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Acute Oral Toxicity of Extract Derived from Fruiting Body of *Phellinus gilvus* in Rats

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ABSTRACT. This study was carried out to investigate the acute oral toxicity of a crude extract derived from fruiting body of *Phellinus gilvus* (PGE) using male and female SD rats. Groups consisted of five male and female rats were treated with a single dose of the test substance intragastrically at 0, 500, 1,000, 2,000, and 5,000 mg/kg, respectively. Clinical signs, body weight change, and food and water consumption change were observed for 14 days after administration. No mortality or abnormal clinical signs in animals were shown during the observation period at the dose used in this study. Also there was no difference in net body weight gain, water and food consumption or gross pathological findings at terminal sacrifice among the groups of rat treated with different doses of the test substance. The results suggested that acute oral toxicity of PGE in rats is very low at the conditions employed in this study and LD_{50} of PGE was estimated to be over 5,000 mg/ml in both sexes of rats.

Keywords: *Phellinus gilvus*, PGE, Acute oral toxicity, Rats, LD_{50}.

INTRODUCTION

The number of mushrooms on Earth is estimated at 140,000, yet maybe only 10% (approximately 14,000 named species) are known (Kirk *et al.*, 2001). For millennia, mushrooms have been valued by humankind as an edible and medical resource. *Phellinus* spp. is known approximately 220 species and is found mainly in tropical America and Africa (Dai *et al.*, 1998). In Korea, it is distributed into 7 species and commonly referred to as Sangwhang. *Phellinus* spp. is a fungus belonging to the Hymenochaetaceae basidiomycetes (Jung *et al.*, 1994). Many kinds of *Phellinus* spp. (e.g. *P. linteus*, *P. igniarius*, *P. pini*, and *P. hartigii*, etc.) are known and they have a variety of medicinal effects (Lee *et al.*, 1996; Rew *et al.*, 2000). Among them, *P. linteus* has been well known as one of the most popular medicinal mushrooms due to its high antitumor activity (Ikekawa *et al.*, 1968; Han *et al.*, 1999) and safety of acute oral toxicity test (Han *et al.*, 2000). *P. gilvus* also has notable biological activities such as antitumor activity, free radical scavenging activity, and proliferation activity of the human fibroblast cells. It, however, did not report about safety of acute oral toxicity test. Furthermore, it is cheaper than the other *Phellinus* spp. because of very short growth period to harvest fruiting body within 3 months unlike the other *Phellinus* spp.. Therefore, it has a possibility which can be developed as a functional food and livestock in future. Recently, *P. linteus* and *P. baumii* were approved for application as a functional food by Korea Food and Drug Administration (KFDA, 2003) but *P. gilvus* is not get an approval.

Therefore, we here report the safety of acute oral toxicity test conducted with crude extract derived from fruiting body of *P. gilvus* (PGE) using male and female SD rats.

MATERIAS AND METHODS

Animal Maintenances

Fifty male and female Sprague-Dawley rats, 4 weeks old, were obtained from Samtako Bio Korea. Rats were

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acclimated in controlled room (temperature: 23±3°C, relative humidity: 50±10%, air circulating frequency: 13~17 times/hr, artificial light: 300 Lux from 7 am to 7 pm, noise: <50 db) for 10 days before experimentation. Rats were housed in a polycarbonate cage (28 cm×42 cm× 18 cm). Free mix (Samtako Bio Korea) and sterilized water were provided ad libitum.

Test substance (PGE)

The fruiting body of *P. gilvus* was supplied by Gyeongbuk Agricultural Technology Administration (Daegu, Korea) and developed rapidly for 3 months in artificial oak sawdust cultures with rice bran (Oak-Sawdust+rice bran 10%) (Jo *et al.*, 2002). The fruiting body of *P. gilvus* was homogenized and extracted using distilled water (1:30) at 100°C for 7 hours. And it was concentrated at 80°C in a rotary evaporator and was freeze-dried. The powder was diluted in distilled water prior to use.

Experimental Designs

A total of 25 male and 25 female rats were randomly assigned to 10 groups. Thus, each group consisted of 5 rats. The highest dose of PGE that could be prepared for oral administration was 5,000 mg/kg. The medium high dose of PGE was 2,000 mg/kg. This preparation was used as the medium high dose followed by sequential dilution to 1,000 and 500 mg/ml, for the medium low dose and the low dose. A single dose of PGE was administered to rat intragastrically using a polyethylene cannula attached to a disposable syringe.

