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Research Section

Toxicological Studies on Procyanidin B-2 for Extern?al Application as a Hair Growing Agent

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Abstract—Procyanidin B-2 [epicatechin-(4$\beta \rightarrow 8$)-epicatechin] is one of condensed tannin that exists widely in plants. We have reported previously that procyanidin B-2 possesses hair epithelial cell growth-promoting activity and stimulates anagen induction in hair cycle progression. To evaluate the safety of topical procyanidin B-2 as a hair growing agent, we examined the mutagenicity, acute subcutaneous injection, primary irritation, skin sensitization, and eye irritation of this compound. Mutagenicity tests using bacteria showed procyanidin B-2 to be non-mutagenic. Chromosomal aberration tests using CHL cells indicated that procyanidin B-2 caused polyploidy but no structural aberrations. In micronucleus tests for mutagenicity using mice, procyanidin B-2 was negative. Acute subcutaneous injection study using rats revealed no symptoms of significant injury. The lethal dose of procyanidin B-2 is greater than 2000 mg/kg (subcutaneous injection). Primary irritation tests using rabbits indicated that procyanidin B-2 containing preparation shows no primary irritation. In the guinea pig maximization test, there was no evidence of sensitization to procyanidin B-2. In primary ocular irritation tests using rabbits, procyanidin B-2 containing preparation and vehicle showed slight irritation of conjunctivae which is assumed to be caused by ethanol. It is suggested that topical procyanidin B-2 is safe and acceptable from the series of toxicological tests.

Keywords: cosmetics; procyanidin; mutagenicity; acute toxicity.

Abbreviations: DNCB = 2,4-dinitrochlorobenzene; FCA = Freund's complete adjuvant; SPF = specific pathogen free; SLS = sodium lauryl sulfate: VAF = virus antibody free.

INTRODUCTION

For the purpose of discovering natural products that possess hair growing activity, we examined a wide range of plant extracts for hair epithelial cell growth-promoting activity. After an extensive search, we have found hair epithelial cell growth-promoting activity and the ability to induce anagen phase in proanthocyanidins extracted from grape seeds (Takahashi *et al*., 1998). We also found that procyanidin oligomers such as procyanidin B-2 [epicatechin-(4$\beta \rightarrow 8$)-epicatechin] (Fig. 1) possess higher hair growing activity than the polymers and monomers (Takahashi *et al*., 1999). Proanthocyanidins are very common in the plant kingdom and are a species of phenolic compounds which take the form of polymers or oligomers built of flavan-3-ol units, such as catechin and epicatechin (Porter, 1994). Proanthocyanidins have been used as skin-protective cosmetics (Wayne *et al*., 1996) and as medicaments for capillary stabilization (Brasseur, 1989; Dartenuc *et al*., 1980) in a partially purified form. We have obtained procyanidin B-2 of high purity from apples with the intent of using this material in cosmetic and medical applications. We report here several toxicological tests for procyanidin B-2 focusing on topical application of this compound as a hair growing agent.

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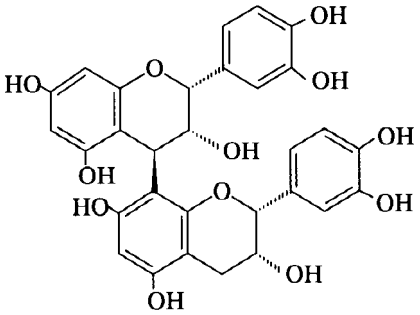

Epicatechin-(4β →8)-epicatechin

Fig. 1. Chemical structure of procyanidin B-2 [epicatechin-(4$\beta \rightarrow 8$)-epicatechin].

