical analysis

For body weight, urinalysis, hematology, clinical chemistry and organ weight data, the significance of intergroup differences was assessed using the Bartlett's test (Bartlett, 1937). If homogeneous, the data were analyzed with the Dunnett's multiple comparison test (Dunnett, 1955), and if not

they were analyzed with the Steel's test (Steel, 1959). For the incidences of histopathological lesions, the significance of differences observed between the control and treated groups was evaluated with the Fisher's exact probability test (Fisher, 1955; Gart et al., 1979). The Mann–Whitney U test was employed for comparison of degrees of change (Gad and Weil, 1989). The levels of significance were set at $P < 0.05$ and 0.01.

3. Results

No deaths were observed in any of the rats during the 90 days of the experiment. Staining of fur and black feces by PCC colorants in the diets was observed in both sexes of the 1.5% and 5.0% groups.

Body weights in the female 5.0% group were significantly increased at week 1 as compared to the control value, but without any consistent relationship to the PCC. The body weights after overnight fasting in both sexes of the PCC treatment groups at autopsy were also similar to those of the respective control groups.

Table 1 summarizes data for average food consumption and average PCC intake. The average food consumption by control and treated animals of both sexes during the treatment periods was similar.

The average PCC intakes for females and males were 336.5 and 317.9 mg/kg body weight/day, respectively, in the 0.5% group, 1030.8 and 941.7 mg/kg body weight/day, respectively, in the 1.5% group, and 3848.9 and 3542.4 mg/kg body weight/day, respectively, in the 5.0% group.

Water consumption in the treated groups of both sexes was similar to the respective control groups (data not shown).

Results of urinalysis performed at week 13 are summarized in Table 2. Brown urine was noted in rats fed 5.0% PCC, this being considered related to the colorant. Significant increase of specific gravity was observed in females of the 1.5% and 5.0% groups and all male groups. Significant decrease of pH in males of the 0.5% and 5.0% groups and increase of K and CL (5.0% group only) ions in both sexes

Table 1
Purple corn color intake data for F344 rats fed a diet containing purple corn color for 90 days

Sex	Dose (%)	No. of rats	Average food consumption (g/animal/day)	Average PCC intake (mg/kg/day)
Male	0	10	14.98	0.00
	0.5	10	15.32	317.85
	1.5	10	15.20	941.69
	5.0	10	16.73	3542.42
Female	0	10	10.23	0.00
	0.5	10	9.73	336.53
	1.5	10	9.99	1030.76
	5.0	10	11.18	3848.88

Table 2
Urinalysis data for F344 rats fed a diet containing purple corn color for 90 days

Sex	Dose (%)	No. of rats	Appearance		Urine volume (g)	pH	Specific gravity	NA (mEq/L)	K (mEq/L)	CL (mEq/L)
			N[a]	B[b]						
Male	0	10	10	0	1.14 ± 0.44	7.273 ± 0.427	1.053 ± 0.013	111.2 ± 37.2	220.6 ± 45.5	127.5 ± 41.4
	0.5	10	10	0	1.00 ± 0.44	$6.837 \pm 0.269^*$	$1.065 \pm 0.012^*$	101.5 ± 37.4	261.0 ± 64.1	142.0 ± 41.9
	1.5	10	10	0	0.90 ± 0.48	6.926 ± 0.451	$1.067 \pm 0.008^{**}$	104.2 ± 21.6	$302.0 \pm 56.0^{**}$	160.6 ± 32.6
	5.0	10	0	10^{**}	0.83 ± 0.39	$6.788 \pm 0.459^*$	$1.072 \pm 0.007^{**}$	112.2 ± 47.4	$348.9 \pm 38.2^{**}$	$213.8 \pm 43.5^{**}$
Female	0	10	10	0	0.90 ± 0.52	7.280 ± 0.676	1.029 ± 0.011	92.3 ± 30.6	110.6 ± 45.2	77.8 ± 23.3
	0.5	10	10	0	0.98 ± 0.49	7.269 ± 0.415	1.045 ± 0.019	116.6 ± 54.8	183.0 ± 81.7	114.9 ± 54.2
	1.5	10	10	0	0.73 ± 0.42	7.551 ± 0.298	$1.048 \pm 0.020^*$	90.1 ± 39.1	$218.9 \pm 102.7^{**}$	108.5 ± 45.5
	5.0	10	0	10^{**}	0.52 ± 0.24	7.057 ± 0.419	$1.058 \pm 0.015^{**}$	115.1 ± 19.4	$238.3 \pm 67.7^{**}$	$146.6 \pm 45.2^{**}$

Values are means ± SD.
*,**: Significantly different from the control group at $P < 0.05$, 0.01, respectively.
[a] Normal.
[b] Brown.

