

## Safety Study of Yeast Hydrolysate with below 10 kDa Molecular Weight in Animal Models

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The aim of this study was to obtain data on the safety of yeast hydrolysate with below 10 kDa molecular weight. The acute [at a single dose of 5000 mg/kg body weight (BW)] and subacute (at a dose of 1000 mg/kg BW for 14 consecutive days) oral toxicity of yeast hydrolysate from *Saccharomyces cerevisiae* was assessed in Sprague-Dawley (SD) rats. The yeast hydrolysate acute treatment via the oral route at the dose of up to 5000 mg/kg did not produce any signs of toxicity or death in the rats. The yeast hydrolysate with below 10 kDa molecular weight did not induce any damage to the internal organs of the rats as examined by hematological and biochemical parameters. The macroscopic analysis of the treated animals did not show significant changes in color and texture. These results show that yeast hydrolysate with below 10 kDa molecular weight possesses very low toxicity as indicated in this SD rat model.

**Key words** — *Saccharomyces cerevisiae*, yeast, safety, acute toxicity, subacute toxicity

## INTRODUCTION

Yeast is a unicellular fungus and probably one of the most commercially exploited microorganisms. The yeast species *Saccharomyces cerevisiae* (*S. cerevisiae*) has been used in baking and fermenting alcoholic beverages for thousands of years.<sup>1)</sup> It is also extremely important as a model organism in modern cell biology research, and is one of the most thoroughly researched eukaryotic microorganisms.<sup>2,3)</sup>

Yeast hydrolysate, which is acquired from *S. cerevisiae* through protein hydrolysis, showed effectiveness in reducing the emotional, physical, and behavioral symptoms of premenstrual syndrome.<sup>4)</sup> Furthermore, yeast hydrolysate has displayed physiological effects on reproductive function<sup>5)</sup> and anti-stress and immunopotentiating activities.<sup>6,7)</sup> Recently, yeast hydrolysate has revealed various physiological effect of according to molecular weight. In Kim *et al.* study,<sup>8)</sup> yeast hydrolysate with 10–30 kDa molecular weight increased longitudinal bone growth and growth hormone secretion in adolescent Sprague-Dawley (SD) rats. And yeast hydrolysate with below 10 kDa molecular weight has displayed the anti-obesity effects in SD rats fed a high fat diet and obese women.<sup>9–11)</sup> It is needed the detailed toxicological information of yeast hydrolysate at the different stage of the molecular weight for safety-in-use of yeast hydrolysate with each molecular weight in an industrial context as a functional material for the health and diet food market.

In our previous study,<sup>12)</sup> we found that the yeast hydrolysate with 10–30 kDa molecular weight was comparatively nontoxic when oral acute and subacute toxicity were examined in SD rats. In this study, we tried to obtain data on the safety of yeast hydrolysate with below 10 kDa molecular weight by assessing its acute and subacute oral toxicity following Organization of Economic Co-operation and Development (OECD) guideline. The acute (at a single dose of 5000 mg/kg body weight)/subacute (at the dose of 1000 mg/kg body weight for 14 consecutive days) oral toxicity of yeast hydrolysate with below 10 kDa was assessed in SD rats of both sexes. In the subacute toxicity study, the effects on biochemical, hematological, and histopathological parameters were evaluated.

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## MATERIALS AND METHODS

**Preparation of Yeast Hydrolysate with below 10 kDa Molecular Weight**—The yeast used was *S. cerevisiae* (IFO 2346; strain Kyokai 6; MCYC 3175; LSUrDNAD1D2) obtained from the Institute for Fermentation Osaka (IFO, Osaka, Japan). *S. cerevisiae* was incubated in medium containing 2% molasses, 0.6% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.1% MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.2% KH<sub>2</sub>PO<sub>4</sub>, 0.03% K<sub>2</sub>HPO<sub>4</sub>, and 0.1% NaCl for 3 days at 30°C. After incubation, the culture was centrifuged at 10000 × *g* for 20 min. The cells were suspended in 20 mM phosphate buffer (pH 7.0) and hydrolyzed with 1000 units of bromelain at 30°C for 4 hr. The hydrolysate was subsequently centrifuged at 10000 × *g* for 20 min. Next, the supernatant was passed through a 10 kDa molecular weight cut-off membrane (Satocon cassette, Sartorius, Gottingen, Germany) and lyophilized.

