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Original Article

Acute toxicity investigation of polysaccharide extracts of *Lentinus polychrous* in rats

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Abstract

Lentinus polychrous (LP) is an edible mushroom that has been increasingly used as a food or a nutritional supplement. The present study investigated acute toxicity of LP in rats. Male and female rats (n = 10/group) were administered a single-dose of 50, 500, and 2,500 mg/kg of polysaccharide extract from either LP fruiting body (PSF) or LP mycelium (PSM). Two control groups, untreated and vehicle (distilled water) administered were included. Body weight, food and water intake, general signs, and mortality were observed for 14 days. Blood samples and organ weights were evaluated at day 14. All treatments caused no effects on food and water consumption, body weight, general signs, gross pathology of organs, organ weight, hematological parameters, or changes in biochemical parameters. We conclude that the NOAEL (no observed-adverse-effect level) dose is greater than 2,500 mg/kg.

Keywords: *Lentinus polychrous*, mushroom, mycelia, fruiting body, acute toxicity

1. Introduction

Lentinus polychrous (LP) is an edible mushroom commonly consumed in the North and Northeast of Thailand. The chemical constituents of LP include proteins, carbohydrates, polysaccharides, reducing sugars, and phenols (Thetsrimuang *et al.*, 2011a). The species has been traditionally used in the treatment of dyspepsia and fever caused by snake or scorpion envenomation. This mushroom has a number of pharmacological effects including antioxidant (Thetsrimuang *et al.*, 2011a; 2011b; Armassa *et al.*, 2009.), anti-inflammatory (Fangkrathok *et al.*, 2013), anti-estrogenic (Fangkrathok *et al.*, 2014.), and cytotoxic effects on cancer cell lines MCF-7, SK-Hep1, and A549 (Thetsrimuang *et al.*, 2011a). Pharmacological studies of LP suggest that it is a good candidate for commercial use as a nutritional supplement, functional food or herbal medicine, and the mushroom has been shown to have several health benefits. However, safe consumption of the mushrooms extract needs support from controlled studies. Some mushrooms have no toxicity, such as *Lignosus rhinoceros* or tiger milk mushrooms (Lee *et al.*, 2011; 2013), *Phylloporia ribis* mycelia (Lu *et al.*, 2014), and *Agaricus blazei* (Kuroiwa *et al.*, 2005). However, some mushrooms such as *Agaricus bisporus* (common button mushroom), *Lentinus edodes* (shitake), and *Pleurotus ostreatus* (oyster mushroom) reportedly have hepatotoxic effects in mice (Nieminen *et al.*, 2009). To our knowledge, there are no safety data available for LP. Therefore, in the

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present study we examined the oral acute toxicity of LP polysaccharide extracts from fruiting bodies (PSF) and mycelia (PSM).

2. Materials and Methods

2.1 Preparation of PSF and PSM

PSF and PSM were prepared as previously described (Fangkrathok *et al.*, 2012). Briefly, the fresh LP fruiting bodies bought from the local market in Khon Kaen province, Thailand were cultivated on potato dextrose agar for 5 days and then the mycelia were transferred to sterile Job's Tears seeds. After 18 days cultivation, the mycelia were dried and milled. The dried mycelial and fruiting body powders were extracted by hot water for 6 h. The debris was separated and then the supernatant was added into 95% ethanol to precipitate the polysaccharides. These polysaccharides were separated and then resuspended in distilled water before freeze drying. The yields of PSF and PSM were 1.90% and 44.04%, respectively.

2.2 Animals

Male and female Wistar rats (6-8 weeks old and 150-250 g of body weight) were supplied by the National Laboratory Animal Center, Mahidol University, Thailand. The animals were housed three rats per cage. Animal rooms were maintained at a temperature of 22±2°C and 12-h light/dark cycle. The rats were fed with standard rat food and water *ad libitum*. The experimental protocol was approved by the Animal Ethics Committee of Khon Kaen University, Khon Kaen, Thailand (Approval Number, AE.KKU. 43/2553). The animals were acclimated for 7 days prior to the experiments. All animals were fasted for 12 h with free access to water before dosing.