Table 3
Hematology data for F344 rats fed a diet containing purple corn color for 90 days

Sex	Dose (%)	No. of rats	RBC ($\times 10^4$/μL)	WBC ($\times 10^2$/μL)	HB (g/dL)	HT (%)	PLT ($\times 10^4$/μL)	PT (s)	APTT (s)
Male	0	10	908.9 ± 18.7	56.8 ± 9.2	15.8 ± 0.2	47.0 ± 1.3	59.5 ± 2.5	10.5 ± 0.8	40.9 ± 6.8
	0.5	10	910.1 ± 16.2	60.4 ± 8.5	15.7 ± 0.3	46.8 ± 1.4	59.5 ± 4.7	10.9 ± 0.4	41.6 ± 9.3
	1.5	10	902.8 ± 26.0	60.4 ± 7.3	15.8 ± 0.3	47.0 ± 0.9	59.2 ± 3.7	11.1 ± 0.4	38.5 ± 7.2
	5.0	10	901.0 ± 19.6	58.6 ± 8.1	16.0 ± 0.2	47.0 ± 1.7	$55.7 \pm 4.4^*$	10.7 ± 0.4	44.9 ± 5.8
Female	0	10	864.4 ± 31.3	48.2 ± 7.1	16.1 ± 0.6	45.3 ± 2.0	70.1 ± 4.1	10.6 ± 0.4	43.7 ± 7.0
	0.5	10	863.7 ± 37.5	54.6 ± 8.3	16.1 ± 0.4	45.4 ± 2.2	$63.4 \pm 6.4^{**}$	9.6 ± 1.8	37.3 ± 14.9
	1.5	10	864.0 ± 36.7	54.0 ± 12.2	16.2 ± 0.3	45.3 ± 1.5	$63.3 \pm 4.8^{**}$	10.1 ± 1.3	45.1 ± 6.4
	5.0	10	837.0 ± 32.0	52.9 ± 8.0	15.9 ± 0.4	44.4 ± 1.5	$58.6 \pm 5.5^{**}$	$9.8 \pm 1.1^*$	45.8 ± 5.3

Values are means ± S.D.
*,**: Significantly different from the control group at $P < 0.05$, 0.01, respectively.

of the 1.5% and 5.0% groups were also observed. Urinary protein was significantly elevated in the 5.0% female group (data not shown).

No ophthalmological abnormalities were found in any animals of the treated or control groups.

Table 3 summarizes hematology data. Platelets were significantly decreased in the 5.0% male group and in all female groups. Significant increase of MCH in the 5.0% male group and significant shortening of PT in the 5.0% female group were found, but without apparent dose-dependence. No statistically significant changes were observed in any of the other parameters examined.

Table 4 summarizes clinical chemistry data. Significant decrease of ALT and BUN in the 5.0% male groups was found, but the clinical significance was not unclear. T-CHO, PL and TG values were significantly decreased for both sexes of rats fed 5.0% PCC. TP and ALB values were significantly decreased in the 1.5% and 5.0% male groups, and the A/G value was significantly increased in the 0.5% and 5.0% female groups. Significant decrease of CA and significant increase of CL were noted in 5.0% males. Significant alteration of AST, NA and K were noted in male or female rats, but without apparent dose-dependence (data not shown). No statistically significant changes were observed in any of the other parameters examined (data not shown).

On gross pathological examination, staining of fur was observed in all animals of both sexes in the 1.5% (blue) and 5.0% (purple) groups. Black material in the stomach, small and large intestine was observed in all animals for both sexes of the 5.0% groups. This was considered due to the anthocyanin colorant. No other treatment-related macroscopic changes were found in treated animals (data not shown).