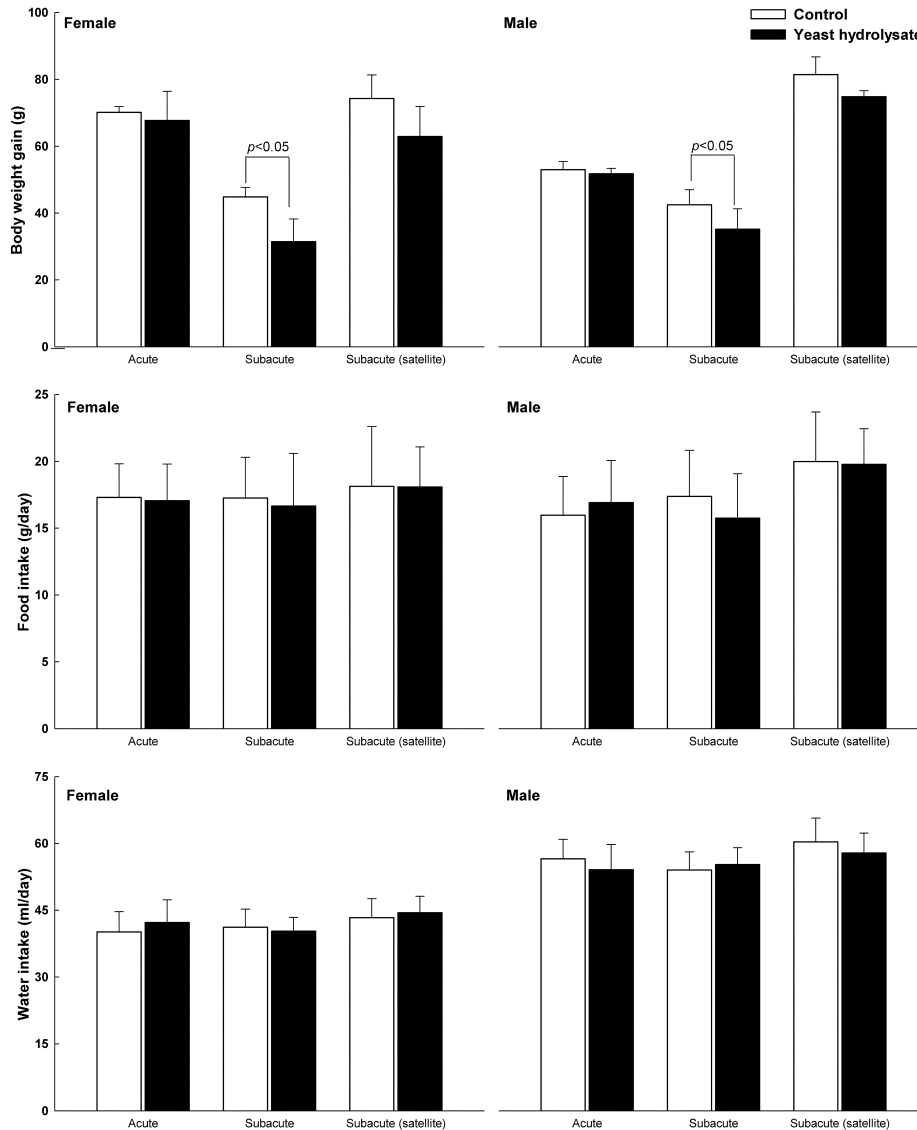
**Experimental Animals**—The experimental protocol was reviewed and approved by the Korea University Animal Care Committee. Female and male SD rats were obtained at 8 weeks of age from Nara Biotech (Kyonggi-do, Korea). They were individually housed in plastic cages with grated stainless steel floors. The colony room was maintained at 24 ± 1°C with 60% atmospheric humidity and a 12 hr light/12 hr dark cycle. The rats had *ad libitum* access to water and to a commercial diet (Samyang Co., Seoul, Korea) containing the following (g/kg of diet): moisture, 80; protein, 230; fat, 35; fiber, 50; carbohydrate, 600; and water.

