The Journal of Applied Pharmacology, 11, 109-111(2003)

Short Communications

Single Oral Toxicity of Jeju Citrus Rind Pectin in Spraque-Dawley Rats

Kyoo Jung SHIM and Se Young CHOUNG*

College of Pharmacy, Kyung Hee University, Seoul, Korea

(Received June 18, 2003; Accepted June 18, 2003)

Abstract–The single oral toxicity of Jeju citrus rind pectin (Jeju pectin) was studied in Spraque-Dawley rats of both sexes. In this study, rats were administrated orally with dosages of 100, 250 and 500 mg/kg of Jeju pectin. We daily examined number of deaths, clinical signs, body weights and gross findings for 14 days after Jeju pectin administration. When we administered different doses of 100, 250 and 500 mg/kg. We found no rats died in both sex after administration. Some clinical signs (decrease locomotor activity, salivation, soft stool, prone position, lacrimation, crouching position, convulsion, ataxic gait, incontinence of urine) were also observed during the experimental period.

Keywords dioxin, acute toxicity, pectin, rat

Consumption of dietary soluble fiber (SF) leads to lowering of plasma LDL cholesterol (LDL-C) (Brown *et al.*, 1999; Liu *et al.*, 1999; Jacobs *et al.*, 1999). Dietary SF intake has been inversely related to the risk of developing coronary heart disease (Appleby *et al.*, 1999; Todd *et al.*, 1999). The intestinal lumen has been widely accepted as the primary site of action of fiber (Schneeman, 1998; Turley, *et al.*, 1991). Amongst those dietary SFs, pectin has been considered as one of the soluble dietary SFs with high effects on hepatic cholesterol homeostasis.

In order to investigate the acute toxicity of Jeju citrus rind pectin, the acute toxicity test was performed following the guidelines and test methods for the safety tests of the drugs provided by the Food and Drug Administration, Korea. Decreased locomotor activity, salivation, soft stool, prone position, lacrimation, crouching position, convulsion, ataxic gait and incontinence of urine of the rats were observed for 14 days in order to gain basic safety data for developing health functional food material by the pectin and enhancing economic progress in Korean agriculture.

MATERIALS AND METHODS

Jeju pectin

Korea New Science and Technology have kindly provided Jeju pectin. Jeju pectin was the extract of Jeju citrus rind (water soluble, no odor and white color fine powder). All the extraction process was done by Korea New Science and Technology. Well-dried powdered raw material (99% of citrus rind pectin) was supplied to the Lab. of Toxicology, College of Pharmacy, Kyung Hee University.

*To whom correspondence should be addressed.

Animal husbandry

Sprague-Dawley rat was purchased from Samtako, Seorang-dong 77-1, Osan City, Kyungki-do. SD rats were acclimatized to laboratory environment for a week.

The animals were reared under the condition below: 23 ± 3°C and 150~300Lux for 12hrs/day. The animal chamber controls temperature and ventilation for optimal rearing condition automatically. The animals were housed in the plastic cage (220×410×220 mm) and fed with the rat-and-mouse food (Samtako Experimental Animals, Korea) and tap water. The food and water was supplied 300 g/day and 480 ml/day, respectively.

Every male and female rat was selected randomly for grouping but each group (five rats, same gender, for each group) was adjusted to similar body weight. Numbering on tail identified each rat in the same group.

Jeju pectin treatment

Sprague Dawley rat(5 week old, 120~140 g), male and female 50 each, was purchased and 80 healthy SD rats (male and female 40 each) were selected. Groups were divided into 4 in each gender and consisted of control, Jeju pectin 500 mg/10 ml in D.W./kg (the maximum possible solubility in water of Jeju pectin) treated group, 250 mg/10 ml/kg and 100 mg/10 ml/kg groups. Ten rats were allotted to each group accord-

ing to their gender. Oral administration was performed with feeding needle at day 0.

Observations

According to the guidelines and test methods on the safety tests of the drugs provided by the Food and Drug Administration, Korea, the acute toxicity of Jeju pectin was investigated. The duration of acute toxicity test was 14 days.