MATERIALS AND METHODS

Isolation of procyanidin B-2 [epicatechin-(4β → 8)-epicatechin]

20 kl of apple juice (*Malus pumila* Miller var. *domestica* Schneider, Fuji variety, commercial juice) was passed through a column (Φ 60 cm × 88.5 cm) filled with Diaion HP-20 resin (Mitsubishi Kasei Co., Tokyo, Japan), equilibrated with demineralized water, then washed with 1000 litres demineralized water and 500 litres 15% (v/v) aqueous methanol. The column was then eluted with 500 litres of 45% (v/v) aqueous methanol and the eluting fraction was evaporated to produce 9450 g of a dry solid. Then, 1465 g of this was dissolved in 25% (v/v) aqueous methanol and passed through a column (Φ 18 cm × 39.3 cm) filled with Sephadex LH-20 (Pharmacia Biotech Co., Uppsala, Sweden) equilibrated with 25% (v/v) aqueous methanol, then washed with 20 litres 25% (v/v) aqueous methanol and 20 litres 50% (v/v) aqueous methanol. 20 litres of 75% (v/v) aqueous methanol was passed through the column and the intended fraction was eluted and evaporated to produce 233 g of a dry solid. Half of this was dissolved in demineralized water and subjected to preparative HPLC (Φ150 mm × 1000 mm, Soken Chemical & Engineering Co., Japan; column packing was ODS silica gel, 50 μm particle size, KE-ODS-50 SRQ, YMC Co., Kyoto, Japan). The eluting conditions were as follows: flow-rate, 645 ml/min; room temperature; mobile phase A = 0.0001% aqueous acetic acid, mobile phase B = methanol; isocratic elution with A = 88%, B = 12%; monitored by a UV detector at a wavelength of 285 nm. A total of 213 g procyanidin B-2 of purity greater than 94% (w/w) in dry weight was obtained from 20 kl apple juice. The product was identified by mass spectrometry, ^{1}H-NMR, and ^{13}C-NMR (Morimoto *et al.*, 1986; Thompson *et al.*, 1972).

Preparation methods of topically applied agents

1% (w/w) procyanidin B-2, 70% (w/w) ethanol, 10%(w/w) 1,3-butylene glycol, 0.5% (w/w) isostearyl *N*-acetylglutamine (Kyowa Hakko Kogyo Co., Japan), 0.25% (w/w) polyoxyethylene (25) glyceryl monopyroglutamate monoisostearate (Nihon Emulsion Co., Japan), 0.1% (w/w) DL-α-tocopherol, 0.05% (w/w) D-biotin, 0.1% (w/w) ascorbyl palmitate, 0.001% (w/w) β-carotene, 0.1% (w/w) sodium citrate and 17.899% (w/w) purified water were uniformly mixed to prepare sample solution. A vehicle without procyanidin B-2 was used as the control.

Toxicological studies

Mutagenicity testing was performed at the Toxicological Research Laboratories, Kyowa Hakko Kogyo Co. (Ube, Yamaguchi, Japan). Other toxicological studies were performed at Panapharm Laboratories (Uto, Kumamoto, Japan). All animals used were kept under standard diet.

Mutagenicity test

Reverse mutation testing was performed using *Salmonella typhimurium* (TA98, TA100 provided from the National Institute of Hygienic Sciences, TA1535 provided from the University of California, and TA1537 provided from the National Institute of Genetics) and *Escherichia coli* (WP2*uvrA* provided by the National Institute of Genetics) with or without S-9 mixture according to the method of Yahagi *et al.* (1977) with slight modification. The concentrations of procyanidin B-2 used were 156, 313, 625, 1250, 2500 and 5000 μg/plate. *In vitro* chromosomal aberration testing was performed using CHL cells (from the Japanese Cancer Research Resources Bank) in the non-activated (Ishidate and Odashima, 1977) or the activated system, with or without S-9 mixture (Matsuoka *et al.*, 1979). Micronucleus testing was performed using mice [Crj: CD-1 (ICR), specific pathogen free (SPF)/virus antibody free (VAF), male, 8 wk old, Charles River Japan Inc., Kanagawa, Japan] at doses of 500, 1000 and 2000 mg/kg by single sc administration (Schmid, 1975).