Clinical signs were observed for 12 hr following treatment of PGE on the day of administration and once everyday thereafter for 14 days. Body weight was measured immediately prior to dosing of PGE and everyday after the treatment. The change of food consumption and water was measured everyday after the treatment. Following the observation period all animals were anesthetized with 5 mg/kg of ketamine HCl intramuscularly (Ketamine®, Yuhan Co., Korea). Autopsy was conducted on every animal and all major organs and tissues including heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testicle were examined for gross lesions.

Body weight, Food, and water consumption changes were compared using ANOVA (Dunnett's test) by SAS program (version 8.1). Differences at $P<0.05$ were considered statistically significant.

RESULTS

Lethality and Clinical Signs

No death among the rats treated with PGE was observed during the observation period of 14 days (Table 1). The high of PGE (5,000 mg/kg) used in this study was the largest one that could be prepared for oral administration to rats. Therefore, the oral LD_{50} of PGE, although it could not be determined precisely, is greater than 5,000 mg/kg.

Rats were observed for any abnormal clinical signs for 12 hr following the treatment. No drug-related abnormal signs were noted in rats regardless of the dose used. Observation of clinical sign was conducted everyday for 14 days. All rats appeared to be entirely healthy and normal during the period (Table 2).

Body Weight Changes

The body weight of rats was monitored for 14 days after the treatment of animals with PGE (Table 3). The mean body weight was slightly increased on the following day in male rats treated with all PGE used in this study (500, 1,000, 2,000, and 5,000 mg/kg), but slightly decreased on the following day in female rats treated with the low dose (500 mg/kg), although the change in

Table 1. Mortality of male and female rats orally treated with PGE

Sex	Dose (mg/kg)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Male	0	*0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	500	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	1,000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	2,000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	5,000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
Female	0	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	500	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	1,000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	2,000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
	5,000	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5

*The number of dead animals/the number of animals per group.

Table 2. Abnormal clinical signs in rats orally treated with PGE

Sex	Dose (mg/kg)	Observations	Range
Male	0	Appears normal	Day 0~Day 14
	500	Appears normal	Day 0~Day 14
	1,000	Appears normal	Day 0~Day 14
	2,000	Appears normal	Day 0~Day 14
	5,000	Appears normal	Day 0~Day 14
Female	0	Appears normal	Day 0~Day 14
	500	Appears normal	Day 0~Day 14
	1,000	Appears normal	Day 0~Day 14
	2,000	Appears normal	Day 0~Day 14
	5,000	Appears normal	Day 0~Day 14

body weight was not statistically significant. The decrease in body weight was slowly recovered, and at terminal sacrifice there was no difference in body weight. Also no difference in net body weight gain among the different dose groups was noted in both sexes.

Food and Water Consumption Changes

Food and water consumption changes of rats were monitored for 14 days after the treatment of animals with PGE (Tables 4, 5). The mean food consumption was slightly decreased on the first day in both male and female rats treated with the high dose (5,000 mg/kg) and medium high dose (2,000 mg/kg), but no mean food consumption were shown in animals regardless of the dose level used in the study from on the second day after treatment, although the change in food consumption was not statistically significant. There was no water consumption change in both male and female

Table 3. Body weight of rats orally treated with PGE (Mean±SD, g)