2.3 Study design

Both male and female rats were used for each oral treatment (n = 10) as follows: groups 1-3 received 50, 500, and 2,500 mg/kg BW of PSF, respectively; groups 4-6 received 50, 500, and 2,500 mg/kg BW of PSM, respectively; group 7 received distilled water as a vehicle, and group 8 was left untreated as the control. PSF and PSM were dissolved in distilled water. All animals were monitored for 14 days after treatment. At the end of day 14, blood samples were withdrawn though cardiac puncture for further analysis.

2.4 Observation and examination methods

2.4.1 General signs

Mortality, general signs, and physical observation of the animals for symptoms such as diarrhea, lethargy, depression, breathing difficulty, equilibrium, convulsions, shuffling, skin and fur problems, salivation, tremors, or coma were noted once daily throughout the entire 14 days of the experimental period. Moreover, the appearance of urine and feces of the rats were observed daily.

2.4.2 Body weight gain and food and water intake

Body weight of each animal was measured daily for 14 days using an electronic balance. Food and water consumption were measured daily for 14 days by weighing the amount of food and water left uneaten using an electronic balance.

2.4.3 Blood analysis

Blood samples obtained from cardiac puncture were analyzed for the usual hematological and biochemical parameters. The hematological parameters included hematocrit (Hct), hemoglobin concentration (Hb), red blood cell count (RBC), white blood cell count (WBC), neutrophil, lymphocyte, monocyte, eosinophil, basophil, and platelets counts, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). The parameters were measured using a Photometric analyzer (Hitachi Automatic Analyzer 912, Japan). Biochemical parameters, measured by Photometric analyzer (Hitachi Automatic Analyzer 912, Japan), were aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), blood urea nitrogen (BUN), creatinine, total bilirubin, direct bilirubin, indirect bilirubin, glucose, triglyceride, cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), sodium (Na^+), potassium (K^+), chloride (Cl^-), and carbon dioxide (CO_2).

2.4.4 Pathological examination

At the end of day 14, the animals were sacrificed. The internal organs of all animals including the brain, heart, liver, kidney, lung, stomach, intestine, testis, ovaries, uterus, and spleen were observed for gross pathological changes (position, size, and color) and weighed.

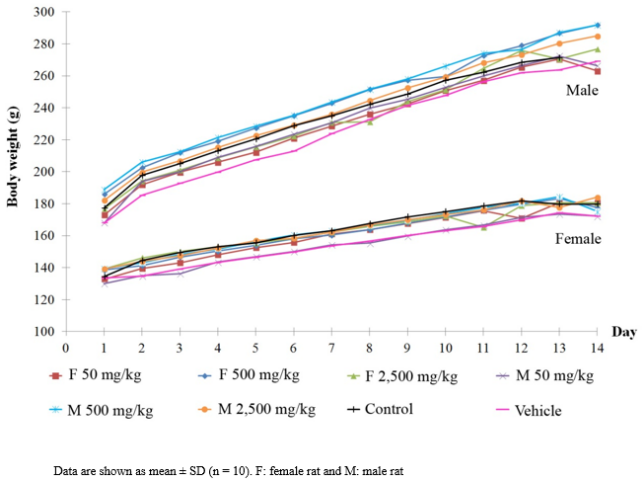
2.5 Statistical analysis

All data were presented as mean±SD. The differences between tested groups and control group were evaluated by independent t-tests using SPSS program version 16.0. Significance was considered at values of $P<0.05$.

3. Results and Discussion

3.1 General signs, body weight, and food consumption

After a single-dose administration of 50, 500, and 2,500 mg/kg of PSF and PSM, all rats survived throughout the observation period of 14 days. There were no abnormalities in clinical signs such as diarrhea, lethargy, depression,



Data are shown as mean ± SD (n = 10). F: female rat and M: male rat

Figure 1. Mean body weight of male and female rats after receiving PSF and PSM at various doses

rolling of the eyes, tears, breathing difficulty, equilibrium sensation, excitement, convulsions, shuffling, dermatitis, bristling of fur, excess salivation, tremors, or coma in any group. The appearance of urine and feces of treated rats, including color and odor, was no different when compared to the control groups.

As shown in Figure 1, the body weight gain of male and female rats after a single-dose administration of 50, 500, and 2,500 mg/kg of PSF and PSM were not significantly different from the control groups (untreated and vehicle groups). Body weights of all rats were increased during the 14 days, from 168.00±16.87 to 291.80±12.12 g and 130.00±8.35 to 184.00±10.58 g for male and female rats, respectively. Male rats in all treatments gained more body weight than those of female rats throughout the 14 days period. There were no treatment-related changes in organ weights when compared to control groups (untreated and vehicle groups). These results indicated that the treatments of PSF and PSM had no effect on the growth of rats.