Acute subcutaneous injection study

Procyanidin B-2 was administered sc to a group of SD rats (Crj: CD, SPF/VAF, 6 wk old, weight 204.2–226.2 g (male), 151.4–175.1 g (female), Charles River Japan Inc., Kanagawa, Japan) at doses of 0, 500, 1000 and 2000 mg/kg (10 ml/kg). Each group consisted of five rats, giving a total test number of 20 male rats and 20 female rats. The procyanidin B-2 sample was dissolved in purified water (injection grade) and injected sc, using a needle, into the neck. The observations were continued for 14 days. On day 14, the rats were sacrificed and examined by autopsy.

Primary irritation test (24-hr closed test)

Procyanidin B-2 preparation and vehicle were topically applied to the dorsal skin of New Zealand White rabbits (Kbs: NZW, SPF, 12 male rabbits, 3.5 months old, weight 2.4–2.8 kg, Kitayama Rabesu Co., Japan) by occlusive patch. The dorsal skin of each rabbit was shaved using an electric shaver, and divided into four 4×4 cm partitions. Two sections were abraded in a cross-hatched pattern using a needle (23G, Terumo Co., Japan) and the rest were left intact. 8% aqueous sodium lauryl sulfate (SLS) was used as the positive control, and physiological saline was used as the negative control. 0.5 ml of each sample, impregnated into a lint cloth (2.5×2.5 cm), was applied to each section, which was then covered with a bandage for 24 hr. At 24.5 and 72 hr after application, the skin state was observed and evaluated according to the method of Draize *et al*. (1944).

Skin sensitization test

Guinea pigs (Crj: Hartley, SPF/VAF, 24 male guinea pigs, 7 wk old, weight 378–494 g, Charles River Japan Inc., Kanagawa, Japan) were used for the test. The dorsal area was shaved using an electric shaver to form a 2×4 cm section. For induction of sensitization, a combination of sc injection and topical application was used.

For the procyanidin B-2 group, sc injection (0.1 ml) of Freund's complete adjuvant (FCA), 1% procyanidin B-2 solution, and 1% procyanidin B-2 + 50% FCA was performed at both sites in the area. 1 wk after the sc injection, the section (2×4 cm) was shaved again and 0.5 g of 10% SLS (in Vaseline) was applied to the section. After 24 hr, the section was wiped with alcohol, and 0.2 ml of 10% procyanidin B-2 impregnated into lint cloth was applied. The area was then covered with a bandage for 24 hr.

The challenge was performed 2 wk after sensitization. Two abdominal sites on the guinea pigs were shaved and 0.1 ml of sample solution (10% procyaninidin B-2 on one side and purified water on another) impregnated into lint cloth (2×2 cm) was topically applied to the sites, which were then covered with a bandage for 24 hr. After 24.5, 48 and 72 hr had elapsed after the challenge, the skin areas of the animals to which the sample had been applied were observed and photographed.

For the positive control, 2,4-dinitrochlorobenzene (DNCB) was used. For induction of sensitization, 0.1% DNCB (in olive oil and in 25% FCA) was injected to the sites (0.1 ml/site) and 1% DNCB (in Vaseline, 0.2 g) was applied. For challenge, 1% DNCB (in Vaseline, 0.1 g) was applied.

The degree of skin sensitization was evaluated according to the method of Magnusson and Kligman (1969).

Primary ocular irritation test

New Zealand White rabbits (Kbs: NZW, SPF, 12 male rabbits, 3.7 wk old, weight 2.5–2.8 kg, Kitayama Rabesu Co., Japan) were used for the test. 100 μl of procyanidin B-2 preparation or vehicle alone was applied to the right eye of each rabbit. The left eye was left untreated as a control. 30 sec after the application of the test solution, the eyes of one half in each group were washed with running water (about 300 ml of tepid water per eye); the eyes of the remaining half were not washed. 1, 24, 48, 72, 96 and 120 hr after the start of the test, the eyes of the rabbits were observed and evaluated according to the method of Draize *et al*. (1944). After observation using a slit lamp, one drop of 2% fluorescein sodium (in water) was applied to the eyes of the rabbits, and after a few secs, the eyes were washed with water (about 20 ml per eye) and the surface of the cornea was observed.