**Acute Toxicity**—According to OECD guidelines for the testing of chemicals, TG420 (OECD) guideline for testing of chemicals, TG420,<sup>13)</sup> the rats were randomly divided into 2 groups per sex. The yeast hydrolysate at a single dose of 5000 mg/kg of body weight was given orally to the treated group (1<sup>st</sup> group), whereas an equal volume of water vehicle was given to the control group (2<sup>nd</sup> group). Observations were made and recorded systematically 1, 2, 4, and 6 hr after test substance administration. The visual observations included changes in the skin, fur, eyes and mucous membranes; the respiratory, circulatory, autonomic, and central nervous systems; as well as somatomotor activity and behavioral patterns. The number of survivors was noted after 24 hr and these rats were then maintained for another 14 days with once daily observations. On day 15, all the rats were fasted for 16–18 hr and then anesthetized with ethyl ether and

sacrificed.

**Subacute Toxicity**—According to OECD TG407,<sup>14)</sup> the rats were divided into 4 groups per sex. The yeast hydrolysate was administered to the treated group (1<sup>st</sup> group) at the dose of 1000 mg/kg of body weight for 14 days, whereas an equal volume of water vehicle was given to the control group (2<sup>nd</sup> group). In order to assess reversibility and the delayed occurrence of toxic effects, satellite groups were employed by giving the yeast hydrolysate (3<sup>rd</sup> group) at the dose of 1000 mg/kg of body weight or an equal volume of water vehicle (4<sup>th</sup> group) for 14 days and then the rats were maintained for another 14 days after treatment. During the period of administration, the animals were weighed and observed daily to detect signs of toxicity. Daily visual observations similar to those performed in the acute toxicity study were made and recorded systematically. At the end of the period, all rats were fasted for 16–18 hr and then anesthetized with ethyl ether and sacrificed.

**Hematological and Biochemical Analysis**—Blood samples were collected from a common carotid artery into heparinized and dry non-heparinized centrifuge tubes. The heparinized blood was used for hematological examinations and the serum separated from the non-heparinized blood was assayed for biochemical analyses. Hematological analysis was performed using an automatic hematological analyzer, KN-21N (Sysmex Co., Kobe, Japan). The parameters included: red blood cell (RBC) count, white blood cell (WBC) count, hematocrit (Hct), hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). For biochemical analysis, the blood was centrifuged at 1500 × *g* for 10 min to obtain the serum and the following parameters were determined using a FUJI DRI-CHEM 3500 analyzer (Fuji Photo Film Co., Osaka, Japan): glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, aspartate aminotransferase (AST), and alanine aminotransferase (ALT).  
**Morphological Study**—After blood collection, the rats were sacrificed for tissue studies. The animals were perfused with saline solution followed by 10% buffered formalin solution for 10 min and then their organs such as the liver, kidneys, spleen, lungs, heart, and sex organs were removed, blotted free of blood, and weighed immediately on an electronic balance for subsequent analysis. The organ weights were expressed in relative terms (g/100 g



**Fig. 1.** Body Weight Gain and Daily Intake of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Acute and Subacute Toxicity

Bars are means  $\pm$  S.D. for 5 rats/group. The group for acute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 5000 mg/kg once followed by no treatment for 14 days. The group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days. The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. The differences between the control and treated groups were evaluated by Student's *t*-test.

of body weight). After fixation in 10% phosphate buffered formalin, the liver and kidneys were processed in a routine manner, embedded in paraffin, and sectioned. Then, to perform light microscopic evaluation, the liver was stained with hematoxylin and eosin (H & E), and the kidneys were stained with periodic acid schiff (PAS).

**Statistical Analysis**—All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 12.0 (SPSS Inc., Chicago, IL, U.S.A.). The differences between the

control and treated groups were evaluated by Student's *t*-test and *p* values less than 0.05 were considered significant. All data are reported as means  $\pm$  standard deviations (S.D.).