Food consumption and water intake of both male and female rats are shown in Table 1. Rats receiving PSF and PSM ingested 95.00±14.76 to 98.49±18.19 and 95.81±18.05 to 106.08±48.51 g/kg BW/day of food, and 188.19±34.73 to 208.50±41.53 and 189.98±21.91 to 199.89±32.83 g/kg BW/day of water for male and female rats, respectively. There were no differences of food consumption or water intake between control groups (untreated and vehicle groups) and treated groups in both sexes.

3.2 Blood analysis

3.2.1 Hematological examinations

Hematological parameters of male and female rats after administration of a single-dose of PSF and PSM are shown in Tables 2 and 3, respectively. Generally, there were no significant differences in the levels of hematological parameters between male rats in all treated groups and control groups, except for lymphocyte level. The lymphocyte level in male rats receiving 2,500 mg/kg of PSM (86.16±2.88%) was significantly higher than those of control groups (78.48±8.13 and 79.35±9.05% for untreated and vehicle groups, respectively). However, this value of lymphocyte count was within the normal range (75.8-92.9%) (Car *et al.*, 2006.). Moreover, the other parameters that would indicate infection such as WBC, neutrophil, monocyte, eosinophil, and basophil levels as well as clinical signs were unchanged. Therefore, the high lymphocyte level of this group has no clinical relevance. For female rats, all hematological parameters of treated groups were not significantly different from control groups.

3.2.2 Blood biochemical parameters

Glucose, creatinine, cholesterol, ALT, Na, K, Cl, and CO_2 levels of male treated groups were not significantly different from controls (Table 4). Other parameters with

Table 1. Food consumption and water intake of male and female rats after treatment with PSF and PSM at various doses.

Groups	Doses (mg/kg)	Food consumption (g/kg BW/day)		Water intake (g/kg BW/day)	
		Male	Female	Male	Female
Untreated		93.55±16.86	98.50±17.92	196.65±26.94	194.85±27.11
Vehicle (distilled water)		100.06±19.3	103.04±30.91	192.07±24.61	199.52±27.02
PSF	50	97.14±17.33	98.68±19.01	208.50±41.53	199.43±26.34
	500	97.52±24.48	101.94±18.41	199.97±20.77	199.89±32.83
	2,500	95.00±14.76	106.08±48.51	192.59±28.13	192.19±61.58
PSM	50	98.49±18.19	100.71±19.36	188.19±34.73	190.27±23.77
	500	94.80±16.35	95.81±18.05	190.44±40.71	190.39±28.83
	2,500	97.87±14.97	97.04±17.08	189.37±41.89	189.98±21.91

Data are shown as mean ± SD (n = 10).

Table 2. Hematological parameters of male rats after treatment with PSF and PSM at various doses.

Parameters	Untreated	Vehicle (distilled water)	PSF			PSM		
			50 mg/kg	500 mg/kg	2,500 mg/kg	50 mg/kg	500 mg/kg	2,500 mg/kg
RBC (10^6/μL)	6.89±1.20	7.04±0.81	7.36±0.74	7.45±0.37	7.58±0.34	7.54±0.57	7.47±0.33	7.20±0.65
WBC (10^3/μ/L)	3.99±1.31	3.31±1.27	3.34±0.90	4.16±0.69	3.87±0.87	3.84±1.07	4.59±1.60	3.47±0.44
Hb (g/dL)	13.07±2.37	13.95±1.59	14.32±1.35	14.51±0.72	14.73±0.59	14.71±0.78	14.53±0.69	13.78±1.12
Hct (%)	42.63±7.95	44.69±5.76	45.80±5.43	45.74±2.07	45.80±1.97	44.83±2.96	46.23±2.09	42.12±4.31
Plt. Count (10^3/μL)	389.00±197.60	398.78±160.08	416.78±189.70	413.10±93.42	356.88±128.31	295.63±145.74	400.10±113.29	323.43±169.53
MCV (fL)	63.57±4.05	63.43±3.29	62.14±2.22	61.42±1.48	60.48±2.68	59.50±1.69	61.94±1.98	58.52±2.32
MCH (pg)	19.49±0.45	19.81±0.39	19.49±0.47	19.50±0.32	19.44±0.61	19.53±0.68	19.47±0.63	19.15±0.45
MCHC (g/dL)	30.75±1.69	31.31±1.48	31.36±1.00	31.72±0.65	32.17±0.75	32.85±0.90	31.43±1.08	32.78±0.94
Neutrophil (%)	14.58±6.22	12.53±5.27	13.91±5.18	14.38±2.40	14.48±4.55	14.36±8.97	14.28±5.42	12.04±3.00
Lymphocyte (%)	78.48±8.13	79.35±9.05	79.74±8.55	78.58±3.61	80.32±4.58	79.22±11.65	79.89±7.13	86.16±2.88*
Monocyte (%)	5.60±3.23	5.37±2.38	4.39±3.57	5.75±1.53	4.14±2.20	4.26±1.65	4.97±2.67	3.69±1.22
Eosinophil (%)	0.83±0.67	1.19±1.11	0.89±1.04	1.29±1.09	0.90±0.71	1.03±0.97	0.86±0.69	0.62±0.51
Basophil (%)	0.05±0.14	0.03±0.08	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Data are shown as mean ± SD (n = 10). * Significantly different from control groups (untreated and vehicle groups) at $P<0.05$.

