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Single dose toxicity studies of sulfated water soluble β-D-glucan in Sprague-Dawley rats

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Abstract

The fungal β-D-glucan is a biological response modifier (BRM), but a major obstacle to the clinical utilization of these BRMs is their relative insolubility in aqueous media. We made soluble sulfated-β-glucan (SGL) from insoluble β-glucan (IGL) by sulfation method. In single dose toxicity study of SGL for 7 days, no negative effects on body weight or food consumption of rats were evident below a dose rate of 2,000 mg kg^{-1} SGL. No clinical pathology, functional/behavioral, or gross observations indicating toxicity were detected. In hematology and biochemistry, statistically significant increases of WBC and neutrophils ($P<0.01$) in male and increase of MCV ($P<0.05$) in females was observed. However, since the changes were not dose–responsive, the effects were considered to be of no toxicological significance. These results suggest that chemically modified sulfated-β-D-glucan was less toxic than the insoluble b-glucan and not considered acutely toxic following peritoneal exposure to 2,000mg kg^{-1} day^{-1} in Sprague-Dawley rats.

Key words

Sulfated-β-D-glucan, *Ganoderma lucidum*, Single dose toxicity

Introduction

Ganoderma lucidum (Fr.) Karst, a popular medicinal mushroom, has been used in traditional medicines in many Asian countries (Wasser, 2002). The chemical constituents of *G. lucidum* include polysaccharides, proteins, nucleosides, fatty acids, sterols, cerebrosides and triterpens (Yeung *et al*., 2004). *Ganoderma* polysaccharides belong to the class of drugs known as biological response modifiers (BRMs) (Shao *et al*., 2004). Special attention has been paid to these fungal polysaccharides as a functional food and a source for the development of biomedical drugs. Polysaccharides represent a structurally diverse class of biological macromolecules with a wide range of physico-chemical properties. The major bioactive *Ganoderma* polysaccharides are β-(1→3) and β-(1→6)-D-glucan (Paterson, 2006; Jianguo and Lina, 2009), which have been shown to slow the growth of sarcoma cells in mice (Cao and Lin, 2004). Moreover, some modified derivatives of (1→3)-a-D-glucan stimulated lymphocyte proliferation and antibody production, and the introduction of carboxymethyl groups at low degrees of substitution could improve the immunostimulating activities (Bao *et al*., 2001). Therefore, modified polysaccharides have attracted more attention, with particular focus on the sulfated derivatives because of their obvious bioactivities (Wang *et al*., 2008). A major obstacle to the clinical utilization of β-glucan BRMs is their relative insolubility in aqueous media. Specifically, (1→3)-β-D-glucan exists as an insoluble microparticulate upon initial isolation from *G. lucidum*. While topical administration of insoluble microparticulate (1→3)-β-D-glucan induces no toxicity, intravenous administration of the microparticulate form is associated with hepatosplenomegaly, granuloma formation, microembolization, and enhanced endotoxin sensitivity (Kuroiwa *et al*., 2005; Sumiya *et al*., 2008). Williams *et al*. (1991, 1992) demonstrated that (1→3)-β-glucan from the yeast *Saccharomyces cerevisiae* is water-soluble and immunologically active. Other studies have shown that several partially synthesized sulfated polysaccharides, including dextran sulfate, are highly inhibitory to the *in vitro* replication of human immunodeficiency virus (HIV) (Yoshida *et al*., 1990). Although several investigations have

reported the optimal culture conditions and medicinal properties of *G. lucidum*, preparation of soluble β-glucan from this mushroom has not been studied in detail for its utilization as BRM. One goal of this study is to develop a water-soluble polysaccharide by the introduction of a sulfated group to enhance the immunomodulatory activity of mushroom polysaccharide. The water-soluble derivatives prepared from the water-insoluble polysaccharide through sulfation and carboxylmethylation reactions have shown relatively higher antitumor and antiviral activities (Nie *et al.*, 2006; Shin *et al.*, 2007; Qiu *et al.*, 2007). The chemical structures of the sulfated β-glucan derivatives from *G. lucidum* have also been identified (Han *et al.*, 2008). In this study, we evaluated the possibility of utilizing sulfated β-glucan as biological response modifier (BRM) and it's single dose toxicity studies in Sprague-Dawley rat.

