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A Single Oral Dose Toxicity Study of Plant Sterol Ester in Sprague-Dawley Rats

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Abstract – The present study was conducted to investigate the acute toxicity of plant sterol ester by a single oral dose in Sprague-Dawley rats. Ten males and 10 females aged 5 weeks were randomly assigned to two groups of 5 rats each and were administered by gavage at dose level of 0 or 20 ml/kg body weight. Parameters measured during the 14-day observation period were mortality, clinical signs, body weight changes, and gross findings. No mortality was observed in the present study. Treatment-related clinical signs, such as pasty stool and diarrhea, were observed on the day of treatment and these signs resulted in soiled fur on day 1 after the treatment. However, no clinical signs were observed on days 2-14 after the treatment. There was no significant difference in body weight changes between the control and treatment groups. At necropsy on day 14 after the treatment, no treatment-related gross findings were observed in the treatment group. Based on these results, it was concluded that a single oral dose of plant sterol ester induced pasty stool and diarrhea in Sprague-Dawley rats at dose level of 20 ml/kg and that the lethal doses were considered to be over 20 ml/kg for both sexes.

Key words □ Plant sterols, plant sterol ester, acute toxicity, diarrhea, rats

Plant sterols (phytosterols), abundant in fat-soluble fractions of plants, are considered to lower serum cholesterol levels, particularly low density lipoprotein (LDL) cholesterol level, by inhibiting absorption of cholesterol in the intestine through competition with cholesterol (Ling and Jones, 1995a). The cholesterol-lowering effect of dietary plant sterols has been studied since 1950s and has been well described in animal and human studies (Lees *et al.*, 1977; Malini and Vanithakumari, 1990; Ling and Jones, 1995b; Jones *et al.*, 1997). In addition to the cholesterol-lowering effect, plant sterols have been suggested to possess several therapeutic activities, such as potent antiinflammatory, antibacterial, antifungal, antigastroulcerative, and antitumor activities (Romero and Lichtenberger, 1990; Janezic and Rao, 1992; Padmaja *et al.*, 1993; Ling and Jones, 1995a). Despite the wide spectrum of biological properties, their use as food additives has been limited by the reason that plant sterols are not soluble in water or in oil. So many researchers have tried to find ways to increase their solubility. For example, plant sterols are esterified with fatty acids to generate plant sterol esters which are soluble in oil (Mattson, 1964, U.S. Patent No. 5,502,045). Recently, Eugene Science Inc. developed an advanced method for preparing fat-soluble plant sterols esterified with unsaturated fatty acids, which is easier to synthesize and does not generate unstable toxic chemical in the process (U.S. Patent application No. 09/431,396).

As a part of safety evaluation studies of test article plant sterol ester, a single oral dose toxicity study was performed in Sprague-Dawley rats. This study was conducted according to the Testing Guidelines for Safety Evaluation of Drugs (KFDA, 1998a) and in compliance with the Good Laboratory Practice Regulations for Nonclinical Laboratory Studies (KFDA, 1998b).

MATERIALS AND METHODS

Animals and housing

Thirteen male and thirteen female Sprague-Dawley rats were obtained from the Toxicology Research Center Breeding

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Facility (KRICT, Taejon, Korea) and used after 8 days of quarantine and acclimatization under SPF conditions. The animal room was maintained at the temperature of 23 ± 3°C, the relative humidity of 50 ± 10%, the air ventilation of 10~20 times/hr, and the light intensity of 150~300 Lux. A 12 hr light/dark cycle was used. The animals were kept in stainless wire cages and were allowed sterilized tap water and commercial rodent chow (Jeil Feed Co, Taejon, Korea) *ad libitum*. This experiment was conducted in facilities approved by the American Association for Accreditation of Laboratory Animal Care (AAALAC); all procedures were approved by our Institutional Animal Care and Use Committee (IACUC).

Test article

The test article plant sterol ester was supplied from Eugene Science Inc. (Seoul, Korea) and was administered with test article only without vehicle. The negative control rats received an equivalent volume of distilled water.

Drug treatment

Because the oral route is a clinically intended route for the test article, the oral administration was selected in the present study. The test article was administered in a single dose to the rats with a dose volume of 20 ml/kg body weight. The application volume was calculated according to the body weight on the treatment day. The rats were fasted overnight prior to dosing and the test article plant sterol ester was administered orally by gavage. After the test article was administered, the rats were fasted for a further 3~4 hours.

Experimental groups

Ten healthy male rats and 10 healthy female rats were randomly assigned to two groups, with 5 males and 5 females in each group.

Selection of dose

Doses of 1.25, 2.5, 5, 10, and 20 ml/kg had been given in a preliminary study to two rats per group. In the study, there were no dead animals and no toxic signs in the groups that received 20 ml/kg or less. On the basis of these results, 20 ml/kg was selected for the limit dose. When considering the specific gravity (0.947 at 40°C) of plant sterol ester, this dose corresponds to about 10 times of the limit test dose recommended by OECD Test Guideline 401 (OECD, 1993).

Clinical observation and mortality

Clinical signs and mortality were checked every hour until 6 hour after dosing and then once a day thereafter up to day 14.

Body weight changes

Individual body weights of animals were measured shortly before the test article administration and on day 1, 3, 7, and 14 after the treatment thereafter.

Necropsy findings

On day 14 after the treatment, all animals were euthanized by carbon dioxide asphyxiation and necropsied with special attention to all vital organs and tissues.