RESULTS

Mutagenicity test

In the reverse mutation test using *S. typhimurium* and *Escherichia coli*, none of the bacterial strains showed an increase in revertants in the presence of procyanidin B-2, with or without metabolic activation (data not shown). In the chromosomal aberration test *in vitro* using CHL cells, procyanidin B-2 did not cause any structural aberrations such as chromosome-type aberrations or chromatid-type aberrations in both the non-activated or activated system, with or without S-9 mixture (data not shown). However, polyploidy was observed in the activated system. The frequency was 15.5% (1.2 mM), 21.5% (2.4 mM), and 23.0% (4.8 mM) with S-9 mixture; 12.0% (0.9 mM) and 11.0% (1.8 mM) without S-9 mixture. In micronucleus testing using *in vivo* mice [Crj: CD-1(ICR)], the frequency of micronucleated polychromatic erythrocytes in the bone marrow of mice given procyanidin B-2 was not significantly different from that of the negative control (data not shown).

Acute subcutaneous injection study

None of the rats died in any of the groups of male and female rats administered with procyanidin B-2 at doses of 0, 500, 1000 or 2000 mg/kg. In the 2000 mg/kg group, a decrease in general activity was observed at 6–24 hr; however, all animals recovered within 48 hr. In the 500, 1000 and 2000 mg/kg administered groups, the injection site showed hair loss appearance (500, 1000, 2000 mg/kg), crust formation (500, 1000, 2000 mg/kg) or swelling (2000 mg/kg) in many of the animals (Tables 1 and 2). The weight of rats treated with 500 (male), 1000 (male and female) and 2000 (male and female) mg/kg decreased on the first day,

Table 1. Clinical signs in male rats after a single subcutaneous administration of procyanidin B-2

		Time after administration																	
		Hours				Days													
Dose	Clinical signs	1	2	3	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 mg/kg	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	0	0	0	0	0	0	0	0	3	3	5	5	5	5	5
	Crust (treated site)	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0
	Loss of hair	0	0	0	0	0	0	0	0	0	0	0	1	3	5	5	5	5	5
1000 mg/kg	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	0	0	0	0	0	0	0	3	0	3	3	3	3	4	4
	Crust (treated site)	0	0	0	0	0	0	0	0	0	0	0	3	2	2	2	2	2	1
	Loss of hair	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	2	2	5
2000 mg/kg	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	3	5	0	0	0	0	2	2	3	3	3	3	4	4	4
	Hypoactivity	0	0	0	3	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	Crust (treated site)	0	0	0	0	0	0	0	0	0	2	2	3	3	3	3	3	3	3
	Swelling (treated site)	0	0	0	0	0	0	0	0	0	2	2	3	3	3	3	3	3	3
	Loss of hair	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2	2

recovered by day 3, and increased thereafter (Table 3). At autopsy on day 14, thickening of the subcutis composed of granulomatous inflammation was observed in 1000 (male, four out of five animals; female, two out of five animals) and 2000 mg/kg (male, five out of five animals; female, five out of five animals) administered groups; and changes in colour (to black grey: composed of accumulation of pigment-laden macrophages) of the duodenal mucosa were observed in the 500 (female, one out of five animals), 1000 (male, four out of five animals; female, five out of five animals) and 2000 (male, five out of five animals; female, five out of five animals) mg/kg groups.