Materials and Methods

β-glucan preparation: *Ganoderma lucidum* IY009 obtained from Il-Yang Pharmaceultical. Co. Ltd. (Korea). Insoluble β-glucan (IGL) and sulfated β-glucan (SGL) from the cultured mycelia of *G. lucidum* were prepared by previously described procedures (Han *et al*., 2008). In brief, IGL was prepared sequentially by using the alkali extraction and ethyl alcohol precipitation method from the cultured mycelia. It was dialyzed with a regenerated cellulose tube (MW cut-off 10,000, Sigma, USA) against distilled water 5 days and then finally dried with a lyophilizer (Il-Shin Inc, Korea). SGL was prepared by modification of the method of Williams *et al.* (1992). IGL was dissolved in Me_2SO contained urea and H_2SO_4. The glucan sulfate solution was purified with an ultrafiltration system (Sartorius. Co. SM 17521) using a 10,000 molecular weight cut-off filters and then lyophilized to dryness (Il-Shin Inc, Korea).

Single dose toxicity study

Experimental design: The animal studies were carried out following the animal ethics guidelines of SCH IACUC (Soonchunhyang Institutional Animal Care and Use Committee). Sprague-Dawley (Bkl: SD) rats were obtained from Dae Han Biolink Co., Ltd. (Korea) at 5 weeks of age (♂ =180-190; ♀ =150-160 g). Animals were acclimatized for 1 week. Before being housed (five per cage) under standard laboratory conditions (temperature: 23 ± 2°C; relative humidity: 50-60%, light-dark cycle: 12/12 h) and received a standard sterilized diet and tap water *ad libitum*. Sterilized litter bedding was changed 3 times a week. Allocation of rats (35♂ + 35♀) to control and treatment groups, their initial weights (♂=188-198; ♀=154-163 g), and SGL administration rationale for single dose (20, 200 and 2,000 mg kg^{-1}) are given in Table 1. Control animals received equal volumes (20 ml kg^{-1}) of water via the same administration route. Animals of both groups were then observed daily for a period of 7 days for clinical observations, body weight and food intake. After 7 days, blood samples were collected for hematological and biochemical examination, and then the animals were sacrificed for pathological examination. All studies were accomplished using good laboratory practices (GLP) for toxicity test guidance on drugs (Notification No. 2000-63, December 11, 2000) issued by the Korea Food & Drug administration (KFDA). The handles and cares of all animals were conformed to National Institutes of Health Guild for the Care and Use of Laboratory Animals (NIH, 1996).

Clinical examinations: Animals were observed twice daily for mortality and a general clinical examination was performed once a day. Detailed clinical observations were made in all animals prior to the first exposure to the test substance and once a week thereafter. All signs of toxicity or morbidity were recorded. The amount of feed and water consumed by mean of each group was recorded daily. Feed intake was calculated as g $group^{-1}$ day^{-1}. The water intake was calculated as ml $group^{-1}$ day^{-1}.

Functional and behavioral investigations: Immediately after SGL injection, the rat was placed in a transparent cage and its behavior was recorded for 30 min after injection. The first 5 min after the injection was considered a conditioning period and behavior was not analyzed. The remaining 25 min were analyzed by two investigators who were blinded to the experimental conditions. Near the end of the administration period, a number of behavioral tests were conducted. To assess neuromuscular function, beam walking and bar grip tests were performed; to determine emotionality, ambulatory activity, and inquisitiveness, the open field test was used; and to assess visual function, the placing response test was performed.

Hematological examinations: Standard hematological tests for erythrocyte count (RBC) (10^{12} l^{-1}), hemoglobin (Hb) concentration (g d l^{-1}), hematocrit (Hct) index (%), mean corpuscular volume (MCV) (fL) and leukocyte count (WBC) (10^9 l^{-1}) were conducted using the automated hematology analyzer (Optifarm Solution Center Co., Korea).

Biochemical examination of blood: Clinical biochemical analysis of blood was conducted before dosing commencement, at the end of the administration period, and at the end of the recovery period in all treated animals. The following parameters: albumin, cholesterol, creatinine, gamma-glutamyl transferase (GGT), urea, L-glutamine 2-oxoglutarate aminotrasferase (ALT), L-asparate, 2-oxoglutarate, aminotransferase (AST), glucose, bilirubin, total protein (TP) and triglyceride (TG) were measured using automated biochemistry analyzers (Hitachi 7180, Hitachi Co., Tokyo, Japan).

