Journal of Microbial & Biochemical Technology - Open Access
www.omicsonline.org JMBT/Vol.2 Issue 2

Research Article OPEN ACCESS Freely available online doi:10.4172/1948-5948.1000024

Safety Evaluation of Yeast Hydrolysate (Notress)

Eun-Young Jung[1,2], Hyun-Sun Lee[1], Ho-Chan Seo[3] and Hyung Joo Suh[1]*

[1]Department of Food and Nutrition, Korea University, Seoul 136-703, Korea
[2]Department of Food and Nutrition, Dongduk Women's University, Seoul 136-714, Korea
[3]Department of Brain Education, University of Brain Education, Cheonan 330-841, Korea

Abstract

The yeast hydrolysate from *Saccharomyces cerevisiae* was evaluated for acute/subacute toxicity on female and male Sprague-Dawley (SD) rats. The single oral dose of the hydrolysate at 5,000 mg/kg did not produce mortality or significant changes in the general behavior and gross appearance of the internal organs of rats. In subacute toxicity study, the hydrolysate was administered orally at a dose of 1,000 mg/kg/day for a period of 14 days. The satellite group was treated with the hydrolysate at the same dose and the same period and kept for another 14 days after treatment. There were no significant differences in organ weights between control and treated group of both sexes. Hematological analysis and blood chemistry revealed no toxicity effects of *S.cerevisiae* hydrolysate. Pathologically, neither gross abnormalities nor histopathological changes were observed. These results show that the hydrolysate possesses very low toxicity as indicated in SD rat model.

Keywords: *Saccharomyces cerevisiae*; Yeast hydrolysate; Acute toxicity; Subacute toxicity

Abbreviations: ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BUN: Blood Urea Nitrogen; GRAS: Generally Recognized As Safe; H & E: Hematoxylin and Eosin; HCT: Hematocrit; Hgb: Hemoglobin; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; MCV: Mean Corpuscular Volume; OECD: Organization of Economic Co-operation and Development; PAS: Periodic Acid Schiff; RBC: Red Blood Cell; WBC: White Blood Cell

Introduction

Technological processes used in food manufacture affect the functional, nutritional and biological properties of food proteins. On the other hand, proteins may be added as functional ingredients to foods to emulsify, bind water or fat, form foams or gels, and alter flavor, appearance, and texture (Anantharaman and Finot, 1993). In recent years, the role of proteins in the diet as physiologically active components has been increasingly acknowledged. Such proteins or their precursors may occur naturally in raw food materials exerting their physiological action direct or upon enzymatic hydrolysis in vitro or in vivo. For example, it has become clear that dietary proteins are a source of biologically active peptides.

Bioactive peptides can be released by enzymatic proteolysis of food proteins and may act as potential physiological modulators of metabolism during the intestinal digestion of the diet. Bioactive peptides usually contain 3–20 amino acid residues and their activity is based on their amino acid composition and sequence (Pihlanto-Leppälä, 2000). The possible regulatory effects of peptides relate to nutrient uptake, immune defense (Chen et al., 1995; Tsuruki et al., 2003), opioid (Pihlanto-Leppälä, 2000), antioxidant (Mendis et al., 2005) and antihypertensive activities (Suetsuna et al., 2004; Suetsuna, 1998).

The development of functional foods is likely to entail the increased use of different protein sources known to contain bioactive components. These protein components may be natural constituents of plant or animal origin or genetically modified or transferred from another source. The introduction into the diet of functional foods supplemented with these compounds may raise the issue that such food products might cause toxicity. Although the bioactive peptides in protein hydrolysates are not known to possess specific allergic or toxic effects, the peptides in protein hydrolysate warrant careful consideration about potential health risks.

There are, however, a great number of scientific and technological issues to be solved before these substances can optimally be exploited for human nutrition and health. Basic research on transgenic production of bioactive proteins and potential side-effects, e.g. allergenicity and toxicity of such proteins, is most important future research needs related to bioactive proteins.