Primary irritation test (24-hr closed test)

In the groups that had been treated with procyanidin B-2 preparation, vehicle and physiological saline, no erythema, oedema or any other reactions were observed in either intact or abraded sites of all rabbits. On the other hand, the group which was administered with 8% SLS showed significant inflammation ranked as "severe irritation" according to the method of Draize *et al.* (1944) (Table 4).

Skin sensitization test

In the skin sensitization test using guinea pigs, none of the four combinations of procyanidin B-2

Table 2. Clinical signs in female rats after a single subcutaneous administration of procyanidin B-2

		Time after administration																	
		Hours				Days													
Dose	Clinical signs	1	2	3	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Control	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
500 mg/kg	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	4	4
	Crust (treated site)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Loss of hair	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3	3	4	4
1000 mg/kg	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	0	0	0	0	0	0	2	2	2	2	3	3	5	5	5
	Crust (treated site)	0	0	0	0	0	0	0	0	0	2	2	2	2	3	3	3	3	3
	Loss of hair	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	4	4
2000 mg/kg	Number of animals	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	Number of animals with abnormality	0	0	0	2	5	0	0	0	0	3	3	4	4	4	4	4	4	4
	Hypoactivity	0	0	0	2	5	0	0	0	0	0	0	0	0	0	0	0	0	0
	Crust (treated site)	0	0	0	0	0	0	0	0	0	3	3	4	4	4	4	4	3	3
	Swelling (treated site)	0	0	0	0	0	0	0	0	0	1	1	2	2	3	3	3	3	3
	Loss of hair	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	3

Table 3. Body weight of rats after a single subcutaneous administration of procyanidin B-2

Sex	Group and dose		Body weight (g) on day 0	1	3	5	7	10	14
Male	Control	n	5	5	5	5	5	5	5
		Mean	217.0	223.0	237.5	256.5	270.0	298.7	322.9
		SD	±5.4	±5.5	±8.2	±12.5	±11.2	±13.5	±17.2
	500 mg/kg	n	5	5	5	5	5	5	5
		Mean	216.5	215.5	232.3	253.1	266.1	293.3	335.2
		SD	±8.1	±8.1	±10.2	±11.5	±14.3	±18.3	±11.3
	1000 mg/kg	n	5	5	5	5	5	5	5
		Me??an	216.7	211.2	227.0	249.7	264.3	294.0	324.1
		SD	±5.9	±6.2	±5.5	±7.6	±7.6	±9.5	±11.0
	2000 mg/kg	n	5	5	5	5	5	5	5
		Mean	215.6	203.8	212.0	233.2	251.5	278.8	306.5
		SD	±4.1	±5.0	±5.3	±5.7	±7.7	±11.8	±15.3
Female	Control	n	5	5	5	5	5	5	5
		Mean	162.2	163.7	172.4	178.8	184.4	201.0	212.6
		SD	±1. 0	±2.6	±3.8	±2.0	±3.9	±3.9	±3.5
	500 mg/kg	n	5	5	5	5	5	5	5
		Mean	161.3	163.0	174.4	182.0	189.0	199.7	213.5
		SD	±6.6	±6.9	±8.2	±9.2	±9.7	±11.3	±12.6
	1000 mg/kg	n	5	5	5	5	5	5	5
		Mean	163.3	159.2	168.5	177.2	183.5	196.9	209.8
		SD	±8.8	±7.6	±7.1	±8.9	±8.3	±12.6	±13.4
	2000 mg/kg	n	5	5	5	5	5	5	5
		Mean	162.2	156.5	164.9	172.7	183.4	197.4	209.7
		SD	±8.9	±8.5	±9.4	±11.3	±15.3	±19.0	±21.0

Table 4. Dermal irritation scores and irritation indices in male rabbits applied topically with procyanidin B-2 preparation