Decreased locomotor activity, salivation, soft stool, prone position, lacrimation, crouching position, convulsion, ataxic gait and incontinence of urine of the rats, and death checks were made daily for 14 days. Dead animals during the observation period were performed biopsy to check the visible adverse change of internal organs.

Body weight change

Body weight change was checked at day 1, 3, 7 and 14 after the oral administration at day 0.

Biopsies

At the termination of the observation period, all the animals were sacrificed under the ether anesthesia and checked all visible symptoms on internal organs. No food was given to the animals for 24 hrs before the biopsy

Statistical analysis

Statistical analyses were made using ANOVA analysis, whereby a value $P < 0.05$ and $P < 0.01$ was considered to be statistically significant.

RESULTS

General symptoms and death ratio

There was no single death of animals till the termination of the experiment (Table I). Decreased locomotor activity, salivation, soft stool, prone position, lacrimation, crouching position, convulsion, ataxic gait and incontinence of urine of the rats were observed for 14 days after single oral administration of Jeju pectin. The 500 mg/kg treated group of male and female showed no particular symptoms except soft stool at day 0 (Table II).

Body weight change

Body weight loss was temporally observed at day 1 in 500 mg/kg treated groups in male and female and was recovered to the normal body weight gaining of the control from day 3 (Table III).

Table I. Mortality of rats orally treated with GBG1

Sex	Dose (mg/kg)	Days after treatment															Final Mortality[a)]
		0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Male	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
Female	Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	250	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10
	500	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/10

[a)] Values are expressed as dead number/total number of animals

Table II. Clinical signs in rats orally treated with GBG1

Sex	Dose (mg/kg)	Observations	Range
Male	Control	Appears normal	Day0(09:10)-Day14(09:30)
	100	Appears normal	Day0(09:10)-Day14(09:30)
	250	Appears normal	Day0(09:10)-Day14(09:30)
	500	Appears normal Soft stool	Day0(09:10)-Day14(09:30) Day0(13:20)-Day0(15:20)
Female	Control	Appears normal	Day0(09:10)-Day14(09:30)
	100	Appears normal	Day0(09:10)-Day14(09:30)
	250	Appears normal	Day0(09:10)-Day14(09:30)
	500	Appears normal Soft stool	Day0(09:10)-Day14(09:30) Day0(12:00)-Day0(15:00)

Table III. Body weight of rats orally treated with GBG1

Sex	Dose (mg/kg)	Days after treatment				
		0	1	3	7	14
Male	Control	153.7 ± 5.5(10)[a)]	177.2 ± 10.3(10)	188.3 ± 10.2(10)	239.7 ± 12.2(10)	298.9 ± 14.8(10)
	100	151.9 ± 7.2(10)	178.2 ± 9.7(10)	186.8 ± 9.4(10)	243.6 ± 9.4(10)	299.2 ± 12.3(10)
	250	149.8 ± 6.9(10)	178.7 ± 11.6(10)	187.7 ± 8.5(10)	240.4 ± 10.6(10)	300.4 ± 11.7(10)
	500	152.2 ± 7.3(10)	171.4 ± 7.3(10)	189.5 ± 7.6(10)	234.9 ± 11.2(10)	296.6 ± 10.2(10)
Female	Control	125.3 ± 8.4(10)	149.2 ± 9.2(10)	164.0 ± 12.1(10)	186.6 ± 11.7(10)	196.8 ± 10.9(10)
	100	123.7 ± 7.2(10)	150.2 ± 8.2(10)	166.8 ± 9.1(10)	185.4 ± 10.3(10)	194.4 ± 10.5(10)
	250	124.2 ± 7.7(10)	147.2 ± 6.4(10)	164.9 ± 13.2(10)	183.3 ± 9.7(10)	200.5 ± 12.3(10)
	500	123.5 ± 6.9(10)	143.2 ± 8.6(10)	169.2 ± 11.5(10)	189.9 ± 8.2(10)	199.8 ± 9.5(10)