Several studies demonstrated that the hydrolysate displayed physiological effects on reproductive function (Beehler et al., 1994), anti-fatigue (Kim et al., 2002), anti-stress (Kim et al., 2002; Suh et al., 2008), immune potentiating activities (Koh et al., 2002), antiobesity activity (Jung et al., 2008; Jung et al., 2009) and growth stimulating activity (Kim et al., 2009). For these reasons, the hydrolysate is receiving remarkable attention as a functional material for the diet food market. Although *S. cerevisiae* is generally recognized as safe (GRAS), the bioactive proteins or peptides from *S. cerevisiae* have not been assured for safety. This study evaluated the acute/subacute toxicity of yeast hydrolysate following Organization of Economic Co-operation and Development (OECD) guideline. The acute (at a single dose of 5,000 mg/kg body weight)/subacute (at the dose of 1,000 mg/kg body weight for 14 consecutive days) oral toxicity of yeast hydrolysate from *S. cerevisiae* was assessed in Sprague-Dawley (SD) rats of both sexes.

***Corresponding author:** Hyung Joo Suh, Department of Food and Nutrition, Korea University, 1 Jenongneung-dong, Sungbuk, Seoul 136-703, Korea, Tel: +82 2 940 2853; Fax: +82 2 940 2850; E-mail: suh1960@korea.ac.kr

Received April 12, 2010; **Accepted** April 29, 2010; **Published** April 29, 2010

Citation: Jung EY, Lee HS, Seo HC, Suh HJ (2010) Safety Evaluation of Yeast Hydrolysate (Notress). J Microbial Biochem Technol 2: 058-063. doi:10.4172/1948-5948.1000024

Material and Methods

Preparation of yeast hydrolysate (Notress)

Saccharomyces cerevisiae IFO 2346 was incubated in medium containing 2% molasses, 0.6% $(NH_4)_2SO_4$, 0.1% $MgSO_4 \cdot 7H_2O$, 0.2% KH_2PO_4, 0.03% K_2HPO_4, and 0.1% NaCl for 3 days at 30°C. After incubation, the culture was centrifuged at 10,000 × *g* for 20 min. The cells were suspended in 20 mM phosphate buffer (pH 7.0) and hydrolyzed with 1,000 units of bromelain at 30°C for 4 h. The hydrolysate was subsequently centrifuged at 10,000 × *g* for 20 min. The supernatant was lyophilized to obtain the yeast hydrolysate (Notress).

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 (Seoul, 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 h light/12 h 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 study

According to OECD guideline for testing of chemicals, OECD TG420 (OECD, 2001a), rats were randomly divided into 2 groups per sex. The yeast hydrolysate at a single dose of 5,000 mg/kg body weight was given orally to treated group (1st group), whereas an equal volume of water vehicle was given to control group (2nd group). Observations were made and recorded systematically 1, 2, 4 and 6 h after test substance administration. The visual observations included changes in the skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous system as well as somatomotor activity and behavioral pattern. The number of survivors was noted after 24 h and these were then maintained for a further 14 days with a once daily observation. On the day 15, all rats were fasted for 16–18 h, then anesthetized with ethyl ether and sacrificed.

Subacute toxicity study

According to OECD TG407 (OECD, 2001b), rats were divided into 4 groups per sex. The yeast hydrolysate was administered to treated group (1st group) at the dose of 1,000 mg/kg body weight for 14 days, whereas an equal volume of water vehicle was given to control group (2nd group). In order to access reversibility and delayed occurrence of toxic effects, satellite group was given the yeast hydrolysate (3rd group) at the dose of 1,000 mg/kg body weight or an equal volume of water vehicle (4th group) for 14 days and kept for other 14 days after treatment. During the period of administration, the animals were weighed and observed daily to detect signs of toxicity. Daily visual observations were made and recorded systematically similar those performed as in the case acute toxicity study. At the end of the period, all rats were fasted for 16–18 h, then anesthetized with ethyl ether and sacrificed.

Hematological and biochemical analysis

Blood samples were collected from a common carotid into heparinized and dry non-heparinized centrifuge tubes. The heparinized blood was used for hematological study and the serum separated from the non-heparinized blood was assayed for biochemical analysis. Hematological analysis was performed using an automatic hematological analyzer KN-21N (Sysmex Co., Kobe, Seoul, Korea). Parameters included: red blood cell (RBC) count, white blood cell (WBC) count, hematocrit (Hct), hemoglobin (Hgb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). For biochemical analysis, blood was centrifuged at 1,500 × *g* for 10 min to obtain serum and the following parameters were determined using FUJI DRI-CHEM 3500 (Fuji Photo Film Co., Osaka, Japan): glucose, blood urea nitrogen (BUN), creatinine, total protein, albumin, total billirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT).