Test	Animal no.	Irritation score[a] 24 hr Erythema I[b]	24 hr Erythema A[c?0]	24 hr Oedema I	24 hr Oedema A	72 hr Erythema I	72 hr Erythema A	72 hr Oedema I	72 hr Oedema A	Mean score[d]	Primary irritation index[e]
	1	0	0	0	0	0	0	0	0	0	
	2	0	0	0	0	0	0	0	0	0	
Procyanidin	3	0	0	0	0	0	0	0	0	0	0.00
B-2 base	4	0	0	0	0	0	0	0	0	0	
	5	0	0	0	0	0	0	0	0	0	
	6	0	0	0	0	0	0	0	0	0	
	1	0	0	0	0	0	0	0	0	0	
Procyanidin	2	0	0	0	0	0	0	0	0	0	
B-2	3	0	0	0	0	0	0	0	0	0	0.00
preparation	4	0	0	0	0	0	0	0	0	0	
	5	0	0	0	0	0	0	0	0	0	
	6	0	0	0	0	0	0	0	0	0	
	7	0	0	0	0	0	0	0	0	0	
	8	0	0	0	0	0	0	0	0	0	
Saline	9	0	0	0	0	0	0	0	0	0	0.00
	10	0	0	0	0	0	0	0	0	0	0
	11	0	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	0	0	0	0
	7	2	2	4	4	4	4	4	4	7.0	
8% sodium	8	2	2	4	4	4	4	4	4	7.0	
lauryl	9	2	2	3	3	2	2	4	4	5.5	6.33
sulfate	10	2	2	2	2	4	4	3	3	5.5	
	11	2	2	4	4	4	4	4	4	7.0	
	12	2	2	3	3	4	4	3	3	6.0	

[a]The degrees of the reaction were as follows (Draize *et al*., 1944).

Erythema formation
1 = Very slight erythema
2 = Well defined erythema
3 = Moderate to severe erythema
4 = Severe erythema

Oedema formation
1 = Very slight oedema
2 = Slight oedema
3 = Moderate oedema
4 = Severe oedema

[b]I = Intact skin.
[c]A = Abraded skin.
[d]Mean score = dermal irritation score (24 hr + 72 hr)/4.
[e]Primary irritation index = Σ mean score/number of animal treated.

Table 5. Contact sensitivity test of procyanidin B-2 in guinea pigs

Group	Number of animals	Sensitizing substances	Challenging substances	Time (hr)	Score[a] –	+	+ +	+ + +	+ + + +	Positive rate (%)
Procyanidin B-2	6	Procyanidin B-2	Procyanidin B-2	24	6					0
				48	6					0
				72	6					0
			Distilled water	24	6					0
				48	6					0
				72	6					0
	6	Non-treatment	Procyanidin B-2	24	6					0
				48	6					0
				72	6					0
			Distilled water	24	6					0
				48	6					0
				72	6					0
DNCB[b]	6	DNCB	DNCB	24			2	4		100
				48					6	100
				72					6	100
			White Vaseline	24	6					0
				48	6					0
				72	6					0
	6	Non-treatment	DNCB	24	6					0
				48	6					0
				72	6					0
			White Vaseline	24	6					0
				48	6					0
				72	6					0

[a]The degrees of the reaction were as follows.
– = no changes
+ = slight erythema
+ + = moderate erythema
+ + + = severe erythema and oedema
+ + + + = crusta or necrosis
[b]DNCB = 2,4-dinitrochlorobenzene.

or non-sensitization following procyanidin B-2 or purified water challenge caused any changes in the skin of any of the animals belonging to these groups. On the other hand, positive control (DNCB sensitization following DNCB challenge) elicited the expected strong response such as erythema, oedema, crusta or necrosis.

Consequently, DNCB ranked as "extreme", whereas procyanidin B-2 ranked as "weak" according to the index of maximization grading (Magnusson and Kligman, 1969) (Table 5).