## RESULTS

### Acute Toxicity

Acute oral toxicity refers to the adverse effects that occur following the oral administration of a

**Table 1.** Hematological Parameters of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Subacute Toxicity

Hematological parameters	Female			
	Group for subacute toxicity <sup>a)</sup>		Satellite group for subacute toxicity <sup>b)</sup>	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
RBC ( $\times 10^6/\mu\text{l}$ )	7.33 $\pm$ 0.52	7.21 $\pm$ 0.19	7.47 $\pm$ 0.30	7.12 $\pm$ 0.17
WBC ( $\times 10^3/\mu\text{l}$ )	8.57 $\pm$ 1.55	8.08 $\pm$ 1.24	8.00 $\pm$ 1.34	7.20 $\pm$ 0.22
Hct (%)	43.77 $\pm$ 3.75	43.98 $\pm$ 2.27	44.73 $\pm$ 2.74	45.10 $\pm$ 1.37
Hgb (g/dl)	13.51 $\pm$ 1.52	13.49 $\pm$ 0.94	14.50 $\pm$ 0.87	14.47 $\pm$ 0.35
MCV (fl)	56.77 $\pm$ 1.45	58.42 $\pm$ 1.26	59.83 $\pm$ 1.37	58.90 $\pm$ 1.20
MCH (pg)	18.34 $\pm$ 0.58	18.21 $\pm$ 0.88	19.33 $\pm$ 0.50	19.47 $\pm$ 0.65
MCHC (g/dl)	31.12 $\pm$ 1.23	31.55 $\pm$ 1.59	32.37 $\pm$ 0.21	32.50 $\pm$ 1.00
Platelets ( $\times 10^3/\mu\text{l}$ )	845.26 $\pm$ 54.42	898.87 $\pm$ 49.99	728.00 $\pm$ 41.52	823.67 $\pm$ 38.94

Hematological parameters	Male			
	Group for subacute toxicity <sup>a)</sup>		Satellite group for subacute toxicity <sup>b)</sup>	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
RBC ( $\times 10^6/\mu\text{l}$ )	7.06 $\pm$ 0.78	7.24 $\pm$ 0.86	7.96 $\pm$ 0.38	7.98 $\pm$ 0.41
WBC ( $\times 10^3/\mu\text{l}$ )	11.21 $\pm$ 1.02	11.00 $\pm$ 0.89	10.47 $\pm$ 0.31	10.82 $\pm$ 0.95
Hct (%)	45.15 $\pm$ 3.35	46.66 $\pm$ 4.32	47.25 $\pm$ 2.35	47.00 $\pm$ 4.48
Hgb (g/dl)	13.99 $\pm$ 0.98	14.28 $\pm$ 1.01	14.97 $\pm$ 0.21	15.00 $\pm$ 0.78
MCV (fl)	57.78 $\pm$ 1.24	57.62 $\pm$ 1.08	58.93 $\pm$ 1.11	58.83 $\pm$ 2.68
MCH (pg)	17.49 $\pm$ 0.52	18.00 $\pm$ 0.15	18.50 $\pm$ 0.52	18.73 $\pm$ 0.06
MCHC (g/dl)	31.07 $\pm$ 1.01	31.22 $\pm$ 1.75	31.27 $\pm$ 0.21	31.90 $\pm$ 1.44
Platelets ( $\times 10^3/\mu\text{l}$ )	721.42 $\pm$ 58.87	705.66 $\pm$ 61.41	692.67 $\pm$ 60.34	695.00 $\pm$ 60.22

Values are means  $\pm$  S.D. for 5 rats/group. *a)* The group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days. *b)* The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. The differences between the control and treated groups were evaluated by Student's *t*-test.

single dose of a substance or multiple doses given within 24 hr. The results indicated that the yeast hydrolysate with below 10 kDa molecular weight acute treatment via the oral route at the dose of up to 5000 mg/kg did not produce any signs of toxicity or death in the rats during 14 days of observation. Therefore, an LD<sub>50</sub> could not be estimated, and it is possibly higher than 5000 mg/kg. LD<sub>50</sub> is a statistically derived single dose of a substance that can be expected to cause death in 50 percent of animals when administered by the oral route.<sup>13)</sup> No significant differences were found between the initial and final body weights of the control and treated rats. A similar absence of toxic effects was observed in the case of food and water consumption (Fig. 1).