Morphological study

After blood collection rats were sacrificed for tissue studies. Rats were perfused with saline solution followed by 10% buffered formalin solution for 10 min and the organs such as liver, kidney, spleen, lung, heart and sex organs were removed, blotted free of blood and weighed immediately on an electronic balance for subsequent analysis. Organ weights were expressed in relative terms (g/100 g of body weight). After fixation in 10% phosphate buffered formalin, liver and kidney were processed in routine manner, embedded in paraffin, and sectioned. Then to perform light microscopic evaluation, the liver was stained with hematoxylin and eosin (H & E), and kidney was stained with periodic acid schiff (PAS).

	Group for acute toxicity[a]		Group for subacute toxicity[b]		Satellite group for subacute toxicity[c]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Female						
Body weight gain (g)	70.17 ± 1.69	68.67 ± 5.24	44.83 ± 2.86	50.20 ±15.27	74.30 ± 6.98	88.70 ± 17.67
Food intake (g/day)	17.30 ± 2.52	16.15 ± 2.22	17.25 ± 3.06	17.15 ± 3.85	18.13 ± 4.48	18.15 ± 3.79
Water intake (ml/day)	40.12 ± 4.55	41.21 ± 3.11	41.18 ± 4.05	42.25 ± 2.21	43.32 ± 4.27	44.45 ± 3.98
Male						
Body weight gain (g)	95.37 ± 4.48	85.87 ±10.38	76.47 ± 8.11	90.33 ± 6.47	146.53 ± 9.55	159.07 ± 21.33
Food intake (g/day)	19.16 ± 3.48	19.55 ± 2.45	20.85 ± 4.14	20.25 ± 4.05	23.98 ± 4.45	23.78 ± 3.33
Water intake (ml/day)	45.22 ± 3.52	44.28 ± 3.69	43.22 ± 3.24	44.47 ± 5.02	48.26 ± 4.29	46.62 ± 4.10

Values are means ± SD for 5 rats/group. [a]The group for acute toxicity was given water vehicle or yeast hydrolysate at 5,000 mg/kg once followed by no treatment for 14 days. [b]The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days. [c]Satellite group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 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.

Table 1: Body weight gain and daily intake of SD rats treated orally with yeast hydrolysate (Notress) for acute/subacute toxicity examinations.

Hematological parameters	Group for subacute toxicity[a]		Satellite group for subacute toxicity[b]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Female				
RBC ($\times10^6$/μl)	7.33 ± 0.52	7.48 ± 0.92	7.47 ± 0.30	7.87 ± 0.24
WBC ($\times10^3$/μl)	8.57 ± 1.55	8.08 ± 1.58	8.00 ± 1.34	7.11 ± 0.90
Hct (%)	43.77 ± 3.75	43.27 ± 3.87	44.73 ± 2.74	39.80 ± 1.13
Hgb (g/dl)	13.51 ± 1.52	14.42 ± 2.74	14.50 ± 0.87	13.60 ± 0.26
MCV (fl)	56.77 ± 1.45	59.82 ± 3.75	59.83 ± 1.37	58.17 ± 1.74
MCH (pg)	18.34 ± 0.58	17.79 ±1.22	19.33 ± 0.50	20.00 ± 0.53
MCHC (g/dl)	31.12 ± 1.23	33.09 ± 1.22	32.37 ± 0.21	33.97 ± 0.68
Platelets ($\times10^3$/μl)	845.26 ± 54.42	856.41 ± 53.39	728.00 ± 41.52	821.45 ± 51.11
Male				
RBC ($\times10^6$/μl)	7.06 ± 0.78	7.62 ± 1.09	7.96 ± 0.38	7.91 ± 0.21
WBC ($\times10^3$/μl)	11.21 ± 1.02	11.92 ± 1.20	10.47 ± 0.31	10.87 ± 1.41
Hct (%)	45.15 ± 3.35	47.12 ± 2.48	47.25 ± 2.35	45.55 ± 0.95
Hgb (g/dl)	13.99 ± 0.98	13.94 ± 1.28	14.97 ± 0.21	14.80 ± 0.10
MCV (fl)	57.78 ± 1.24	57.77 ± 2.25	58.93 ± 1.11	58.47 ± 0.55
MCH (pg)	17.49 ± 0.52	17.59 ± 1.36	18.50 ± 0.52	18.70 ± 0.44
MCHC (g/dl)	31.07 ± 1.01	31.24 ± 1.13	31.27 ± 0.21	32.30 ± 0.66
Platelets ($\times10^3$/μl)	721.42 ± 58.87	742.49 ± 66.03	692.67 ± 60.34	707.89 ± 55.47