The relative organ weight data are given in Table 5. Slight but significant decrease in the relative liver and thymus weights in the 5.0% male group and significant increase of relative pituitary weights in the 5.0% male group were noted. Significant decrease of thymus weights in the 0.5% males and the pituitaries in the 1.5% females were also found but these were considered to be incidental, since dose-dependence was lacking. Relative salivary glands weights for both sexes of rats fed 5.0% were significantly increased. Significant changes were also found in the heart, kidney and thyroid data, but without apparent dose-dependence.

No treatment-related histopathological changes were observed in any organs or tissues of animals given 5.0% PCC in the diet. Abnormalities observed sporadically in the control and highest dose groups are listed in Table 6.

4. Discussion

In the present 90-day feeding study of PCC at the dose of 5.0% in F344 rats, no mortalities occurred and systemic toxicity was not evident. Staining of fur and black feces, brown urine and black material in the stomach, small intes-

Table 4
Clinical chemistry data for F344 rats fed a diet containing purple corn color for 90 days

Sex	Dose (%)	No. of rats	ALT (U/L)	BUN (mg/dL)	T-CHO (mg/dL)	PL (mg/dL)	TG (mg/dL)	TP (g/dL)	ALB (g/dL)	A/G	CA (mg/dL)	CL (mEq/L)
Male	0	10	56.6 ± 5.1	22.8 ± 1.2	62.1 ± 4.5	119.9 ± 7.7	84.7 ± 22.4	6.2 ± 0.1	2.71 ± 0.06	0.767 ± 0.024	10.0 ± 0.2	101.6 ± 0.9
	0.5	10	56.0 ± 5.3	22.1 ± 2.0	60.4 ± 4.9	118.0 ± 9.1	85.6 ± 18.3	6.2 ± 0.1	2.71 ± 0.07	0.776 ± 0.033	10.1 ± 0.2	101.0 ± 2.5
	1.5	10	56.9 ± 5.9	21.8 ± 1.2	59.1 ± 5.4	114.7 ± 8.7	90.2 ± 18.2	6.1 ± 0.1*	2.65 ± 0.05*	0.764 ± 0.016	10.0 ± 0.2	102.1 ± 0.9
	5.0	10	50.6 ± 5.4*	20.4 ± 2.1**	54.8 ± 5.2**	103.5 ± 9.4**	61.1 ± 14.8*	6.0 ± 0.1**	2.62 ± 0.06**	0.785 ± 0.030	9.9 ± 0.2*	102.9 ± 0.8**
Female	0	10	44.8 ± 18.0	21.6 ± 1.4	77.7 ± 6.4	153.3 ± 12.3	33.4 ± 13.2	5.8 ± 0.2	2.70 ± 0.07	0.860 ± 0.021	9.6 ± 0.3	102.1 ± 2.0
	0.5	10	42.6 ± 4.0	21.9 ± 1.9	73.9 ± 6.1	148.2 ± 12.1	32.0 ± 7.8	5.6 ± 0.3*	2.62 ± 0.13	0.889 ± 0.028*	9.2 ± 0.6	100.1 ± 3.0
	1.5	10	40.4 ± 2.2	21.3 ± 2.0	75.4 ± 6.7	147.2 ± 12.7	35.4 ± 10.2	5.7 ± 0.2	2.68 ± 0.08	0.885 ± 0.036	9.5 ± 0.2	100.2 ± 1.2
	5.0	10	45.0 ± 7.8	21.4 ± 1.3	63.1 ± 5.0**	128.0 ± 8.4*	20.8 ± 7.0**	5.7 ± 0.2	2.70 ± 0.08	0.896 ± 0.028*	9.7 ± 0.2	102.6 ± 1.7

Values are means ± SD.

*,**: Significantly different from the control group at $P < 0.05$, 0.01, respectively.