Pathology examinations

Gross pathology: Prior to post-mortem examination, all animals were weighed and thereafter sacrificed via ethyl ether inhalation. All the animals were subjected to full necropsy procedures, including examination of the following organs and/or sites: External surface of body; all orifices; cranial cavity; cervical tissues and organs; thoracic cavity and its contents, organs, and tissues; and the abdominal cavity and its contents, organs and tissues.

Organ weights: To evaluate of the organ toxicity by SGL, organ wet weight of liver, kidney, spleen and heart were measured. Immediately after dissection, the harvested organs were trimmed of any adherent tissues and weighed in closed Petri dishes in order to avoid drying effects. Organ weights were expressed as absolute values (g).

Statistical procedures : Standard deviation of means were calculated for body weights, food consumption, organ weights, hematological and biochemical parameters. Analysis of variance (ANOVA) was used for statistical evaluation of the data. When a significant dose-effect was found, further statistical analysis using Dunnett's multiple comparisons were used for a detailed assessment of data obtained and for an assessment of their mutual relationship.

Results and Discussion

Mean body weights of males and females were measured daily. As seen in Fig. 1, 2, body weight decreased more than in control during the first 2 days in the intraperitoneal injection group (2,000 mg kg^{-1} b. wt.) but then increased again. However, no significant differences in animal weights were observed between any of the intravenous injection treatment groups and the control groups. As shown in Fig. 3, 4, no significant difference in mean weekly food consumption was observed between any of the groups, nor any correlation was observed between food consumption and the dose of SGL. Sumiya *et al*. (2008) reported no differences in body weight change and food consumption compared with the control group when rats were intraperitoneally injected with 2,000 mg $kg^{-1}day^{-1}$ glucan obtained from Himematsutake extract for 3 months. Similarly, Kuroiwa *et al*. (2005) showed that rats treated with water extract of *Agaricus blazei* at a sub-acute level, *i.e.*, intraperitoneal injection of 2,965 mg kg^{-1} day^{-1}, were similar to the control group. These reports paralleled our results, which showed non-toxic effects in treatment groups (2,000 mg kg^{-1} day^{-1}) receiving the glucan extracted from *G. lucidum* and its chemically sulfated -β-glucan.

In a pre-clinical safety evaluation by Williams *et al*. (1988), the authors investigated the effects of a soluble β-glucan preparation isolated from *S. cerevisiae*. The authors reported that acute single administration of the soluble β-glucan preparation at intravenous doses of up to 1,000 mg kg^{-1} b. wt. in male. ICR/HSD mice had no effect on mortality, and no effect on Harlan Sprague–Dawley rats at a dose of 500 mg kg^{-1} b.wt. Differential species-specific effects were observed during repeat intraperitoneal dosing, where soluble β-glucan administration (250 mg kg^{-1} b.wt. day^{-1}) over 7 days resulted in no effect on weight gain in male ICR/HSD mice, yet produced a significant 10% decrease in weight gain in Sprague–Dawley rats and guinea pigs (Williams *et al*., 1988). In the present study, individual clinical examinations in male and female groups indicate that SGL was well tolerated throughout the treatment period, with no mortality or signs of morbidity observed. The only clinical change noted was moderate diarrhea and transient weight loss during days 1~2 in one

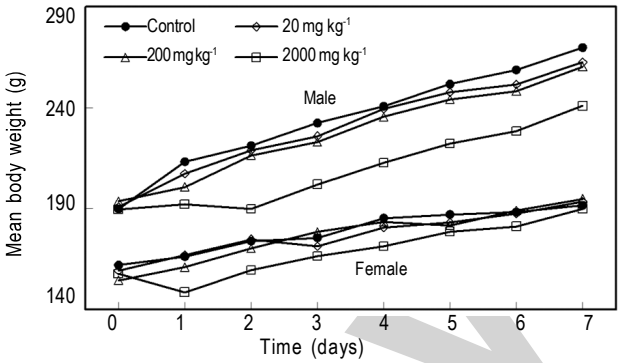

Fig. 1 : Changes in body weights of male and female rats given single doses of sulfated-β-glucan by intraperitoneal injection. The data represent the mean of 5 animals in each group (P<0.01)