Values are means ± SD for 5 rats/group. [a]The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days. [b]The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days followed by no treatment for 14 days. RBC; red blood cell, WBC; white blood cell, Hct; hematocrit, Hgb; hemoglobin, MCV; mean corpuscular volume, MCH; mean corpuscular hemoglobin, MCHC; mean corpuscular hemoglobin concentration. The differences between the control and treated groups were evaluated by Student's *t*-test.

Table 2: Hematological parameters of SD rats treated orally with yeast hydrolysate (Notress) for subacute toxicity examinations.

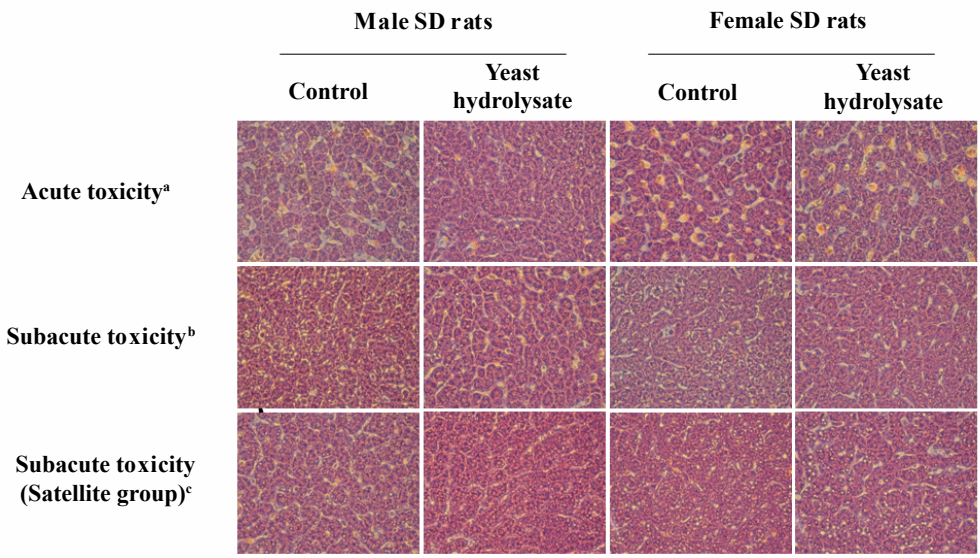

Figure 1: Representative microscopic findings in the liver of SD rats treated orally with yeast hydrolysate by hematoxylin-eosin (H & E) staining (×400). [a]The group for acute toxicity was given water vehicle or yeast hydrolysate at 5,000 mg/kg once, followed by no treatment for 14 days. [b]The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days. [c]Satellite group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days followed by no treatment for 14 days.

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 12.0 (SPSS Inc., IL, USA). The differences between control and treated groups were evaluated by Student's *t*-test. The *p* values less than 0.05 were considered significant. All data were reported as means ± standard deviations (SD).

Result

Acute and subacute toxicity

Acute oral toxicity refers to the adverse effects that occur following the oral administration of a single dose of a substance or multiple doses given within 24 hours (OECD, 2001a). The results indicated that the yeast hydrolysate acute treatment via the oral route at the dose of up to 5,000 mg/kg did not produce any signs of toxicity or death in the rats during 14 days of observation. Therefore, an LD_{50} could not be estimated, and it is possibly higher than 5,000 mg/kg. LD_{50} 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 (OECD, 2001a). 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 (Table 1).