### Subacute Toxicity

No toxicity signs, such as piloerection, alterations in locomotor activity or diarrhea, or deaths, were recorded during the 14 consecutive days of yeast hydrolysate with below 10 kDa molecular weight treatment via the oral route at doses of 1000 mg/kg. The body weight gain of the rats

treated with yeast hydrolysate with below 10 kDa molecular weight was significantly lower compared to that of the control ( $p < 0.05$ ); yeast hydrolysate with below 10 kDa molecular weight induced a decrease in body weight gain in relation to the control group ( $p < 0.05$ ). There were no significant differences in daily food and water intake between the control and treated rats (Fig. 1).

### Hematological and Biochemical Parameters

The hematological profiles of the treated and control groups are presented in Table 1. No statistically significant differences were recorded in any of the hematological parameters analyzed. Table 2 shows the biochemical profiles of the treated and control groups. The BUN test is a measurement of the amount of nitrogen in the blood in the form of urea, and BUN values are affected by dietary protein.<sup>15)</sup> The BUN values of the control rats were slightly lower than the normal range of BUN values for SD rats, and the BUN values of the rats treated with yeast hydrolysate with below 10 kDa molecular weight, which is a rich source of protein and

**Table 2.** Blood Biochemical Parameter of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Subacute Toxicity

Blood biochemical parameter	Female			
	Group for subacute toxicity <sup>a)</sup>		Satellite group for subacute toxicity <sup>b)</sup>	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Glucose (mg/dl)	89.67 ± 8.02	94.67 ± 13.65	114.00 ± 9.70	116.33 ± 10.23
BUN (mg/dl)	8.57 ± 0.50	10.37 ± 0.32**	13.40 ± 1.40	13.45 ± 1.22
Creatinine (mg/dl)	0.30 ± 0.01	0.30 ± 0.01	0.27 ± 0.05	0.28 ± 0.05
Total protein (g/dl)	5.27 ± 0.06	6.00 ± 0.10	5.81 ± 0.13	5.84 ± 0.14
Albumin (g/dl)	3.53 ± 0.12	3.80 ± 0.01	3.79 ± 0.15	3.80 ± 0.13
Total bilirubin (mg/dl)	0.47 ± 0.06	0.53 ± 0.15	0.58 ± 0.08	0.58 ± 0.09
AST (U/l)	85.33 ± 5.08	89.33 ± 6.21	90.00 ± 6.97	92.92 ± 7.80
ALT (U/l)	27.67 ± 4.16	29.33 ± 2.53	29.33 ± 4.18	30.92 ± 4.23

Blood biochemical parameter	Male			
	Group for subacute toxicity <sup>a)</sup>		Satellite group for subacute toxicity <sup>b)</sup>	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Glucose (mg/dl)	102.00 ± 8.89	102.33 ± 4.93	118.67 ± 4.04	125.00 ± 6.24
BUN (mg/dl)	7.03 ± 0.32	11.80 ± 0.26**	13.00 ± 0.56	13.43 ± 0.31
Creatinine (mg/dl)	0.30 ± 0.01	0.37 ± 0.06	0.30 ± 0.01	0.30 ± 0.01
Total protein (g/dl)	5.40 ± 0.20	5.63 ± 0.15	5.60 ± 0.20	5.83 ± 0.06
Albumin (g/dl)	3.50 ± 0.01	3.60 ± 0.17	3.53 ± 0.06	3.62 ± 0.10
Total bilirubin (mg/dl)	0.47 ± 0.06	0.47 ± 0.05	0.47 ± 0.06	0.53 ± 0.06
AST (U/l)	95.00 ± 5.01	98.33 ± 13.02	96.33 ± 8.13	99.33 ± 6.79
ALT (U/l)	37.33 ± 2.16	35.00 ± 2.61	41.67 ± 2.58	39.01 ± 2.00

Values are means ± S.D. for 5 rats/group. *a)* The group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days. *b)* The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate with below 10 kDa molecular weight at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. \*\*Significantly different from the control,  $p < 0.01$ . The differences between the control and treated groups were evaluated by Student's *t*-test.

amino acids, were increased to normal range.

### Morphological Parameters

The representative microscopic findings in the liver and kidneys of the rats for the acute and subacute oral treatments of yeast hydrolysate with below 10 kDa molecular weight are shown in Figs. 2 and 3. The macroscopic analysis of the treated animals did not show significant changes in color and texture when compared to the control group in both the male and female rats. Also, the microscopical findings did not suggest histological alterations in the liver and kidneys.