Table 3. Hematological parameters of female rats after treatment with PSF and PSM at various doses.

Parameters	Untreated	Vehicle (distilled water)	PSF			PSM		
			50 mg/kg	500 mg/kg	2,500 mg/kg	50 mg/kg	500 mg/kg	2,500 mg/kg
RBC (10^6/μL)	7.16±0.40	7.34±0.44	7.43±0.22	7.56±0.77	7.33±0.56	7.07±0.62	7.65±0.98	7.16±0.38
WBC (10^3/μ/L)	2.02± 0.38	2.31±0.69	2.51±0.58	2.46±0.60	2.27±0.66	2.39±0.33	2.56±0.80	2.65±0.70
Hb (g/dL)	14.16±0.67	14.35±0.84	14.74±0.45	14.96±1.37	14.34±0.80	14.16±1.17	14.95±1.57	14.20±0.51
Hct (%)	46.30±3.84	45.55±4.06	43.82±1.56	46.41±4.60	44.97±2.80	43.46±1.14	45.45±5.72	44.77±2.14
Plt. Count (10^3/μL)	436.14±130.44	553.80±176.88	534.10±115.89	447.90±205.00	438.89±159.29	481.20±184.40	440.10±205.39	438.30±75.13
MCV (fL)	62.65±2.33	62.01±2.85	59.02±1.68	61.44±159.29	61.43±3.18	58.87±0.92	59.46±1.52	62.64±3.98
MCH (pg)	19.80±0.60	19.55±0.30	19.86±0.51	19.82±0.43	19.60±0.47	20.05±0.33	19.61±0.63	19.85±0.60
MCHC (g/dL)	30.70±1.53	31.61±1.54	33.64±0.41	32.27±0.89	31.96±1.51	34.57±0.82	33.00±1.00	31.77±1.47
Neutrophil (%)	11.77±3.81	8.99±1.97	10.98±2.48	9.82±2.66	11.82±4.35	8.92±3.66	11.58±4.69	8.52±3.72
Lymphocyte (%)	82.27±3.63	84.78±2.67	84.40±2.79	84.69±4.66	81.69±6.71	85.25±5.08	82.66±5.47	85.09±4.40
Monocyte (%)	5.00±2.00	5.37±1.55	3.87±1.28	4.72±2.96	5.42±2.32	4.36±2.13	4.48±1.52	4.93±1.95
Eosinophil (%)	0.96±0.54	0.86±0.30	0.75±0.27	0.64±0.47	1.07±0.84	0.96±0.49	1.05±0.82	1.00±0.46
Basophil (%)	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Data are shown as mean ± SD (n = 10). * Significantly different from control groups (untreated and vehicle groups) at $P<0.05$.

