The Journal of Applied Pharmacology, 11, 200-203(2003)

A Single Oral Dose Toxicity Study of Bamboo Leaf Water Extract in Sprague-Dawley Rats

Dong Ho SHIN, Pan Gu JANG, Ki Seok OH, Jae Ha KIM[1], Hee Jong CHUNG[2] and Jong Choon KIM*

College of Veterinary Medicine, Chonnam National University, Gwangju 500-757, Korea

[1]College of Medicine, Chonnam National University, Gwangju 500-746, Korea

[2]College of Agriculture and Life Science, Chonnam National University, Gwangju 500-757, Korea

(Received August 21, 2003; Accepted September 19, 2003)

Abstract – The present study was carried out to investigate the potential acute toxicity of bamboo leaf water extract by a single oral dose in Sprague-Dawley rats. Twenty male and female rats aged 5 weeks were randomly assigned to four groups of 5 rats each and were administered singly by gavage at dose levels of 0, 1250, 2500, or 5000 mg/kg body weight. Mortalities, clinical findings, and body weight changes were monitored for the 14-day period following the administration. At the end of 14-day observation period, all animals were sacrificed and complete gross postmortem examinations were performed. Throughout the study period, no treatment-related deaths were observed. There were no adverse effects on clinical signs, body weight, and gross finding at any dose tested. The results showed that the single oral administration of bamboo leaf water extract did not induce any toxic effect at a dose level of below 5000 mg/kg in rats and that the minimal lethal dose were considered to be over 5000 mg/kg body weight for both sexes.

Key words □ bamboo leaf water extract, acute toxicity, rats

Modern people focus much more on the qualitative improvement of their life as the level of their life has been improved. Chemical compounds are used frequently to treat diseases and furthermore to prevent them, but they much often show not only the intended pharmacological effects but also show undesirable side effects. Recently people have paid their attention to the development of alternative therapy and functional food additives by natural substances that can improve health and prevent diseases, while having little side effects even used in a long term.

Bamboo is a plant of Gramineae which ranges largely in the temperate regions including Korea, the subtropical regions and the tropics. From the ancient times, bamboo sheath, branch, leaf, bamboo shoot and bamboo shavings have been largely used as herbs (Kim *et al.*, 2001). Particularly, bamboo leaves have been used for folk remedies against fever, bleeding, sweating, palsy and high blood pressure, and reported to have sterilizing, antifungal, antioxidant and anticancer effects (Shibata *et al.*, 1975; Tsunoda *et al.*, 1998; Hu *et al.*, 2000; Kwon et al., 2001). Recently we have reported that administration of bamboo leaf water extract showed a decrease in the disturbance of spleen and jejunal crypt cells induced by X-radiation in mice (Shin *et al.*, 2003). Accordingly, bamboos increasingly have the use value as a herb or a health supplement. But the potential adverse effects of bamboos have never been studied yet.

As a part of safety evaluation of the test article, bamboo leaf water extract, an acute oral dose toxicity study was conducted to investigate the potential acute toxicity after single oral administration of bamboo leaf water extract in Sprague-Dawley rats. The present study was carried out according to the test guidelines from the Korea Food and Drug Administration (KFDA) and Organisation for Economic Cooperation and Development (OECD) guidelines for the testing of chemicals.

MATERIALS AND METHODS

Animal husbandry and maintenance

Twenty-four Sprague-Dawley rats of each sex were obtained from the Bio Genomics (Seoul, Korea) at 4 weeks of age and used after one week of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 23 ± 3°C and a relative humidity of 50 ± 10% with artificial lighting from 08:00 to 20:00 and with 13~18 air changes per hour. Only

*To whom correspondence should be addressed.

healthy animals were assigned to the study. The animals were kept in stainless wire cages and were allowed sterilized tap water and commercial rodent chow (Samyang Feed Co, Wonju, Korea) ad libitum. The animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996).

Test article and preparation

One hundred g of fresh bamboo leaves (*Phyllostachys nigra* Var. *henesis Strapf*) was collected and extracted three times with 1000 ml of distilled water at 60°C of water bath for 4 hours and filtered. The extract fluid was concentrated under reduced pressure and dried on a lyophilizer. The test article was used after dissolution in sterilized distilled water immediately before treatment and that of lower group was prepared by stepwise dilution of that of the high dose group. The vehicle control rats received the same volume of distilled water alone.