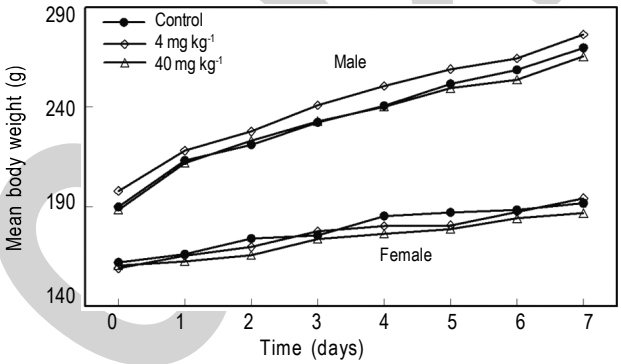

Fig. 2 : Changes in body weights of male and female rats given single doses of sulfated-β-glucan by intravenous injection. The data represent the mean of 5 animals in each group (P<0.01)

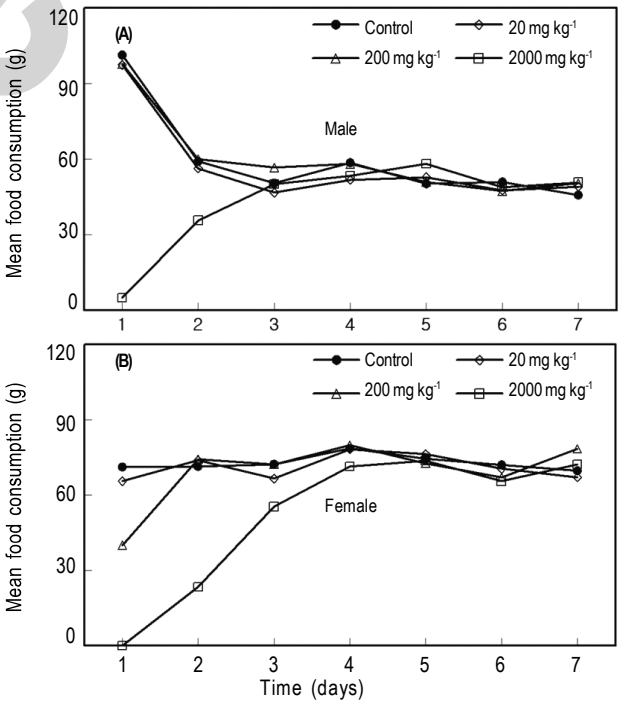

Fig. 3: Food consumption of male and female rats given single doses of sulfated-β-glucan by intraperitoneal injection. The data represent the mean of each group (P<0.01)

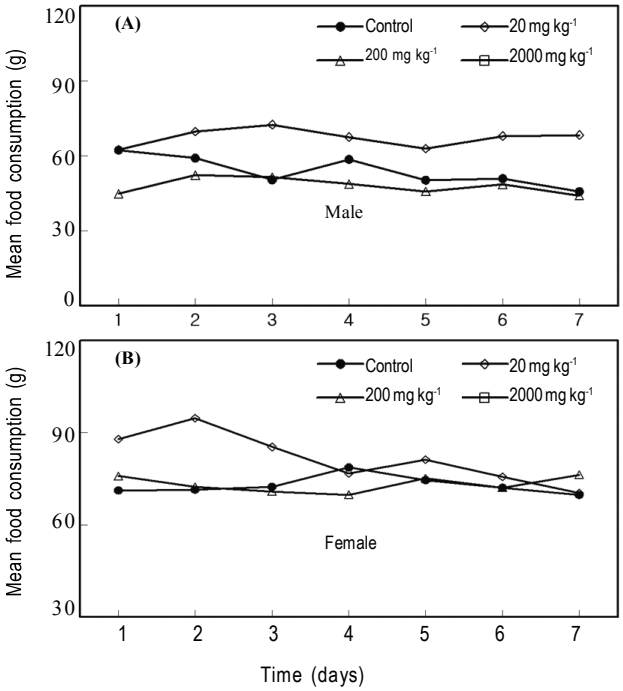

Fig. 4: Food consumption of male and female rats given single doses of sulfated-β-glucan by intravenous injection. The data represent the mean of each group (P<0.01)

male receiving high-dose (2,000 mg kg^{-1} b.wt.) of SGL. No evidence of diarrhea was observed in any other treated males, nor was diarrhea observed in any treated females; thus, the effect in this single high-dose male was appeared to be of no toxicological significance. There was no evidence of any compound-related effects in the ophthalmic examinations. A number of functional and behavioral tests were performed near the end of the treatment period. No evidence of neuromuscular impairment was observed in control and SGL. In addition, no differences between treated rats and controls in emotionality, ambulatory activity, or inquisitiveness were observed in the placing response and open field behavior tests.