No toxicity signs, such as piloerection, alterations in locomotor activity or diarrhea, or deaths, were recorded during the 14 consecutive days of yeast hydrolysate treatment via the oral route at doses of 1,000 mg/kg. For the female rats, no statistically significant differences were recorded for body weight gain,

Blood biochemical parameters	Group for subacute toxicity[a]		Satellite group for subacute toxicity[b]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Female				
Glucose (mg/dl)	89.67 ± 8.02	91.50 ± 1.50	114.00 ± 9.70	112.33 ± 7.02
BUN (mg/dl)	8.57 ± 0.50	12.57 ± 1.31**	13.40 ± 1.40	15.47 ± 0.55
Creatinine (mg/dl)	0.30 ± 0.01	0.33 ± 0.06	0.27 ± 0.05	0.27 ± 0.06
Total protein (g/dl)	5.27 ± 0.06	6.10 ± 0.10	5.81 ± 0.13	6.20 ± 0.26
Albumin (g/dl)	3.53 ± 0.12	3.93 ± 0.06	3.79 ± 0.15	4.00 ± 0.10
Total bilirubin (mg/dl)	0.47 ± 0.06	0.57 ± 0.12	0.58 ± 0.08	0.77 ± 0.15
AST (U/l)	85.33 ± 5.08	94.33 ± 8.39	90.00 ± 6.97	94.67 ± 1.53
ALT (U/l)	27.67 ± 4.16	28.33 ± 3.51	29.33 ± 4.18	30.00 ± 2.65
Male				
Glucose (mg/dl)	102.00 ± 8.89	105.00 ± 13.45	118.67 ± 4.04	111.33 ± 11.02
BUN (mg/dl)	7.03 ± 0.32	11.50 ± 0.70**	13.00 ± 0.56	12.87 ± 1.23
Creatinine (mg/dl)	0.30 ± 0.01	0.33 ± 0.06	0.30 ± 0.01	0.23 ± 0.06
Total protein (g/dl)	5.40 ± 0.20	5.77 ± 0.06	5.60 ± 0.32	5.80 ± 0.10
Albumin (g/dl)	3.50 ± 0.01	3.80 ± 0.10	3.53 ± 0.06	3.73 ± 0.06
Total bilirubin (mg/dl)	0.47 ± 0.06	0.47 ± 0.07	0.47 ± 0.06	0.53 ± 0.06
AST (U/l)	95.00 ± 5.01	104.67 ±6.43	96.33 ± 8.13	101.33 ± 7.08
ALT (U/l)	37.33 ± 2.16	34.00 ± 1.73	41.67 ± 2.58	42.33 ± 3.21

Values are means ± SD for 5 rats/group. [a]The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days. [b]The satellite group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days followed by no treatment for 14 days. BUN; lood urea nitrogen, AST; aspartate aminotransferase, ALT; alanine aminotransferase. **Significantly different from control, $p<0.01$. The differences between the control and treated groups were evaluated by Student's *t*-test.

Table 3: Blood biochemical parameters of SD rats treated orally with yeast hydrolysate (Notress) for subacute toxicity examinations.