significant differences included, BUN levels of male rats treated with 50 and 2,500 mg/kg of PSF (21.71±3.64 and 17.19 ±2.89 mg/dl, respectively) and 500 and 2,500 mg/kg of PSM (20.08±2.27 and 19.88±3.33 mg/dl, respectively), which were significantly lower than those of control groups (26.49±2.96 and 25.87±2.66 mg/dl for untreated and vehicle groups, respectively). For lipid profiles of male rats, HDL values of the lowest dose of PSF and PSM (19.00±2.45 and 19.00±2.94 mg/dl, respectively) were significantly lower than those of control groups (22.63±1.41 and 23.50±3.06 mg/dl for untreated and vehicle groups, respectively). In contrast, LDL values of these groups (26.90±6.12 and 27.60±5.85 mg/dl, respectively) were significantly higher than those of control groups (17.63 ±9.77 and 24.30±13.09 mg/dl for untreated and vehicle groups, respectively). Moreover, triglyceride values of these groups (79.10±19.08 and 75.90±26.61 mg/dl, respectively) were markedly lower than those of control groups (115.25± 35.80 and 106.80±24.55 mg/dl for untreated and vehicle groups, respectively). Nevertheless, the decrease of HDL and triglyceride and the increase of LDL values were not dose-dependent. These phenomena indicated that these polysaccharide extracts might affect lipid metabolism; this needs further study. In addition, there were no detectable gross pathological changes in abdomen and other internal organs. Therefore, the effect of LP on lipid metabolism should be further investigated.

For biochemical parameters of liver function, only the AST levels of male rats receiving the highest dose of PSF (330.75±123.03 U/L) and PSM (304.56±70.19 U/L) were markedly higher than that of control groups (177.00±86.09 and 178.17±60.49 U/L for untreated and vehicle groups, respectively). Although the AST levels in treated and control rats were higher than the previous reported values in Sprague Dawrey rats (77-157 U/L, Car *et al.*, 2006), it is unlikely that the levels indicate hepatotoxicity of PSF and PSM in this study. In contrast, ALP values of male rats treated with 50 and 2,500 mg/kg of PSM (190.80±31.44 and 180.30±27.68 U/L, respectively) were significantly lower than those of

Table 4. Blood biochemical parameters of male rats after treatment with PSF and PSM at various doses.

Parameters	Untreated	Vehicle (distilled water)	PSF			PSM		
			50 mg/kg	500 mg/kg	2,500 mg/kg	50 mg/kg	500 mg/kg	2,500 mg/kg
Glucose (mg/dl)	161.63±37.20	168.70±59.28	151.60±55.40	163.50±40.04	164.50±41.37	143.90±48.47	182.10±35.84	169.60±42.52
BUN (mg/dl)	26.49±2.96	25.87±2.66	21.71±3.64*	23.69±1.60	17.19±2.89*	24.93±6.60	20.08±2.27*	19.88±3.33*
Creatinine (mg/dl)	0.43±0.10	0.47±0.07	0.48±0.08	0.42±0.04	0.46±0.05	0.49±0.06	0.40±0.05	0.47±0.05
Cholesterol (mg/dl)	63.13±4.52	69.30±6.90	61.60±7.68	64.50±8.61	67.90±6.62	61.60±8.64	63.00±11.01	69.20±7.21
Triglyceride (mg/dl)	115.25±35.80	106.80±34.55	79.10±19.08*	122.00±40.93	97.30±31.65	75.90±26.61*	128.80±40.37	116.70±39.46
HDL (mg/dl)	22.63±1.41	23.50±3.06	19.00±2.45*	22.50±3.75	24.30±2.91	19.00±2.94*	21.70±2.67	22.20±2.10
LDL (mg/dl)	17.63±17.63	24.30±13.09	26.90±6.12*	17.70±7.60	24.00±9.94	27.60±5.85*	15.50±6.42	23.60±10.89
AST (U/L)	177.00±86.09	178.17±60.49	182.80±60.57	168.30±61.14	330.75± 123.03*	187.60±30.06	160.60±35.45	304.56 ±70.19*
ALT (U/L)	40.25±15.28	49.80±12.19	47.00±9.64	43.70±7.39	37.30±6.55	46.00±13.50	38.00±6.63	48.80±19.66
ALP (U/L)	229.38±44.56	238.00±40.22	207.30±40.21	238.60±35.71	233.60±41.51	190.80±31.44*	212.20±24.15	180.30±27.68*
Total bilirubin (mg/dl)	4.41±1.04	3.68±1.06	3.83±1.39	2.76±1.25*	3.43±0.77	3.52±1.29	3.25±1.06*	5.00±1.44
Direct bilirubin (mg/dl)	0.35±0.16	0.32±0.15	0.35±0.12	0.19±0.10	0.19±0.06	0.36±0.18	0.20±0.09	0.67±0.33*
Indirect bilirubin (mg/dl)	4.06±1.01	3.36±1.03	3.48±1.34	2.57±1.18*	3.24±0.80	3.16±1.18	3.05±1.00	4.33±1.17
Na (mEq/L)	148.13±2.17	145.50±2.20	148.56±2.07	146.80±2.15	147.30±1.57	146.70±4.64	147.78±2.11	144.10±3.03
K (mEq/L)	5.63±0.33	6.23±1.75	6.41±0.77	5.10±0.70	4.95±0.82	6.34±1.49	5.59±0.73	6.68±0.77
Cl (mEq/L)	106.75±2.60	106.50±2.45	107.44±3.00	106.10±2.18	105.50±1.72	107.90±2.33	105.89±2.20	105.00±1.33
CO_2 (mEq/L)	13.51±1.51	12.25±3.03	12.77±0.78	14.00±2.24	14.29±2.16	12.27±1.68	14.31±2.19	15.66±2.33