Hematology and clinical chemistry data are presented in Tables 2-5. Although sporadic, statistically significant increases in WBC and neutrophils (P<0.01) occurred in males at the highest dose (2,000 mg kg^{-1} b.wt.), and did not exceed the maximums of physiological and historical control data ranges. According to Sumiya *et al.* (2008), female mice treated with the glucan (1,000 mg kg^{-1} day^{-1}) extracted from *Himematsutake* exhibited increases in the number of lymphocytes and decreases in leukocytes after two-week exposure (1,000-2,000 mg kg^{-1} day^{-1}). Since the decline was within the historical control, the effect was considered to be of no clinical significance. A statistically significant increase in MCV (P<0.05) was also observed in females, although the changes in RBC, Hct, and blood clotting time

Table- 1: Experimental design of a single-dose intraperitoneal and intravenous injection of sulfated β-glucan in rats

Route	Group	No. of animal	Initial weight (g)	Dose		
				Total dosage (ml kg^{-1})	No. of dose	Injection volume (ml kg^{-1})
Intraperitoneal	Control					
	Male		190			
	Female	5	156	-	1	20
	Group 1					
	Male		191			
	Female	5	159	20	1	20
	Group 2					
	Male		194			
	Female	5	155	200	1	20
	Group 3					
	Male		190			
	Female	5	158	2000	1	20
Intravenous	Control					
	Male		190			
	Female	5	156	-	1	20
	Group 1					
	Male		188			
	Female	5	161	4	1	20
	Group 2					
	Male		198			
	Female	5	163	40	1	20

Table- 2 : Hematological parameters observed in the single dose toxicity by intraperitoneal injection of sulfated β-D-glucan in rats

Analysis at day 7	Dose (mg kg^{-1} b.wt. day^{-1})			
	Control (0)	Low-dose (20)	Mid-dose (200)	High-dose (2000)
Males				
RBC (m µl^{-1})	6.05±0.45	5.86±0.33	5.69±0.56	5.86±0.84
MCV (fl)	54.97±2.03	56.23±2.05	54.43±2.32	54.53±1.72
Hct (%)	33.27±2.66	33.13±1.39	30.90±1.65	31.87±4.01
WBC (k µl^{-1})	4.07±1.85	6.80±1.55	5.71±1.58	8.59±0.92**
Hb (g µl^{-1})	13.83±1.21	13.77±0.64	12.43±1.21	12.57±0.55
Neutrophil (%)	16.89±2.13	19.89±3.37	18.31±4.56	20.35±7.57
Eo (%)	0.92±0.46	1.03±0.53	0.87±0.60	0.88±0.74
Ba (%)	0.31±0.20	0.38±0.10	0.24±0.27	0.47±0.49
Ly (%)	77.30±2.23	73.39±3.23	77.87±6.07	72.96±4.76
Mo (%)	4.58±0.76	5.31±0.30**	2.77±1.07	5.34±3.56
Females				
RBC (m µl^{-1})	5.07±1.20	4.53±2.17	5.81±0.59	4.39±0.70
MCV (fl)	54.73±1.82	52.63±1.92	53.47±1.21	51.83±2.25**
Hct (%)	27.87±7.09	23.63±10.98	31.1±3.72	23.40±2.19
WBC (k µl^{-1})	4.59±0.91	4.21±1.89	3.13±1.08	2.91±0.92
Hb (g/µl)	11.47±1.64	9.77±4.28	13.27±5.75	10.0±1.75
Neutrophil (%)	14.53±2.48	8.48±3.0	14.89±6.04	23.37±3.46
Eo (%)	2.10±0.73	0.48±0.29	2.21±1.36	1.09±1.05
Ba (%)	0.93±0.3	0.23±0.28*	1.25±0.58	0.7±0.7
Ly (%)	79.53±3.13	88.59±2.49	79.01±8.57	72.68±2.76
Mo (%)	2.91±1.6	2.23±0.6	2.64±0.67	2.15±1.5