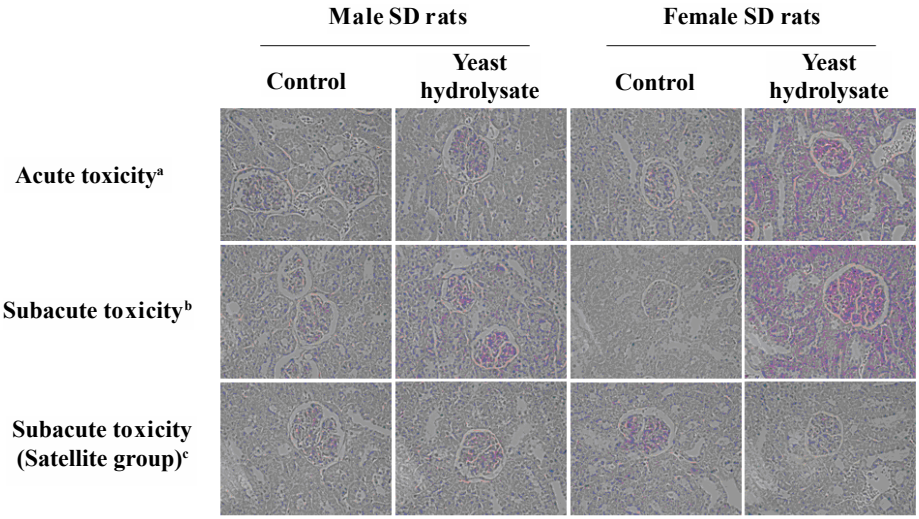

Figure 2: Representative microscopic findings in the kidneys of SD rats treated orally with yeast hydrolysate by periodic acid Schiff (PAS) staining (×400). [a]The group for acute toxicity was given water vehicle or yeast hydrolysate at 5,000 mg/kg once followed by no treatment for 14 days. [b]The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days. [c]Satellite group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days followed by no treatment for 14 days.

although the body weight gain of the female rats treated with yeast hydrolysate was slightly lower compared to that of the control. There were no significant differences in daily food and water intake between the control and treated rats (Table 1).

Hematological and biochemical parameters

The hematological profiles of the treated and control groups are presented in Table 2. No statistically significant differences were recorded in any of the hematological parameters analyzed.

Table 3 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 (Sarwar et al., 1999). 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, which is a rich source of protein and 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 are shown in Figure 1 and Figure 2. 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.

Generally, the reduction of internal organ weight is a simple

Relative organ weight (g/100 g of body weight)	Group for acute toxicity[a]		Group for subacute toxicity[b]		Satellite group for subacute toxicity[c]	
	Control	Yeast hydrolysate	Control	Yeast hydrolysate	Control	Yeast hydrolysate
Female						
Liver	3.93 ± 0.35	3.73 ± 0.10	3.77 ± 0.16	3.31 ± 0.31	3.72 ± 0.20	3.12 ± 0.39
Kidney	0.98 ± 0.05	0.90 ± 0.07	0.89 ± 0.05	0.85 ±0.09	0.84 ± 0.11	0.82 ± 0.09
Spleen	0.32 ± 0.01	0.28 ± 0.03	0.26 ± 0.03	0.26 ± 0.03	0.23 ± 0.02	0.20 ± 0.04
Lung	0.51 ± 0.02	0.54 ± 0.01	0.48 ± 0.03	0.45 ± 0.05	0.42 ± 0.04	0.44 ± 0.05
Heart	0.38 ± 0.01	0.39 ± 0.01	0.34 ± 0.01	0.39 ± 0.03	0.30 ± 0.01	0.35 ± 0.05
Ovary	0.08 ± 0.01	0.09 ± 0.02	0.06 ± 0.01	0.07 ± 0.05	0.05 ± 0.01	0.05 ± 0.01
Male						
Liver	4.77 ± 0.38	4.48 ± 0.45	3.80 ± 0.13	3.21 ± 0.13	3.37 ± 0.10	3.23 ± 0.09
Kidney	1.06 ± 0.13	1.00 ± 0.08	0.87 ± 0.07	0.87 ± 0.09	0.82 ± 0.01	0.79 ± 0.05
Spleen	0.38 ± 0.08	0.34± 0.09	0.24 ± 0.01	0.26 ± 0.03	0.20 ± 0.01	0.24 ± 0.02
Lung	0.44 ± 0.01	0.41 ± 0.01	0.41 ± 0.02	0.45 ± 0.05	0.36 ± 0.04	0.38 ± 0.05
Heart	0.38 ± 0.01	0.40 ± 0.03	0.34 ± 0.01	0.39 ± 0.04	0.36 ± 0.01	0.32 ± 0.04
Testis	0.76 ± 0.09	0.80 ± 0.10	0.71 ± 0.07	0.73 ± 0.09	0.68 ± 0.05	0.72 ± 0.06

Values are means ± SD for 5 rats/group. [a]The group for acute toxicity was given water vehicle or yeast hydolysate at 5,000 mg/kg once followed by no treatment for 14 days. [b]The group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 mg/kg daily for 14 days. [c]Satellite group for subacute toxicity was given water vehicle or yeast hydrolysate at 1,000 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.

Table 4: Relative organ weights of SD rats treated orally with yeast hydrolysate (Notress) for acute/subacute toxicity examinations.

and sensitive index of toxicity after exposure to a toxic substance (Teo et al., 2003). In the present study, the relative internal organ weights of the rats were not altered by the yeast hydrolysate (Figure 2). Furthermore, gross examination of the internal organs of all rats revealed no detectable abnormalities. The yeast hydrolysate did not induce any damage to the internal organs as examined by blood parameters. Thus, it can be concluded that yeast hydrolysate is virtually nontoxic.