Data are shown as mean ± SD (n = 10). * Significantly different from control groups (untreated and vehicle groups) at $P<0.05$.

Table 5. Blood biochemical parameters of female rats after treatment with PSF and PSM at various doses.

Parameters	Untreated	Vehicle (distilled water)	PSF			PSM		
			50 mg/kg	500 mg/kg	2,500 mg/kg	50 mg/kg	500 mg/kg	2,500 mg/kg
Glucose (mg/dl)	146.14±29.85	117.70±17.63	151.90±29.80	123.40±32.48	110.50±15.09	169.40±44.38	139.70±19.43	156.30±33.51
BUN (mg/dl)	26.66±6.30	26.18±4.44	26.76±3.48	22.62±3.24	24.67±3.27	24.04±6.54	25.09 8.89±	22.17±3.87
Creatinine (mg/dl)	0.49±0.11	0.46±0.07	0.45±0.05	0.48±0.09	0.43±0.07	0.45±0.07	0.51±0.06	0.49±0.22
Cholesterol (mg/dl)	72.86±11.38	69.00±10.98	70.60±8.77	78.10±9.68	69.60±11.75	78.10±14.75	72.90±7.77	79.70±12.58
Triglyceride (mg/dl)	70.86±27.39	65.40±26.88	57.70±25.13	60.40±14.82	75.40±26.38	66.50±32.89	56.10±25.91	56.90±12.83
HDL (mg/dl)	47.71±5.96	42.20±5.18	46.50±6.06	50.10±5.07	38.30±10.01*	46.50±3.21	44.30±7.12	47.60±5.83
LDL (mg/dl)	16.5±2.89	17.22±8.77	12.50±5.80	15.90±7.74	16.16±10.30	20.33±9.11	17.40±5.66	22.98±10.65
AST (U/L)	447.14±162.12	434.00±143.40	482.80±114.69	427.00±161.63	407.75±71.27	445.57±91.60	414.88±156.74	337.80±133.71
ALT (U/L)	89.71±24.78	85.10±33.62	82.70±15.83	78.00±18.61	66.20±17.18	77.80±18.27	85.00±39.74	64.00±20.31*
ALP (U/L)	177.29±74.90	165.20±57.28	165.30±19.56	129.90±29.62*	126.10±42.74*	164.80±41.21	135.50±42.90	153.80±28.69
Total bilirubin (mg/dl)	3.99±1.11	3.82±0.89	3.46±0.44	4.13±1.07	4.33±0.71	4.07±1.11	4.62±0.93	3.87±0.84
Direct bilirubin (mg/dl)	0.46±0.33	0.67±0.18	0.46±0.10	0.58±0.37	0.71±0.15	0.50±0.23	0.63±0.27	0.49±0.19
Indirect bilirubin (mg/dl)	3.53±0.94	3.15±0.76	3.00±0.48	3.55±0.85	3.62±0.72	3.57±1.08	3.84±0.63	3.38±0.77
Na (mEq/L)	143.71±2.36	144.10±2.28	142.90±1.52	142.00±2.31	142.67±1.41	143.60±2.07	141.38±2.77	143.00±1.66
K (mEq/L)	5.87±1.24	6.64±0.96	6.44±0.71	6.31±1.07	6.19±0.94	6.81±1.29	6.90±1.25	5.93±0.90
Cl (mEq/L)	113.14±3.13	108.50±2.46	110.00±2.21	109.00 1.83	108.44 3.61	112.20±4.26	112.38±5.90	111.33±6.82
CO_2 (mEq/L)	11.91±1.41	12.20±1.68	11.90±1.77	13.93±2.80	13.72±2.08	11.27±2.68	13.03±2.88	11.74±1.92