Table 5
Relative organ weight data for F344 rats fed a diet containing purple corn color for 90 days

Sex	Dose (%)	No. of rats	B.W.[a] (g)	Relative organ weights (%)						
				Heart	Liver	Kidneys	Thymus	Pituitary	Thyroids	Salivary glands
Male	0	10	295.7 ± 17.3	0.298 ± 0.004	2.47 ± 0.09	0.65 ± 0.01	0.052 ± 0.004	0.0023 ± 0.0005	0.0058 ± 0.0009	0.162 ± 0.011
	0.5	10	299.5 ± 9.9	0.285 ± 0.007**	2.44 ± 0.09	0.63 ± 0.03*	0.048 ± 0.003**	0.0023 ± 0.0005	0.0064 ± 0.0010	0.157 ± 0.008
	1.5	10	300.0 ± 13.9	0.292 ± 0.009	2.41 ± 0.06	0.65 ± 0.03	0.049 ± 0.005	0.0025 ± 0.0005	0.0070 ± 0.0007**	0.160 ± 0.011
	5.0	10	288.1 ± 13.2	0.292 ± 0.011	2.30 ± 0.07**	0.65 ± 0.02	0.047 ± 0.004*	0.0029 ± 0.0003**	0.0065 ± 0.0011	0.174 ± 0.010*
Female	0	10	162.4 ± 8.4	0.332 ± 0.014	2.23 ± 0.10	0.68 ± 0.03	0.093 ± 0.009	0.0069 ± 0.0009	0.0079 ± 0.0014	0.187 ± 0.010
	0.5	10	159.5 ± 6.2	0.320 ± 0.012	2.24 ± 0.10	0.68 ± 0.03	0.093 ± 0.010	0.0066 ± 0.0011	0.0075 ± 0.0016	0.191 ± 0.011
	1.5	10	163.1 ± 6.4	0.323 ± 0.017	2.18 ± 0.11	0.65 ± 0.03*	0.096 ± 0.013	0.0060 ± 0.0008*	0.0080 ± 0.0011	0.188 ± 0.014
	5.0	10	160.6 ± 8.0	0.320 ± 0.016	2.19 ± 0.10	0.69 ± 0.04	0.089 ± 0.006	0.0062 ± 0.0008	0.0077 ± 0.0008	0.199 ± 0.013*

Values are means ± SD.
*,**: Significantly different from the control group at $P < 0.05$, 0.01, respectively.
[a] Body weight data after overnight fasting.

Table 6
Histopathological findings for F344 rats fed a diet containing purple corn color for 90 days

Organ/ findings	No. of rats affected/group of 10			
	Male		Female	
Purple corn color in diet (%)	0	5.0	0	5.0
No. of rats	10	10	10	10
Pituitary				
Remnant, Rathke's pouch (1)	0	0	0	1
Cyst, pars distalis (1)	0	0	0	1
Adrenals				
Accessory adrenocortical tissue (1)	0	0	2	1
Kidneys				
Eosinophilic body, proximal tubule (1)	10	10	0	0
Regeneration (1)	1	0	0	0
Mineralization, medulla (1)	2	0	10	10
Oviducts				
Dilatation, lumen (1)	–	–	1	0
Uterus				
Dilatation, lumen (1)	–	–	1	1

Figure in parentheses indicate the grade of lesions; (1) slight.
The other organs that were examined were excluded from this table, since no histological alterations were observed in them.
Histopathological examination was not performed for the low and intermediate dose groups.

tine and large intestine were observed, but these were simply due to coloring by the test material in the diet.

Urinalysis revealed significant increase of specific gravity in the 1.5% and 5.0% females and all male groups, as well as significantly increased K (1.5% and 5.0%) and CL (5.0%) in both sexes. However, these changes were considered to be explained by the tendency for decrease of urine volume noted in these groups. Urinary pH was significantly decreased in the 0.5% and 5.0% male groups, but this change was noted in male rats only, and also there was no clear dose-relationship. Significant increase of urinary protein was noted in the 5.0% female group. This is known to occur with renal failure, but there was no evidence of toxicity in the clinical chemistry and histopathologically, and also no clear dose-relationship. Therefore, this was not considered to be toxicological significant. No effects of test materials in urinalysis have been shown in toxicity studies of anthocyanin food colors.

On hematological analysis, platelets were significantly decreased in all female groups and in the 5.0% males. Such reduction in platelets could be due to decreased production in the bone marrow or increased intravascular coagulation. However, other parameters, such as RBC, WBC and reticulocyte counts did not differ among the groups and no alterations were evident regarding the histopathology of the hematopoietic organs.