Discussion

According to the OECD guidelines (OECD, 2001b), if an acute toxicity test at a single dosage level of at least 5,000 mg/kg produces no observable toxic effects, then a full study using three dose levels is not considered necessary and a dose of 1,000 mg/kg of body weight given once daily for 14 days can be used to evaluate subacute toxicity.

The present investigation found that yeast hydrolysate at a high dose of 1,000 mg/kg daily for 14 days did not cause toxicity signs such as piloerection, alterations in locomotor activity, or death. According to the OECD guidelines (OECD, 2001a) for the testing of chemicals, the results of this acute toxicity study indicate that yeast hydrolysate is fairly non-toxic.

In the subacute and acute toxicity study, no significant differences were found between the initial and final body weights of the control and treated rats (Table 4). However, the yeast hydrolysate containing the 30-10 kDa molecular weight peptides (YGF) induced significant increases in body weight in relation to the control group in the female rats ($p<0.05$). This increase in body weight, which was higher than that of the control group, may be due to appetite stimulation by the yeast hydrolysate, leading to increased food consumption and observed body weight (data was not showed). For the female rats, no statistically significant differences were recorded for body weight gain, although the body weight gain of the female rats treated with yeast hydrolysate was slightly lower compared to that of the control. However, in the male rats, yeast hydrolysate containing the below 10 kDa molecular weight peptides (Eatless) induced a significant decrease in body weight gain in relation to the control group ($p<0.05$).

In previous report (Jung et al., 2008; Jung et al., 2009), the results indicated that yeast hydrolysate containing below 10 kDa molecular weight peptides (Eatless) reduced body weight gain and body fat in normal diet-fed rats and increased the lipid energy metabolism by altering the expression of NOS and VIP neurons. Our previous studies (Kim et al., 2009) reported the body weight gain, food intake and food efficiency ratios of the N-control (saline), P-control (foremilk 1 g/kg of BW) and YH-1 and YH-2 (yeast hydrolysate containing 30-10 kDa molecular weight, YGF, 0.5 and 1 g/kg of BW, respectively) groups following the 4 week experimental period. The YH-1 and YH-2 groups showed significant increases in body weight gain compared with the N-control group ($p < 0.05$); however, there was no significant difference in the body weight gain between the YH-1 and YH-2 groups. However, yeast hydrolysate containing non-fractionated peptide (Notress) did not showed weight changes.

Correspondingly, these data are within the normal limits established under laboratory control and determined by Lillie et al. (1996). Thus, it can be concluded that yeast hydrolysate is virtually nontoxic. Overall, the yeast hydrolysate in this study was found to be comparatively nontoxic when oral acute and subacute toxicity were examined in SD rats.

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_{50} is 1,868 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, 1,868 mg/kg (Heimbach et al., 2007).

In the present study, no deaths or signs of toxicity were observed in the rats that received yeast hydrolysate up to an oral acute dose of 5,000 mg/kg, thus establishing its safety for use. In conclusion, these results show that yeast hydrolysate possesses very low toxicity, as indicated in our rat model. However, a chronic toxicity study is needed to further support the safe use of this yeast hydrolysate.

References

1. Anantharaman K, Finot PA (1993) Nutritional aspects of food proteins in relation

to technology. Food Rev Int 9: 629-655. » CrossRef » PubMed » Google Scholar

2. Beehler JW, Millar JG, Mulla MS (1994) Protein hydrolysates and associated bacterial contaminants as oviposition attractants for the mosquito Culex quinquefasciatus. Med Vet Entomol 8: 381-385. » CrossRef » PubMed » Google Scholar

3. Chen J, Suetsuna K, Yamauchi F (1995) Isolation and characterization of immunostimulative peptides from soybean. J Nutr Biochem 6: 310-313. » CrossRef » PubMed » Google Scholar

4. Heimbach JT, Sebestyen G, Semjen G, Kennepohl E (2007) Safety studies regarding a standardized extract of fermented wheat germ. Int J Toxicol 26: 253-259. » CrossRef » PubMed » Google Scholar