Primary ocular irritation test

In the eye irritation test using New Zealand White rabbits, procyanidin B-2 preparation and vehicle alone ranked equally as "minimally irritating" according to the Kay and Calandra method (Kay and Calandra, 1962). No changes were observed in the cornea or iris, but slight irritation of the conjunctivae was observed. In the study using fluorescein sodium, some damage was observed to the surface of the cornea in all groups (both washed and non-washed; both procyanidin B-2 preparation and vehicle) 1 hr after administration. However, these symptoms became attenuated after 24 hr and disappeared over the next 3–4 days. No difference was observed between the two groups of procyanidin B-2 preparation and vehicle alone (Table 6).

DISCUSSION

Mutagenicity tests using *S. typhimurium* and *E. coli* showed procyanidin B-2 to be non-mutagenic. Yu and Swaminathan (1987) have also reported that procyanidin B-2 is non-mutagenic to bacteria. Chromosomal aberration tests using a mammalian cell line (CHL cells) indicated that procyanidin B-2 caused polyploidy but no structural aberrations. Popp and Schimmer (1991) report that polyploidy is induced by procyanidin trimers and tetramers such as procyanidin C-1 and procyanidin D but not by procyanidin dimers such as procyanidin B-2 in human lymphocyte cultures. In micronucleus tests for mutagenicity using *in vivo* mice, procyanidin B-2 was negative (this study).

The results of these tests indicate that procyanidin B-2 induced only polyploidy in chromosomal aberration tests *in vitro*.

Table 6. Primary ocular irritation test of procyanidin B-2 preparation in rabbits

Test substance	Treatment	No. of animals	Tissue	Ocular irritation score[a] 1 h[b]	1 d	2 d	3 d	4 d	5 d	Evaluation[c]
Procyanidin B-2 preparation	Non-washed eye	3	Cornea	0	0	0	0	0	0	Minimally irritating
			Iris	0	0	0	0	0	0	
			Conjunctivae	10.7	6.7	4.0	1.3	0	0	
			M.T.S.[d]	10.7	6.7	4.0	1.3	0.0	0.0	
	Washed eye	3	Cornea	0	0	0	0	0	0	Minimally irritating
			Iris	0	0	0	0	0	0	
			Conjunctivae	8.0	6.0	4.0	1.3	0	0	
			M.T.S.	8.0	6.0	4.0	1.3	0.0	0.0	
Procyanidin B-2 base	Non-washed eye	3	Cornea	0	0	0	0	0	0	Minimally irritating
			Iris	0	0	0	0	0	0	
			Conjunctivae	10.7	6.7	4.0	2.0	0.7	0	
			M.T.S.	10.7	6.7	4.0	2.0	0.7	0.0	
	Washed eye	3	Cornea	0	0	0	0	0	0	Minimally irritating
			Iris	0	0	0	0	0	0	
			Conjunctivae	9.3	6.7	2.0	0	0	0	
			M.T.S.	9.3	6.7	2.0	0.0	0.0	0.0	

[a]According to Draize *et al.* (1944).
[b]Time after application.

The acute sc injection study using SD rats revealed no symptoms of significant injury caused by procyanidin B-2 except for inflammation of injection sites. The lethal dose of procyanidin B-2 is greater than 2000 mg/kg (sc injection) in both male and female animals. Primary irritation tests using New Zealand White rabbits indicated that procyanidin B-2 preparation and vehicle show no primary irritation. Skin sensitization tests using guinea pigs suggested that the skin sensitizing activity of procyanidin B-2 is extremely low. In primary ocular irritation tests of eyes using New Zealand White rabbits, procyanidin B-2 containing preparation and vehicle alone showed equally slight irritation of conjunctivae and some effect on the surface of the cornea. However, no difference was observed between the two groups, so it is assumed that these stimuli were caused by ethanol (Weil and Scala, 1971) or other compounds contained in both agents. The degree of irritation was lower than that of ethanol, so it is concluded that both agents are safe for use as hair tonic.

The results noted from these tests are acceptable and suggest the safety of the procyanidin B-2 preparation by topical application to the extent evaluated.

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