## DISCUSSION

Generally, the reduction of internal organ weight is a simple and sensitive index of toxicity after exposure to a toxic substance.<sup>16)</sup> In this study, the relative internal organ weights of the rats were not altered by the yeast hydrolysate with below 10 kDa

molecular weight (Table 3). Furthermore, gross examination of the internal organs of all rats revealed no detectable abnormalities. Yeast hydrolysate with below 10 kDa molecular weight did not induce any damage to the internal organs as examined by blood parameters. Thus, it can be concluded that yeast hydrolysate with below 10 kDa molecular weight is virtually nontoxic.

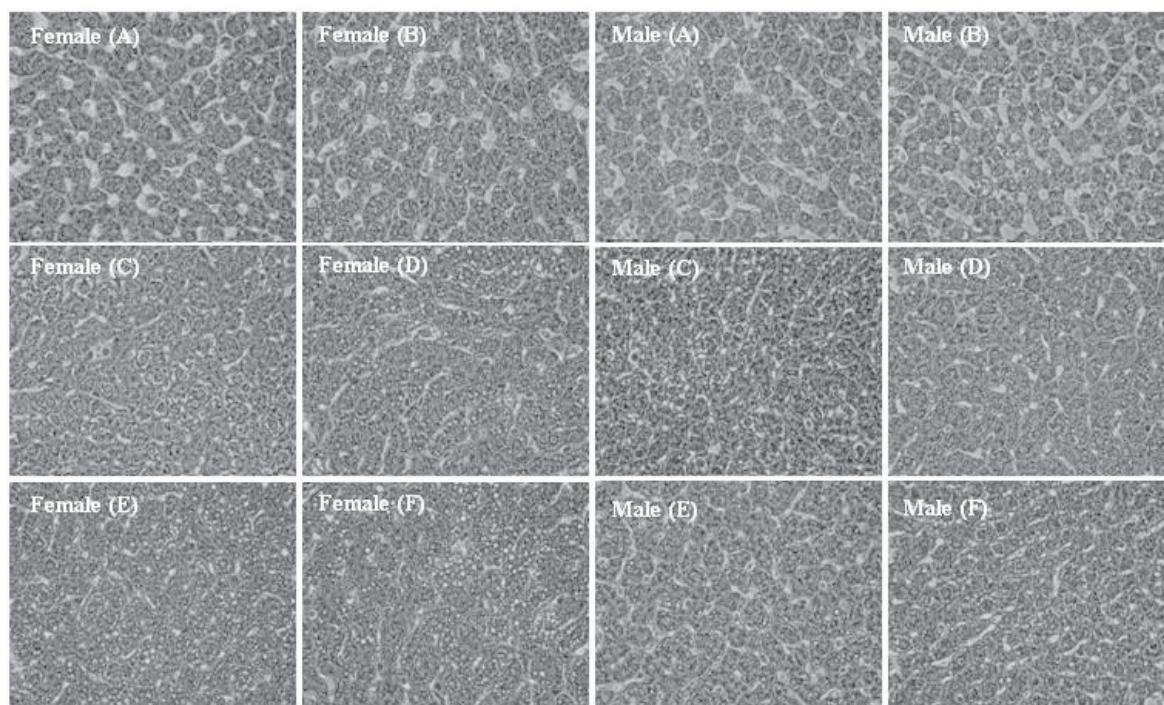
Substances fermented by *S. cerevisiae* are generally recognized as safe (GRAS). For example, the acute oral toxicity of wheat germ powder fermented by *S. cerevisiae* was found to very low, in which the LD<sub>50</sub> is 1868 mg/kg, the highest dose tested in mice and rats. Its subacute toxicity is also very low, as no toxicity was observed at the highest dose tested in rats, 1868 mg/kg.<sup>17)</sup>

Kim *et al.*<sup>11)</sup> tried to assess the anti-obesity effect of yeast hydrolysate with below 10 kDa molecular weight and found that it reduced body weight in SD rats fed a high fat diet. To investigate the effects of yeast hydrolysate with below 10 kDa molecular weight on appetite regulation mechanisms in