Sex	Dose (mg/kg)	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Male	0	132.3 ±3.2	147.3 ±2.9	154.7 ±3.2	165.4 ±4.1	174.3 ±5.1	182.3 ±5.1	190.4 ±5.8	196.0 ±7.2	205.5 ±6.1	213.2 ±5.7	223.1 ±6.8	232.6 ±9.1	232.4 ±9.1	243.7 ±9.9	248.3 ±9.4
	500	134.4 ±3.0	149.7 ±3.5	159.6 ±3.7	170.2 ±3.8	180.2 ±2.8	189.1 ±4.6	196.9 ±4.5	202.8 ±6.5	212.2 ±6.1	218.4 ±6.8	228.3 ±8.6	238.3 ±9.4	241.8 ±9.8	250.6 ±9.9	260.6 ±9.8
	1,000	134.5 ±8.7	149.4 ±9.3	159.3 ±9.7	167.5 ±10.9	179.0 ±11.6	187.3 ±11.4	196.0 ±12.5	202.4 ±12.8	209.1 ±11.9	216.0 ±12.1	223.0 ±11.9	231.1 ±12.2	237.3 ±11.9	242.1 ±11.4	255.5 ±10.8
	2,000	134.0 ±6.7	147.8 ±3.4	157.6 ±4.3	167.2 ±4.3	177.2 ±5.4	185.2 ±5.2	193.3 ±5.4	201.5 ±6.6	208.8 ±8.1	216.3 ±10.5	224.1 ±9.5	231.5 ±10.0	236.8 ±9.7	243.4 ±12.0	248.6 ±10.6
	5,000	134.8 ±5.7	149.0 ±6.9	156.3 ±8.1	166.5 ±8.7	175.9 ±9.7	183.9 ±8.8	191.8 ±9.0	199.7 ±11.9	206.6 ±10.9	215.5 ±10.7	224.1 ±11.2	230.9 ±11.4	237.6 ±9.7	244.5 ±10.6	248.8 ±10.4
Female	0	114.6 ±4.2	125.8 ±5.0	131.8 ±5.6	136.6 ±5.2	144.4 ±3.4	156.9 ±7..6	151.2 ±6.1	154.2 ±7.2	157.7 ±8.3	161.2 ±8.6	165..4 ±8.1	169.6 ±6.6	172.8 ±7.5	177.6 ±7.1	179.5 ±7.5
	500	111.9 ±5.1	123.5 ±3.8	129.9 ±3.8	136.2 ±5.1	142.9 ±4.5	146.1 ±5.1	149.8 ±4.2	153.9 ±6.6	154.3 ±5.9	157.4 ±7.4	159.9 ±6.0	162.6 ±6.7	166.6 ±6.0	167.2 ±8.5	173.5 ±6.4
	1,000	111.0 ±4.4	123.0 ±2.5	131.5 ±3.1	137.0 ±3.4	143.0 ±6.3	147.8 ±5.2	153.8 ±5.3	153.9 ±8.9	158.6 ±8.7	162.6 ±8.9	166.2 ±8.6	172.2 ±9.9	173.9 ±10.0	178.9 ±12.1	180.5 ±11.2
	2,000	114.2 ±1.9	125.8 ±3.9	131.3 ±3.0	136.5 ±4.4	141.9 ±4.6	146.5 ±5.1	151.7 ±5.9	153.1 ±3.5	158.4 ±6.3	162.6 ±4.0	164.5 ±7.4	169.7 ±6.9	170.7 ±10.1	174.5 ±7.1	178.5 ±8.9
	5,000	111.8 ±3.6	125.0 ±4.0	131.0 ±5.0	135.1 ±6.2	142.9 ±6.4	148.1 ±7.1	151.8 ±8.0	154.4 ±8.7	158.1 ±8.5	163.7 ±7.8	166.5 ±10.4	169.3 ±11.7	169.8 ±11.2	174.9 ±10.0	178.9 ±12.5

Table 4. Food consumption of rats orally treated with PGE (300 g-g of residual in cage/the number of animals per groups)

Sex	Dose (mg/kg)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Male	0	20.2	16.3	20.3	16.0	19.7	18.9	19.2	20.3	19.3	21.7	18.9	21.6	20.3	22.2
	500	21.0	17.3	21.3	16.3	22.1	20.8	19.5	22.0	18.9	21.2	19.9	22.1	21.1	22.3
	1,000	20.5	17.9	20.2	17.2	20.3	19.3	20.2	19.5	18.3	19.8	18.5	20.4	18.5	20.7
	2,000	19.8	16.9	20.5	17.1	20.2	19.7	20.9	19.9	19.5	21.6	19.0	21.3	21.0	21.3
	5,000	15.5	16.9	20.2	14.7	20.8	19.5	19.8	20.7	19.8	22.2	18.4	22.8	21.0	21.2
Female	0	17.4	13.2	15.2	12.1	16.4	15.2	14.4	15.2	14.8	15.5	13.5	14.1	16.3	16.2
	500	16.9	13.7	16.7	13.0	15.4	14.7	14.0	13.6	12.7	13.8	11.6	15.0	13.3	14.5
	1,000	15.4	14.7	16.6	13.3	17.0	15.6	14.1	14.0	14.6	14.7	13.4	14.9	15.7	16.4
	2,000	16.3	13.5	16.0	12.9	15.8	16.3	14.6	14.9	14.2	14.4	13.9	15.7	15.7	16.0
	5,000	17.2	14.6	16.9	13.6	17.1	15.7	15.3	15.4	14.6	15.9	12.1	15.3	14.0	15.0

Table 5. Water consumption of rats orally treated with PGE (480 ml-ml of residual in cage/the number of animals per groups)