Selection of doses and experimental groups

In a dose-range finding study, there were no dead animals at dose levels of 5000 mg/kg or below. Based on the results, dose of 5000 mg/kg which was a base line of practically nontoxic was selected for the highest dose in this study. Doses of 2500 and 1250 mg/kg were selected as middle and low doses, respectively, using a common ratio of 2. In addition, a vehicle control group was added to determine the effects of vehicle.

Treatment

The rats were fasted overnight prior to dosing and the test article was administered orally by gavage. The test article was administered singly at a dose volume of 20 ml/kg body weight. The application volume was calculated according to the fasted body weight on the treatment day. After the test article was administered, the rats were fasted for a further 3-4 hours.

Mortality and clinical observation

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

Individual bodys 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 overdose and necropsied with special attention to all vital organs and tissues.

Statistical analysis

Body weight values were presented by means ± S.D. Because no mortality was observed in the present study, statistical analysis for calculating the LD_{50} value was not performed.

RESULTS

Mortality

No animal in both sexes was found dead by the treatment of test article during the testing period (Table I). Therefore, it was estimated that the minimal lethal dose of the test article is considered to be over 5000 mg/kg in both sexes.

Clinical observation

During the study period, only a single female in the 2500 mg/kg group showed reddish tears from the eyes from days 1 to 3 after treatment. But this finding was not observed from day 4 after treatment (Table I).

Body weight

There were no notable changes which could be attributed to the treatment of test article (Table II).

Gross finding

At necropsy on day 14 after treatment, no treatment-related effects were found in any dose group tested (Table III).

DISCUSSION

The present study was conducted to investigate the potential acute toxicity of bamboo leaf water extract administered by gavage to Sprague-Dawley rats at dose levels of 0, 1250, 2500,

Table I. Mortality and clinical signs in rats treated with bamboo leaf water extract

Dose (mg/Kg)	Mortality		Clinical signs	
	Male	Female	Male	Female
0	0/5[a]	0/5	-[b]	-
1250	0/5	0/5	-	-
2500	0/5	0/5	-	Reddish tear[c]
5000	0/5	0/5	-	-

[a] Values are expressed as number of dead animals/total number of animals.
[b] No clinical signs were observed.
[c] A single female showed the sign from days 1 to 3 after administration.

Table II. Changes of body weights in rats treated with bamboo leaf water extract

Sex	Days after treatment	Dose (mg/Kg)			
		0	1250	2500	5000
Male	0	187.8 ± 16.3[a]	189.6 ± 16.2	187.6 ± 13.3	193.0 ± 13.2
	1	211.9 ± 20.1	215.8 ± 14.6	215.5 ± 16.8	218.6 ± 13.5
	3	219.8 ± 17.8	228.1 ± 12.6	213.4 ± 14.2	237.3 ± 16.4
	7	230.1 ± 19.5	238.2 ± 12.0	236.3 ± 11.9	252.4 ± 15.0
	14	287.4 ± 20.7	287.7 ± 12.4	266.2 ± 14.4	281.1 ± 21.3
	Weight gain	99.6 ± 6.3	98.2 ± 8.3	78.6 ± 4.5	88.1 ± 8.4
Female	0	145.3 ± 7.4	143.6 ± 6.5	143.0 ± 5.4	147.2 ± 8.3
	1	159.1 ± 9.0	161.4 ± 8.1	156.2 ± 6.2	164.0 ± 7.8
	3	163.8 ± 10.6	161.1 ± 10.1	156.3 ± 7.1	149.0 ± 8.0
	7	179.1 ± 10.8	189.7 ± 16.6	177.8 ± 14.2	169.9 ± 11.6
	14	194.8 ± 9.0	207.4 ± 23.3	212.3 ± 14.9	202.7 ± 12.2
	Weight gain	49.5 ± 5.9	63.8 ± 8.4	69.3 ± 9.7	55.5 ± 4.6

[a] Values are presented as means ± S.D.