Statistical analysis

The data obtained in this study were analyzed using a LabCat system (Innovative Programming Associates Inc., New Jersey, USA). Body weight values were presented by mean ± S.D.

RESULTS

Mortality

No animal in both sexes was found dead by the treatment of test article during the observation period of 14 days (Table I).

Clinical findings

Treatment-related clinical signs, such as pasty stool, diarrhea, and soiled fur, were observed in the treatment group (Table II).

Table I. Mortality of SD rats after a single oral administration of plant sterol ester

Sex	Dose (mg/kg)	No. of rats	Hours after treatment						Days after treatment														Final mortality
			1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Male	0	5	0*	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5 (0%)
	20	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5 (0%)
Female	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5 (0%)
	20	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/5 (0%)

*Number of dead animals.

Table II. Clinical findings of SD rats after a single oral administration of plant sterol ester

Sex	Dose (ml/kg)	No. of rats	Findings	Hours after treatment						Days after treatment													
				1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Male	0	5	Appears normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	20	5	Appears normal	5	5	5	0	0	0	0	5	5	5	5	5	5	5	5	5	5	5	5	5
			Pasty stool	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Diarrhea	0	0	0	0	4	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Soiled fur	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Female	0	5	Appears normal	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
	20	5	Appears normal	5	5	5	2	0	0	0	5	5	5	5	5	5	5	5	5	5	5	5	5
			Pasty stool	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Diarrhea	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
			Soiled fur	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0

Number of animals with normal or abnormal clinical signs.

Table III. Body weight changes of SD rats after a single oral administration of plant sterol ester

Sex	Dose (ml/kg)	No. of rats	Days after treatment					Body weight gain
			0	1	3	7	14	
Male	0	5	129.8 ± 3.58	154.1 ± 4.83	174.4 ± 6.51	206.5 ± 6.15	254.8 ± 8.85	125.0 ± 6.60
	20	5	127.1 ± 3.10	152.3 ± 4.04	173.1 ± 3.64	203.7 ± 6.12	259.7 ± 7.76	132.5 ± 5.61
Female	0	5	112.0 ± 2.67	134.6 ± 4.35	150.5 ± 5.30	168.5 ± 7.48	193.2 ± 6.43	81.2 ± 4.07
	20	5	112.9 ± 2.21	134.7 ± 3.53	148.8 ± 5.09	168.7 ± 7.60	194.8 ± 13.61	81.9 ± 12.31

Values are mean ± S.D. (g).

Table IV. Necropsy findings of SD rats after a single oral administration of plant sterol ester

Sex	Dose (ml/kg)	No. of rats	Findings	Frequency
Male	0	5	No gross finding	5/5 (100%)
	20	5	No gross finding	5/5 (100%)
Female	0	5	No gross finding	5/5 (100%)
	20	5	No gross finding	5/5 (100%)

Pasty stool was found in 5 males and 3 females at 4 hours after the treatment and in 1 male rat at 5 hours after the treatment. Diarrhea was observed in 4 males and 5 females at 5 hours after the treatment and in all animals of both sexes at 6 hours after the treatment. Soiled fur was seen in all treatment animals of both sexes on day 1 after the treatment. However, these signs were not observed from day 2 after the treatment. There were no clinical signs in the negative control group.

Body weight changes

No changes were observed in body weight which could be attributed to the treatment with plant sterol ester (Table III).

Gross findings

At necropsy on day 14 after the treatment, no treatment-related effects were discovered in any treatment animals (Table IV).

DISCUSSION

The test article in this study is a plant sterol ester which is being developed by Eugene Science Inc. In order to assess the acute toxicity of plant sterol ester which is esterified with fatty acids, 10 male and 10 female Sprague-Dawley rats were randomly assigned to two groups of 5 rats each and were administered by gavage at dose level of 0 or 20 ml/kg body weight.

No mortality was observed in the treatment group of both sexes during the observation period of 14 days. Therefore, it was estimated that the lethal doses of plant sterol ester were more than 20 ml/kg in both sexes. Clinical signs observed included pasty stool, diarrhea, and soiled fur in the treatment group. It was considered that these clinical signs were induced by test article plant sterol ester and that pasty stool and diarrhea caused soiled fur on day 1 after the treatment. However, because these findings were not observed on days 2-14 after the treatment, we considered that these findings were not a toxicological effect. These results observed in the present

study are similar to the findings of Pollak (1985), who showed that dietary phytosterols caused diarrhea in animals and humans. Previous studies revealed that the oral administration of a large volume of test articles, such as corn oil and highly lipid soluble chemicals, resulted in increased gastrointestinal motility and decreased time for absorption of the test article in the gastrointestinal tract, causing transient diarrhea (Alexander *et al.*, 1987; Chan and Hayes, 1989; Mascolo, 1994). Because plant sterols are abundant in fat-soluble fractions of plants such as corn and bean, pasty stool and diarrhea found in the present study were regarded as a result of the treatment of test article. There were no adverse effects on body weight changes and necropsy findings at 20 ml/kg plant sterol ester. When considering the specific gravity (0.947 at 40°C) of the test article, this dose level is equivalent to about 247 times of human clinical dose, i.e., 76.7 mg/kg/day, when the body weight of normal adult is assumed to be 60 kg.

Based on these results, it was concluded that a single oral dose of plant sterol ester induced pasty stool and diarrhea in Sprague-Dawley rats and that the lethal doses were considered to be over 20 ml/kg for both sexes.

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