Sex	Dose (mg/kg)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14
Male	0	32.0	22.0	30.0	25.0	27.0	27.0	24.0	27.0	30.0	32.0	27.0	27.0	28.0	28.0
	500	38.0	24.0	36.0	27.0	31.0	29.0	28.0	31.0	31.0	39.0	32.0	34.0	34.0	32.0
	1,000	31.0	24.0	28.0	25.0	27.0	27.0	26.0	25.0	27.0	31.0	27.0	28.0	28.0	28.0
	2,000	34.0	26.0	31.0	27.0	33.0	26.0	30.0	29.0	27.0	35.0	29.0	28.0	27.0	28.0
	5,000	30.0	26.3	31.3	27.5	25.0	25.0	35.0	25.0	28.8	31.3	22.5	30.0	26.3	27.5
Female	0	30.0	23.0	26.0	24.0	26.0	24.0	23.0	25.0	29.0	32.0	24.0	25.0	28.0	27.8
	500	30.0	24.0	29.0	25.0	27.0	27.0	26.0	25.0	27.0	31.0	27.0	28.0	28.0	28.2
	1,000	28.0	24.0	26.0	21.0	24.0	22.0	18.0	19.0	23.0	26.0	20.0	22.0	23.0	24.0
	2,000	26.0	22.0	27.0	23.0	25.0	29.0	22.0	26.0	28.0	28.0	26.0	28.0	27.0	28.0
	5,000	30.0	22.0	26.0	24.0	24.0	21.0	21.0	20.0	22.0	25.0	20.0	20.0	21.0	24.0

Table 6. Gross pathological findings of rats orally treated with PGE

Sex	Dose (mg/kg)	Pathological findings	Frequency
Male	0	No gross findings	0/5*
	500	No gross findings	0/5
	1,000	No gross findings	0/5
	2,000	No gross findings	0/5
	5,000	No gross findings	0/5
Female	0	No gross findings	0/5
	500	No gross findings	0/5
	1,000	No gross findings	0/5
	2,000	No gross findings	0/5
	5,000	No gross findings	0/5

*Pathological finding incidences/the number of animals per group.

rats regardless of the dose of PGE used.

Gross Pathological Findings

At the end of the observation period all rats were sacrificed and autopsied. All major organs including heart, lung, liver, stomach, intestine, kidney, adrenal gland, spleen, and ovary or testis were examined grossly. There were no abnormal lesions in both male and female rats regardless of the dose of PGE used (Table 6).

DISCUSSION

Acute single oral toxicity of a crude extract derived from fruiting body of *P. gilvus* was examined using male and female SD rats in the present study. This PGE was administered to rats at a dose of 0, 500, 1,000, 2,000, and 5,000 mg/kg intragastrically. As the results, no mortality was observed in rats treated with PGE at a dose of 5,000 mg/kg, the largest one which could be prepared for oral administration. Therefore, the LD_{50} of PGE, although it could not be determined precisely, is greater than 5,000 mg/kg. During the observation period of 14 days, no abnormal clinical signs were shown in animals regardless of the dose level used in the study. The body weight of male rats treated with all PGE used in this study (500, 1,000, 2,000, and 5,000 mg/kg) appeared to increase transiently following administration of PGE and female rats treated with the low dose (500 mg/kg) appeared to decrease transiently, too, although the difference was statically insignificant. The decrease in body weight was slowly recovered and there was no difference in body weight at terminal sacrifice. And, there was not a difference in the final body weight or in net body weight gain for the observation period among the groups of 0, 1,000, 2,000, and 5,000 mg/kg in female rats. But, the increasing rate of mean body weight was slightly decreased on the following day treated with extract of *P. linteus* during the observation period of 14 days (Han *et al.*, 2001). Thus, we may think that *P. gilvus* used in this study may have higher safety than *P. linteus*. in acute oral toxicity.

The mean food consumption of male rats treated with the high dose (5,000 mg/kg) and medium high dose (2,000 mg/kg) appeared to decrease transiently on the first day after administration of PGE, although the difference was statically insignificant. But, the mean food consumption was constantly decreased on the following day treated with extract of *P. linteus* at high dose groups compared with control group (Han *et al.*, 2001). Thus, we also think that *P. gilvus* used in this study has a little higher safety than *P. linteus* in acute oral toxicity. There was no water consumption change in both male and female rats regardless of the dose of PGE used. At autopsy all the major organs were examined for gross lesions. But the test substances did not induce any abnormal changes at the doses used in this study.

In summary, the crude extract derived from fruiting body of *P. gilvus* did not induce any death, changes in body weight, abnormal clinical signs, or changes in food and water consumption when given at a dose as large

as 5,000 mg/ml. From the observed results in the study, we can suggest that acute single oral toxicity of PGE is very low under the conditions employed in this study.

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