Data are shown as mean±SD (n = 10). * Significantly different from control groups (untreated and vehicle groups) at $P<0.05$.

control groups (229.38±44.56 and 238.00±40.22 U/L for untreated and vehicle groups, respectively). The increased AST values without increment of ALP and ALT levels and liver weights indicate the low potential to cause hepatotoxic effect of these extracts. Direct bilirubin values of rats treated with 2,500 mg/kg of PSM (0.67±0.33 mg/dl) were higher than those of control groups (0.35±0.16 and 0.32±0.15 mg/dl for untreated and vehicle groups, respectively, $P<0.05$). The increase of direct bilirubin may suggest that the liver cannot clear bilirubin properly and the bile duct may be blocked (Walker *et al.*, 1990.). Moreover, total bilirubin and indirect bilirubin levels at this dose (5.00±1.44 and 4.33±1.17 mg/dl, respectively) were not significantly different when compared to untreated rats (4.41±1.04 and 4.06±1.01 mg/dl, respectively) and vehicle groups (3.68±1.06 and 3.36±1.03 mg/dl, respectively, $P<0.05$). Therefore, the increase of direct bilirubin of rats treated with 2,500 mg/kg of PSM may not have been due to the hepatotoxicity of LP mycelium and

Table 6. Organ weights of male rats given PSF and PSM at various doses

Organ weights (g/kg BW)	Untreated rats	Vehicle (distilled water)	PSF			PSM		
			50 mg/kg	500 mg/kg	2,500 mg/kg	50 mg/kg	500 mg/kg	2,500 mg/kg
Brain	7.07±0.61	7.51±0.65	7.27±0.31	7.21±0.83	7.40±0.47	7.10±0.33	6.96±0.27	6.97±0.32
Heart	4.10±0.46	4.06±0.25	3.98±0.74	4.02±0.39	4.27±0.29	4.28±0.61	3.94±0.28	4.23±0.37
Liver	49.24±10.08	46.82± 8.02	37.57±2.46*	49.46±3.56	39.94±2.66*	37.94±2.06*	44.34±3.17*	38.98±2.22*
Kidneys	8.50±1.16	8.97±0.76	7.98±0.34	8.84±0.93	8.21±0.78	7.67±0.90	8.28±0.43	7.77±0.50
Lung	7.16±0.82	7.31±0.80	6.49±0.84	8.41±2.99	6.75±0.84	7.19±2.62	7.02±0.93	6.79±1.11
Stomach	8.44±3.32	7.02±0.78	6.58±1.00	6.53±0.66	6.26±0.61	6.44±0.89	6.29±0.67	6.29±0.37
Intestine	29.48±3.07	30.02±2.43	28.07±1.30	30.20±1.83	28.24±1.27	29.91±1.99	29.45±0.95	29.14±1.56
Testis	14.68±1.48	14.07± 1.45	14.28±1.57	13.63±0.81	13.96±1.28	13.61±1.11	13.03±0.86	13.87±1.10
Spleen	3.36±0.50	3.29±0.46	3.41±0.31	3.05±0.26	3.02±0.36	3.05±0.31	3.36±0.36	3.28±0.46

Data are shown as mean ± SD (n = 10). * Significantly different from control groups (untreated and vehicle groups) at $P<0.05$.