Table III. Gross findings of necropsy in rats treated with bamboo leaf water extract

Dose (mg/Kg)	Male	Female
0	0/5[a]	0/5
1250	0/5	0/5
2500	0/5	0/5
5000	0/5	0/5

[a] Values are expressed as number of abnormal animals/total number of animals.

or 5000 mg/kg body weight. The results showed that a single oral dose of bamboo leaf water extract produced no adverse effect on mortality, clinical sign, body weight change and necropsy finding in rats.

Reddish tear observed in the female of the 2500 mg/kg group was not considered to be related to treatment of test article since this finding occurred in a very low incidence and did not exhibit a dose-response relationship. It is well known that this finding is common for normal Sprague-Dawley rats and can result from the diverse factors such as stress, disease, and fighting (Kim et al., 2002; Kim et al., 2003). There were no treatment-related effects on mortality, body weight change and necropsy finding in any treated group.

Based on the results, it was concluded that a single oral dose of bamboo leaf water extract did not induce any toxic effect in Sprague-Dawley rats at dose levels of 5000 mg/kg or below, and that the minimal lethal dose was considered to be over 5000 mg/kg body weight for both sexes. This test showed that bamboo leaf water extract is a safe natural substance that has no toxicity even in the maximum dose, and furthermore presented the feasibility as herb.

ACKNOWLEDGEMENTS

This study was supported by a grant from the 2002 Agricultural R&D Promotion Center, Korea.

REFERENCES

Hu, C., Zhang, Y. and Kitts, D. D. J. (2000). Evaluation of antioxidant and prooxidant activities of bamboo *Phyllostachys nigra* var. *Henonis* leaf extract in vitro. *Agric. Food Chem.*, **48**, 3170-3176.

KFDA (Korea Food and Drug Administration). (1998). Guidelines for Toxicity Studies of Drugs, Notification No. 1998-116, Seoul, Korea

Kim, J. C., Kang, B. H., Shin, C. C., Kim, Y. B., Lee, H. S., Kim, C. Y., Han, J., Kim, G. S., Chung, D. W. and Chung, M. K. (2002). Subchronic toxicity of plant sterol esters administered by gavage to Sprague-Dawley rats. *Food Chem. Toxicol.*, **40**, 1569-1580.

Kim, J. C., Shin, D. H., Kim, S. H., Ahn, T. H., Kang, S. S., Jang, B. S., Kim, C. Y. and Chung, M. K. (2003). Developmental toxicity evaluation of the new fluoroquinolone antibacterial DW-116 in rats. *Teratogen. Carcinogen. Mutagen.*, **23(Suppl. 1)**, 123-136.

Kim, N. K., Cho, S. H., Lee, S. D. (2001). Functional properties and antimicrobial activity of bammbo (*Phyllostachys spp.*) extracts. *Kor. J. Postharvest Sci. Technol.*, **8**, 475-480.

Kwon, M. H., Hwang, H. J., Sung, H. C. (2001). Identification and antioxidant activity of novel chlorogenic acid derivatives from bamboo (*Phyllostachys edulis*). *Agric. Food Chem.*, **49**, 4646-4655.

NRC (National Research Council). 1996. Guide for the Care and Use of Laboratory Animals. National Research Council. National Academy, Washington, USA.

OECD (Organisation for Economic Co-operation and Development). (1995). OECD Guidelines for Testing of Chemicals, Guideline 421: Reproduction/ Developmental Toxicity Screening Test, Paris, France.

Shibata, M., Yamatake, M., Sakamoto, M., Kanamori, K., Tak-

agi, K., Okabe, S. (1975). Pharmacological studies on bamboo grass. Nippon Yakurigaku Zasshi, **71**, 481-485.
Shin, D. H., Kim, J. C., Kim, S. R., Oh, H., Park, I. C., Oh, K. S., Chung, H. J., Jang, J. S., Kim, S.H. (2003). The effects of *Phyllostachys nigra var. henonis Strapf* leaf extract in the gamma-irradiated mice. *Kor. J. Vet. Res.*, **43**, 49-55.
Tsunoda, S., Yamamoto, K., Sakamoto, S., Inoue, H., Nagasawa, H. (1998). Effects of Sasa Health, extract of bamboo grass leaves, on spontaneous mammary tumourigenesis in SHN mice. *Anticancer Res.*, **18**, 153-158.