[a)](n)=number of animals

Table IV. Gross observation of necropsy in rats orally treated with GBG1

Sex	Dose (mg/kg)	Observation	Frequency	
			Decedents	Survivors
Male	Control	N.G.F[a)]	0/0[b)]	10/10
	100	N.G.F	0/0	10/10
	250	N.G.F	0/0	10/10
	500	N.G.F	0/0	10/10
Female	Control	N.G.F	0/0	10/10
	100	N.G.F	0/0	10/10
	250	N.G.F	0/0	10/10
	500	N.G.F	0/0	10/10

[a)]No Gross Findings. [B)]Values are expressed as animal numbers

Biopsies

No gross finding was observed at day 14 in all Jeju pectin oral administrated groups of male and female (Table IV).

DISCUSSION

After the single oral dose of 100, 250 and 500 mg/kg of Jeju citrus rind pectin, death ratio, general symptoms, body weight change were checked for 14 days and biopsy was performed at day 14 to observe toxicity of the pectin.

There was no single death of animals till the termination of the experiment. The pectin treated male and female animals showed no particular symptoms during the experiment periods except soft stool (500 mg/kg treated group) at day 0.

The temporally observed body weight loss at day 1 in 500 mg/kg treated both male and female groups was recovered to normal body weight gaining of that of control from day 3, indicating no body weight loss by the pectin. In addition, there were no gross findings from the biopsies of all animals examined.

From these facts, single oral administration of Jeju citrus rind pectin showed no approximate lethal dose at the maximum possible solubility in water (500 mg/10 ml of water/kg). However, this was not enough supporting safety data. Additional experiment at the dose of 2 g/day of pectin is badly needed to show safety (according to the toxicity guidelines) for any commercially available material for health functional food and what not. With the dose of 2 g/day might induce gastro-intestinal side effects. Temporary body weigh loss was found at day 1. However, commonly found gross findings at the lethal dose that might produce progressive decreased inactivity and eventually led to coma (Torsoni *et al.*, 2002) were not observed.

REFERENCES

Appleby PN, Thorogood M, Mann JI, Key TJA. (1999) The Oxford vegetarian study: an overview. *Am J Clin Nutr* **70**(Suppl.),525S-31S.

Brown L, Rosner B, Willet WC, Sacks FM. (1999) Cholesterol-lowering effects of dietary fiber: a meta-analysis. *Am J Clin Nutr* **69**, 30-42

Jacobs DR, Meyer KA, Kushi LH, Folsom AR. (1999) Whole-grain intake may reduce the risk of ischemic heart disease death in postmenopausal women: the Iowa Womens Health Study. *Am J Clin Nutr* **68**, 248-57

Liu S, Stampfer MJ, Hu FB, Giovannucci E, Rimm E, Manson JE, *et al.* (1999) Whole-grain consumption and risk of coronary heart disease: results from the Nurses Health Study. *Am J Clin Nutr* **70**, 412-9

Schneeman BO. (1998) Fiber, inulin and oligofructose: similarities and differences. *J Nutr* **129**(Suppl.),1424S-7S.

Todd S, Woodward M, Tunstall-Pedoe H, Bolton-Smith C. (1999) Dietary, antioxidant vitamins and fiber in the etiology of cardiovascular disease and all-causes mortality: results from the Scottish Heart Health Study. *Am J Epidemiol* **150**, 1073-80.

Torsoni AS., Figueiredo de Barros B., Toledo JC, Jr., Haun M., Krieger MH., Tfouni E. and Franco WD. (2002) Hypotensive Properties and Acute Toxicity of *trans*-$[Ru(NH_3)_4P(OEt)_3(NO)](PF_6)_3$, a New Nitric Oxide Donor, Nitric Oxide, Volume **6**, Issue 3, May, Pages 247-254

Turley SD, Daggy BP, Dietschy JM. (1991) Cholesterol Lowering action of psyllium mucilloid in the hamster: sites and possible mechanisms of action. *Metabolism* **40**, 1063-73.