**Table 3.** Relative Organ Weights of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Subacute Toxicity

Relative organ weight (g/100 g of body weight)	Group for acute toxicity <sup>a)</sup>		Group for subacute toxicity <sup>b)</sup>		Satellite group for subacute toxicity <sup>c)</sup>	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate	Control	Yeast hydrolysate
<b>Female</b>						
Liver	3.93 ± 0.35	4.02 ± 0.24	3.77 ± 0.16	3.79 ± 0.14	3.72 ± 0.20	3.73 ± 0.10
Kidney	0.98 ± 0.05	0.99 ± 0.09	0.89 ± 0.05	0.87 ± 0.04	0.84 ± 0.11	0.86 ± 0.06
Spleen	0.32 ± 0.01	0.30 ± 0.01	0.26 ± 0.03	0.26 ± 0.03	0.23 ± 0.02	0.25 ± 0.05
Lung	0.51 ± 0.02	0.49 ± 0.03	0.48 ± 0.03	0.47 ± 0.02	0.42 ± 0.04	0.43 ± 0.04
Heart	0.38 ± 0.01	0.36 ± 0.03	0.34 ± 0.01	0.34 ± 0.01	0.30 ± 0.01	0.32 ± 0.02
Ovary	0.08 ± 0.01	0.09 ± 0.01	0.06 ± 0.01	0.07 ± 0.01	0.05 ± 0.01	0.04 ± 0.01
<b>Male</b>						
Liver	4.77 ± 0.38	4.88 ± 0.44	3.80 ± 0.13	3.72 ± 0.26	3.37 ± 0.10	3.31 ± 0.29
Kidney	1.06 ± 0.13	1.05 ± 0.08	0.87 ± 0.07	0.89 ± 0.01	0.82 ± 0.01	0.86 ± 0.06
Spleen	0.40 ± 0.08	0.36 ± 0.09	0.24 ± 0.01	0.20 ± 0.05	0.20 ± 0.01	0.19 ± 0.04
Lung	0.44 ± 0.01	0.45 ± 0.03	0.41 ± 0.02	0.40 ± 0.01	0.36 ± 0.04	0.38 ± 0.01
Heart	0.38 ± 0.01	0.36 ± 0.03	0.34 ± 0.01	0.34 ± 0.01	0.30 ± 0.01	0.32 ± 0.02
Testis	0.76 ± 0.09	0.74 ± 0.08	0.71 ± 0.07	0.72 ± 0.04	0.68 ± 0.05	0.69 ± 0.04

Values are means ± S.D. for 5 rats/group. *a)* The group for acute toxicity was given water vehicle or yeast hydrolysate at 5000 mg/kg once followed by no treatment for 14 days. *b)* The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1000 mg/kg daily for 14 days. *c)* The satellite group for subacute toxicity was given the water vehicle or yeast hydrolysate at 1000 mg/kg daily for 14 days followed by no treatment for 14 days. The differences between the control and treated groups were evaluated by Student's *t*-test.