Interestingly, on clinical chemistry analysis, T-CHO, PL and TG were significantly lowered in both sexes of the 5.0% group. Igarashi et al. (1990) reported that anthocyanins lower serum cholesterol levels in rats and studies have

shown that this is also the case with phenolic compounds (Fukuyo et al., 1986; Muramatsu et al., 1986). It is believed that these effects are in fact beneficial. Recently, Tsuda et al. (2003) also reported that dietary PCC suppressed mRNA levels of enzymes involved in fatty acid and triacylglycerol synthesis in mice. The results provide a biochemical and nutritional basis for the use of PCC as a new dietary ingredient in dietary supplement. Lipid-lowering effects with PCC may be considered in the same light. It has been known that the decrease of cholesterol and triglyceride was induced by the peroxisome proliferators, HMG-CoA reductase inhibitor, bile acid sequestrants and taurine. (Endo et al., 1976; Erkelens, 1990; Nishimura et al., 2003). Toxicologically, decrease in serum lipids may generally reflect induced hyperthyroidism or impaired hepatic protein synthesis. However, there was no evidence in the clinical chemistry and histopathological examination. Therefore, the observed changes in T-CHO, PL and TG were not considered to be toxicologically significant. A/G values were significantly increased in the 5.0% female group, although TP and ALB were not affected by the treatment. Therefore, it is unlikely that this change was due to treatment-related toxicity. TP and ALB were here found to be slightly but significantly lowered in 1.5% and 5.0% males and this is known to generally occur with liver injury and/or renal failure. However, there was no histopathological evidence in support of liver injury or renal failure. The concentration of CL ions was significantly increased in males of the 5.0% group. However, closely related factors, such as NA and K ions, were not changed in any groups. Therefore, this finding was considered to be incidental. CA ions were significantly but slightly lowered in 5.0% males and such decrease can occur with decrease of parathyroid and/or thyroid functions (Tanimoto, 1988), but there was no evidence of functional changes in the parathyroid or thyroid histopathologically. Furthermore, organ weight analysis demonstrated no changes, and CA ion values in the 5.0% male group were within our control background data. Therefore, the decrease of CA is considered to have no toxicological significance.

Relative liver and thymus weights were significantly lowered in the 5.0% male group, while relative pituitary weights were increased. The changes of liver and thymus weight were slight, and did not appear to be related to any adverse effects of the treatment, since no alterations were found on histopathological examination of the liver, thymus or pituitary. Relative salivary gland weights were significantly increased in both sexes of the 5.0% group. However, these changes are considered not to be associated with the treatment since they were very slight and no alteration was apparent on histopathological assessment.

On histopathological examination, renal mineralization at the junction of inner and outer strips of the outer medulla was observed, especially in female rats. The incidence and severity of the mineralization in this study is similar to the spontaneous lesion in F344 female rats (Dixon et al., 1995). It has been reported that the development of the mineralization was related to dietary calcium and phosphorus ratio (Rao, 2002). However, no changes in the clinical chemistry were observed in this study, and were considered spontaneous in nature.

Purple corn color, purple sweet potato color, red cabbage color, perilla color, and grape-skin color, for example, have found wide application as anthocyanin colors. They are widely distributed in the human diet, and we ingest large amounts of anthocyanins daily. Regarding toxicity of anthocyanins, we have conducted the 90-day toxicity study of purple sweet potato color (Sano et al., 1996) and red cabbage color (unpublished data), and no toxicity was evident, but other reports are relatively limited. It has been reported that PCC or anthocyanin may have benefits for the prevention of obesity (Tsuda et al., 2003), and in the present study, lipid-lowering effects with PCC were apparent. Moreover, it is known that the natural anthocyanin, PCC, purple sweet potato color and red cabbage may have some hope for colorectal cancer prevention (Hagiwara et al., 2001, 2002). Therefore, the given potential for prevention effects on life-style related disease use as a new dietary ingredient in dietary supplement can be envisaged.

In conclusion, from the present results the no-observed-adverse-effect level (NOAEL) for PCC was estimated to be 5.0% in diet for both sexes (male: 3542 mg/kg/day, female: 3849 mg/kg/day). Therefore, the results of the current study provide strong support for the safety of conventional use of PCC.

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