Table 7. Organ weights of female rats given PSF and PSM at various doses

Organ weights (g/kg BW)	Untreated rats	Vehicle (distilled water)	PSF			PSM		
			50 mg/kg	500 mg/kg	2,500 mg/kg	50 mg/kg	500 mg/kg	2,500 mg/kg
Brain	10.38±0.56	10.80±0.52	10.45±0.81	10.63±0.62	10.51±0.70	10.48±0.41	10.54±1.19	10.16±0.43
Heart	4.72±0.66	5.47±1.74	5.05±0.55	4.96±0.64	4.55±0.57	4.83±0.52	5.15± 0.63	4.76±0.78
Liver	48.10±5.19	46.97±8.35	50.73±2.66	43.99±4.11	45.74±4.14	41.42±4.66	41.31±4.29	49.09±5.38
Kidneys	10.92±1.34	11.47±1.56	11.32±0.54	10.83±0.99	10.20±1.01	11.07±0.94	10.59±0.85	10.42±0.88
Lung	9.26±1.74	9.38±1.69	10.63±2.01	10.43±2.65	12.65±2.95*	8.21± 1.56	10.01±3.62	13.31±3.19*
Stomach	8.83±2.33	8.37±1.23	8.35±0.90	8.92±1.19	7.75±0.94	8.04±0.90	9.07±2.00	8.48±1.43
Intestine	38.42±3.60	38.02±3.17	39.51±1.22	36.80±2.33	38.00± 3.64	36.76±3.80	35.95±3.03	36.90±1.75
Ovaries and uterus	4.36±0.67	5.25±0.73	5.64±1.80	4.70±0.59	4.74±1.46	4.44±1.26	5.16±1.42	3.02±0.70
Spleen	3.62±0.39	3.60±0.24	3.69±0.38	3.58±0.41	3.70±0.53	3.71±0.45	3.44±0.63	3.84±0.39

Data are shown as mean ± SD (n = 10). * Significantly different from control groups (untreated and vehicle groups) at $P<0.05$.

needs further study.

In the case of female rats, no significant changes of biochemical parameters were observed in all treatment groups compared to the control groups (untreated and vehicle groups) as shown in Table 5. There were some parameters lower than those of the control groups ($P<0.05$). For example, HDL value of female rats received 2,500 mg/kg of PSF (38.30±10.01 mg/dl) was significantly lower than those of the control groups (47.71±5.96 and 42.20±5.18 mg/dl for untreated and vehicle groups, respectively). The effects of PSF and PSM on lipid profiles in female and male rats were different. PSF and PSM at the lowest dose significantly affected lipid profiles of male rats while these effects were not shown in female rats. Saito *et al.* (2014) also reported that gender affected lipid profiles of rat. Sex hormones may be involved in lipid metabolism of rats that received PSF and PSM. Further studies of this phenomenon are needed. The ALT value of female rats treated with 2,500 mg/kg of PSM (64.00±20.31) was significantly lower than the control groups (89.71±24.78 and 85.10±33.62 U/L for untreated and vehicle groups, respectively). Moreover, ALP values of female rats treated with 500 and 2,500 mg/kg of PSF (129.90±29.62 and 126.10±42.74 U/L, respectively) were significantly lower than those of the control groups (177.29±74.90 and 165.20±57.28 U/L for untreated and vehicle groups, respectively).

3.3 Relative organ weight and gross pathology

Organ weights of male and female rats treated with a single-dose of PSF and PSM at various doses are listed in Tables 6 and 7, respectively. In general, there were no significant differences in any organ weight or appearance in either sex, except for the liver weight of male rats receiving 50 and 2,500 mg/kg of PSF (37.57± 2.46 and 39.94±2.66 g/kg BW, respectively) and 50, 500, and 2,500 mg/kg of PSM (37.94± 2.06, 44.34±3.17, and 38.98±2.22 g/kg BW, respectively), which were markedly lower than those of untreated and vehicle groups (49.24±10.08 and 46.82±8.02 g/kg BW, res-

pectively) at $P<0.05$. The lower liver weight of male rats was also the information to support the low potential of hepatotoxicity by LP as mentioned that PSF and PSM caused the increase of AST levels. Moreover, lung weights of female rats given the highest dose of both PSF and PSM (12.65± 2.95 and 13.31±3.19 g/kg BW, respectively) were slightly higher than those of control groups (9.26±1.74 and 9.38± 1.69 g/kg BW for untreated and vehicle groups, respectively).

4. Conclusions

Neither PSF nor PSM at any doses (50, 500, and 2,500 mg/kg) resulted in acute toxicity, as shown by the survival, body weight, general clinical signs, gross anatomy and organ weight, hematological parameters as well as clinical biochemical parameters throughout the experimental period of 14 days. Since there was no toxicity at the highest doses of PSF and PSM, their NOAEL (no observed-adverse-effect level) doses are thus higher than 2,500 mg/kg.

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