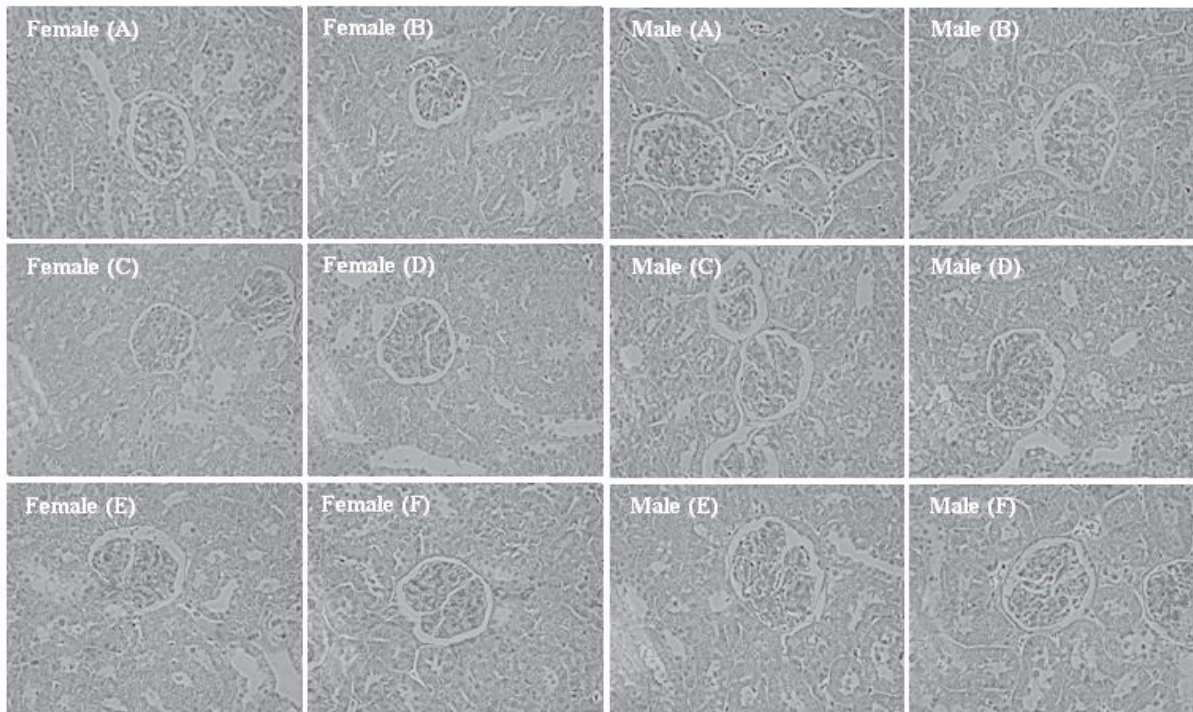
**Fig. 2.** Representative Microscopic Findings in the Liver of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Acute and Subacute Toxicity (Hematoxylin-eosin Stain, × 400)

(A) Water vehicle group for acute toxicity, (B) Yeast hydrolysate group for acute toxicity, (C) Water vehicle group for subacute toxicity, (D) Yeast hydrolysate group for subacute toxicity, (E) Water vehicle (satellite) group for subacute toxicity and (F) Yeast hydrolysate (satellite) group for subacute toxicity.

the central nervous system, Jung *et al.*<sup>10)</sup> measured nitric oxide synthase (NOS) expression and vasoactive intestinal peptide (VIP) immunoreactivity in

the paraventricular nucleus (PVN) and ventromedial hypothalamic nucleus (VMH) of the hypothalamus using histochemical methods. They sug-





**Fig. 3.** Representative Microscopic Findings in Kidneys of SD Rats Treated Orally with Yeast Hydrolysate with below 10 kDa Molecular Weight for Acute and Subacute Toxicity (Periodic Acid Schiff Stain,  $\times 400$ )

(A) Water vehicle group for acute toxicity, (B) Yeast hydrolysate group for acute toxicity, (C) Water vehicle group for subacute toxicity, (D) Yeast hydrolysate group for subacute toxicity, (E) Water vehicle (satellite) group for subacute toxicity and (F) Yeast hydrolysate (satellite) group for subacute toxicity.

gested that administering yeast hydrolysate with below 10 kDa molecular weight to SD rats reduced body weight gain by altering neuropeptide Y (NPY) and tryptophan hydroxylase (TPH) expression. Furthermore, Jung *et al.*<sup>18)</sup> examined the anti-obesity effects of yeast hydrolysate with below 10 kDa molecular weight on obese women. The results also showed that this yeast hydrolysate with below 10 kDa molecular weight caused a higher reduction of body weight compared to placebo. Therefore, it is considered that due to the anti-obesity effect of yeast hydrolysate with relative low molecular weight, the body weight gains of the rats treated with yeast hydrolysate with below 10 kDa molecular weight were lower compared to those of the control ( $p < 0.05$ ).

In the present study, no deaths or signs of toxicity were observed in the rats that received yeast hydrolysate with below 10 kDa molecular weight up to an oral acute dose of 5000 mg/kg, thus establishing its safety for use. In conclusion, these results show that yeast hydrolysate with below 10 kDa molecular weight possesses very low toxicity, as indicated in our rat model. However, a chronic toxicity study is needed to further support the safety-

in-use of this yeast hydrolysate with below 10 kDa molecular weight

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