Red blood cell (RBC), Mean corpusucular volume (MCV), Hematocrit (Hct), White blood cell (WBC), Eosinophil (Eo), Basophil (Ba), Lymphocyte (Ly), Monocyte (Mo). Statistical significance relative to control indicated as * P<0.05 and ** P<0.01 by Dunnett's t-test

Table- 3 : Hematological parameters observed in the single dose toxicity by intravenous injection of sulfated β-D-glucan in rats

Analysis at day 7	Dose (mg kg^{-1} b. wt. day^{-1})		
	Control (0)	Low-dose (4)	High-dose (40)
Males			
RBC (m µl^{-1})	6.05±0.45	6.12±0.21	5.75±0.45
MCV (fl)	54.97±2.03	55.70±2.61	56.73±3.78**
Hct (%)	33.27±2.66	34.10±1.67	32.50±0.78
WBC (k µl^{-1})	4.07±1.85	10.34±2.65**	6.03±1.33
Hb (g µl^{-1})	13.83±1.21	14.15±0.88	15.33±2.92
Neutrophil (%)	16.89±2.13	17.28±2.57	14.92±2.57
Eo (%)	0.92±0.46	0.95±0.19	1.35±0.19
Ba (%)	0.31±0.20	0.31±0.10	0.49±0.10
Ly (%)	77.30±2.23	78.03±1.60	78.42±1.60
Mo (%)	4.58±0.76	3.44±1.52	4.81±1.52
Females			
RBC (m µl^{-1})	5.07±1.20	6.15±0.22	6.02±0.31
MCV (fl)	54.73±1.82	53.65±1.59	54.13±2.20
Hct (%)	27.87±7.09	33.03±0.96	32.60±2.69
WBC (k µl^{-1})	4.59±0.91	5.82±2.34	3.07±0.65**
Hb (g µl^{-1})	11.47±1.64	13.38±0.40	13.13±1.03
Neutrophil (%)	14.53±2.48	12.57±4.38	13.40±1.21
Eo (%)	2.10±0.73	1.50±0.85	0.41±0.28
Ba (%)	0.93±0.3	0.56±0.31	0.76±0.41
Ly (%)	79.53±3.13	82.91±4.07	82.17±2.41
Mo (%)	2.91±1.6	2.65±0.32	3.26±1.03

Red blood cell (RBC), Mean corpusucular volume (MCV), Hematocrit (Hct), White blood cell (WBC), Eosinophil (Eo), Basophil (Ba), Lymphocyte (Ly), Monocyte (Mo). Statistical significance relative to control indicated as ** P<0.01 by Dunnett's t-test

Table- 4 : Serum chemistry profiles obtained in the single dose toxicity by intraperitoneal injection of sulfated β-D-glucan in rats