5. Jung EY, Suh HJ, Kim SY, Hong YS, Kim MJ, et al. (2008) Appetite suppressive effects of yeast hydrolysate on nitric oxide synthase (NOS) expression and vasoactive intestinal peptide (VIP) immunoreactivity in hypothalamus. Phytother Res 22: 1417-1422. » CrossRef » PubMed » Google Scholar

6. Jung EY, Kang DH, Suh HJ, Chang UJ (2009) Effects of yeast hydrolysate on neuropeptide Y (NPY) and tryptophan hydroxylase (TPH) immunoreactivity in rats. Phytother Res 23: 619-623. » CrossRef » PubMed » Google Scholar

7. Kim JM, Kim SY, Jung EY, Bae SH, Suh HJ (2009) Yeast hydrolysate induces longitudinal bone growth and growth hormone release in rats. Phytother Res 23: 731-736. » CrossRef » PubMed » Google Scholar

8. Kim KM, Yu KW, Kang DH, Suh HJ (2002) Anti-stress and anti-fatigue effect of fermented rice bran. Phytother Res 16: 700-702. » CrossRef » PubMed » Google Scholar

9. Koh JH, Yu KW, Suh HJ (2002) Biological activities of Saccharomyces cerevisiae and fermented rice bran as feed additives. Lett Appl Microbiol 35 : 47-51. » CrossRef » PubMed » Google Scholar

10. Lillie LE, Temple NJ, Florence LZ (1996) Reference values for young normal Sprague-Dawley rats: weight gain, hematology and clinical chemistry. Hum Exp Toxicol 15: 612-616. » CrossRef » PubMed » Google Scholar

11. Mendis E, Rajapakse N, Kim SK (2005) Antioxidant properties of a radicals scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. J Agric Food Chem 53: 581-587. » CrossRef » PubMed » Google Scholar

12. Organisation of Economic Co-operation and Development (OECD) (2001a) The OECD guideline for testing of chemical: 407 repeated dose oral toxicity-rodent: 28-day or 14-day Study. OECD: Paris, France. » CrossRef » PubMed » Google Scholar

13. Organisation of Economic Co-operation and Development (OECD) (2001b) The OECD Guideline for Testing of Chemical: 420 Acute Oral Toxicity. OECD: Paris, France. » CrossRef » PubMed » Google Scholar

14. Pihlanto-Leppälä A (2000) Bioactive peptides derived from bovine whey proteins: Opioid and ace-inhibitory. Trends Food Sci Tech 11: 347-356. » CrossRef » PubMed » Google Scholar

15. Sarwar G, L'Abbe MR, Trick K, Botting HG, Ma CY (1999) Influence of feeding alkaline/heat processed proteins on growth and protein and mineral status of rats. Adv Exp Med Biol 459: 161-177. » CrossRef » PubMed » Google Scholar

16. Suetsuna K, Maekawa K, Chen JR (2004) Antihypertensive effects of Undaria pinnatifida (wakame) peptide on blood pressure in spontaneously hypertensive rats. J Nutr Biochem 15: 267-272. » CrossRef » PubMed » Google Scholar

17. Suetsuna K (1998) Isolation and characterization of angiotensin I-converting enzyme inhibitor dipeptides derived from Allium sativum L (garlic). J Nutr Biochem 9: 415-419. » CrossRef » PubMed » Google Scholar

18. Suh HJ, Kim SY, Chang UJ, Kim JM (2008) Anti-stress effects of chewing gum prepared with yeast hydrolysate. Eur Food Res Technol 227: 331-336. » CrossRef » PubMed » Google Scholar

19. Teo SK, Stirling DI, Thomas SD, Evans MG, Khetani VD (2003) A 90-day oral gavage toxicity study of d-methylphenidate and d,l-methylphenidate in beagle dogs. Int J Toxicol 22: 215-226. » CrossRef » PubMed » Google Scholar

20. Tsuruki T, Kishi K, Takahashi M, Tanaka M, Matsukawa T, et al. (2003) Soymetide an immunostimulating peptide derived from soybean β-conglycinin, is an fMLP agonist. FEBS Lett 540: 206-210. » CrossRef » PubMed » Google Scholar