Analysis at day 7	Dose (mg kg^{-1} b.wt. day^{-1})			
	Control (0)	Low-dose (20)	Mid-dose (200)	High-dose (2000)
Males				
Creatinine (mg dl^{-1})	0.4±0.2	0.4±0.1	0.3±0.1	0.3±0.1
Urea (mg dl^{-1})	16.0±2.0	16.0±1.0	15.0±1.0	14.7±1.2
ALT (u l^{-1})	100.7±4.2	79.0±12.8	78.7±3.5	99.3±10.3
AST (u l^{-1})	528.7±1.9	501.0±75.2	338.7±86.0	545.5±23.3
Glucose (mmol l^{-1})	20.7±8.6	20.0±16.5	23.0±11.4	28.5±0.7
TP (g L^{-1})	7.6±0.5	7.3±1.5	7.0±0.8	7.8±0.6
Bilirubin (mg dl^{-1})	2.2±1.4	2.2±2.7	1.8±1.5	2.2±1.7
GGT (u l^{-1})	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Albumin (g dl^{-1})	4.5±0.4	4.1±0.8	4.0±0.4	4.3±0.5*
Cholesterol (mg dl^{-1})	96.3±3.8	93.3±7.4	89.3±5.8	97.7±7.5*
TG (u l^{-1})	98.3±5.0	74.0±20.0	71.3±3.5*	84.7±14.6
Females				
Creatinine (mg dl^{-1})	0.2±0.1	0.3±0.1	0.2±0.1	0.2±0.1
Urea (mg dl^{-1})	21.0±4.4	17.7±1.5	19.7±2.1	11.0±3.2
ALT (u l^{-1})	62.3±8.5	71.0±15.9	71.0±11.0	75.0±3.0
AST (u l^{-1})	279.7±64.0	229.0±119.4	203.7±93.7	203.7±137.7*
Glucose (mmol l^{-1})	31.7±13.1	16.0±13.5	22.7±20.1	20.7±10.7
TP (g/l)	8.6±1.2	7.3±0.1	7.6±0.6	7.7±0.5
Bilirubin (mg dl^{-1})	4.0±3.0	1.1±0.3	2.1±1.6	1.9±0.9
GGT (u l^{-1})	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Albumin (g dl^{-1})	5.1±0.8	4.4±0.3	4.6±0.2	4.1±0.3*
Cholesterol (mg dl^{-1})	104.7±4.7	95.3±3.8	104.0±8.9	93.0±6.2
TG (u l^{-1})	52.7±14.2	49.3±17.0	44.7±17.6	37.3±2.1

L-alanine: 2-oxoglutarate aminotransferase (ALT), L-aspartate: 2-oxoglutarate aminotransferase (AST), c-glutamyl transpeptidase (GGT), Triglycerides (TG), Total protein (TP). Statistical significance relative to control indicated as * P<0.05 by Dunnett's t-test

Table- 5 : Serum chemistry profiles obtained in the single dose toxicity by intravenous injection of sulfated β-D-glucan in rats

Analysis at day 7	Dose (mg kg^{-1} b.wt. day^{-1})		
	Control (0)	Low-dose (4)	High-dose (40)
Males			
Creatinine (mg dl^{-1})	0.4±0.2	0.3±0.1	0.3±0.0
Urea (mg dl^{-1})	16.0±2.0	14.3±0.6	13.0±0.0
ALT (u l^{-1})	100.7±4.2	76.9±2.9	73.0±9.0
AST (u l^{-1})	528.7±1.9	341.0±50.1	388.0±15.0
Glucose (mmol l^{-1})	20.7±8.6	10.3±2.1	9.0±2.0
TP (g l^{-1})	7.6±0.5	7.0±0.2	7.0±0.2
Bilirubin (mg dl^{-1})	2.2±1.4	0.5±0.2*	1.2±0.5
GGT (u l^{-1})	0.0±0.0	0.0±0.0	0.0±0.0
Albumin (g dl^{-1})	4.5±0.4	3.9±0.2	4.2±0.4
Cholesterol (mg dl^{-1})	96.3±3.8	104.0±11.5	110.0±10.0*
TG (u l^{-1})	98.3±5.0	42.3±13.1	57.5±13.5
Females			
Creatinine (mg dl^{-1})	0.2±0.1	0.3±0.06	0.2±0.1
Urea (mg dl^{-1})	21.0±4.4	13.0±1.73	18.0±1.7
ALT (u l^{-1})	62.3±8.5	81.0±7.0	70.3±11.5
AST (u l^{-1})	279.7±64.0	368.3±69.5	295.0±83.9
Glucose (mmol l^{-1})	31.7±13.1	11.7±9.07	15.3±4.7
TP (g l^{-1})	8.6±1.2	7.8±0.1	7.8±0.3
Bilirubin (mg dl^{-1})	4.0±3.0	1.8±0.5	1.8±0.5
GGT (u l^{-1})	0.0±0.0	0.0±0.0	0.0±0.0
Albumin (g dl^{-1})	5.1±0.8	4.6±0.45	4.8±0.5
Cholesterol (mg dl^{-1})	104.7±4.7	100.3±3.79	107.0±10.4
TG (u l^{-1})	52.7±14.2	48.3±10.5	34.7±12.5

L-alanine: 2-oxoglutarate aminotransferase (ALT), L-aspartate: 2-oxoglutarate aminotransferase (AST), c-glutamyl transpeptidase (GGT), Triglycerides (TG), Total protein (TP). Statistical significance relative to control indicated as * P<0.05 by Dunnett's t-test

Table- 6 : Summary of organ weight obtained in single dose intraperitoneal and intravenous injection of sulfated β-D-glucan in rat

Route	Sex	Dose (mg kg^{-1} b. wt. day^{-1})	Final body weight (g)	Organ (g)				
				Heart	Liver	Spleen	Kidney	
							Left	Right
Intrapritoneal		0	269.76±7.72	1.24±0.11	12.48±0.89	0.77±0.05	1.35±0.04	1.37±0.13
	Male	20	262.49±5.67	1.13±0.14	11.92±0.39	0.77±0.21	1.31±0.04	1.33±0.09
		200	260.21±4.78	1.28±0.29	12.54±0.49	0.95±0.17*	1.28±0.07	1.28±0.08
		2000	240.92±12.05	1.07±0.08	11.73±1.15	0.76±0.04	1.11±0.06*	1.09±0.10
		0	191.63±2.70	1.04±0.11	8.59±0.16	0.57±0.07	1.02±0.09	1.04±0.11
	Female	20	193.43±6.55	1.13±0.01	9.39±0.43	0.56±0.10	1.08±0.08	0.96±0.02
		200	194.84±3.62	2.33±0.2	8.85±0.53	0.61±0.04	1.02±0.07	0.82±0.22
		2000	189.85±7.90	1.08±0.06	11.34±1.72	0.72±0.24	0.99±0.06	0.95±0.02
Intravenous		0	269.76±7.72	1.24±0.11	12.48±0.89	0.77±0.05	1.35±0.04	1.37±0.13
	Male	4	276.48±21.40	1.13±0.06	12.24±1.10	0.81±0.05	1.27±0.17	1.26±0.10
		40	265.37±7.53	1.08±0.13	11.99±0.68	0.79±0.08	1.36±0.06	1.26±0.03
		0	191.63±2.70	1.04±0.11	8.59±0.16	0.57 ±0.07	1.02±0.09	1.04±0.11
	Female	4	197.39±7.15	1.07±0.03	9.11±0.21*	0.61 ±0.17	1.02±0.04	0.93±0.03
		40	186.73±9.83	1.06±0.07	8.17±0.68	0.54 ±0.02	0.94±0.04	0.90±0.07

Statistical significance relative to control indicated as * $P<0.05$ by Dunnett's *t*-test

seen in males were not observed in the female test groups. Similar to the hematological evaluation, isolated statistically significant changes were observed bilirubin, glucose, cholesterol, LDL-cholesterol, urea, and total protein; however, these changes were within historical control range, were not dose–responsive, and did not occur in both sexes and the effects were considered to be of no toxicological significance. According to Kuroiwa *et al.* (2005), biological examination of mice treated with hot extract of *Agaricus blazei* at 5% dose rate revealed an increase in MCV, but it was statistically insignificant. Furthermore, Delaney *et al.* (2003) reported that in 5% glucan-treated female and male rats, concentration of hemoglobin gradually decreased for 14 days and thereafter recovered to control levels. In the hematological studies of male rats treated with 2,000 mg kg^{-1} of SGL, there was no significant toxicity effects observed.

No pathological symptoms were present in rats subjected to SGL treatment based on necropsy after the recovery period. Overall, gross necropsy examination of animal organs and tissues revealed no pathological alterations attributable to SGL.

Organ weights in males belonging to the group receiving intraperitoneal SGL administration were not statistically significant different in heart, liver and spleen, although the weight of the kidney of males was lower in proportion to concentration (Table 6). A significant increase in weights of the liver and spleen ($P<0.05$) compared with those in the control group was observed in the group of females treated with SGL at a dose of 2,000 mg kg^{-1} b.wt. day^{-1}. But, these results suggest that due to random biological variation they are of no toxicological significance. Moreover, these findings are consistent with the results of Feletti *et al*. (1992), who did not observe any evidence of increase in spleen weights or histopathological effects at similar doses of yeast β-glucan administered by gavage over a period of 52 weeks. In terms of organ weights in male and female rats administered intravenous SGL, all dose groups and the control group did not show significant differences. Therefore, these data indicate that the systemic administration of SGL, over a wide dose range, does not induce mortality or significant toxicity.

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