Single-dose oral toxicity of fermented rice extracts (FREs): A 14-day observation

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Abstract: The aim of present research was to determine the acute oral toxicity of fermented rice extracts (FREs), in female and male ICR mice. To investigate the toxicity and identify target organs, FREs were orally administered once to male and female ICR mice at doses of 0 (vehicle control), 500, 1000, or 2000 mg/kg body weight (BW). Effects on mortality, BW, and clinical signs were monitored over 14 days, including changes in the weights and histopathological characteristics of 14 organs, as described in the Korea Food and Drug Administration (KFDA) Guidelines (2009-116, 2009). No treatment-related mortality was observed during the 14-day observation period in either gender. In addition, no FRE-related change was observed in BW or organ weight (OW), clinical indicators, or histopathological findings in this study. Our results suggest that the FRE is non-toxic in mice and is therefore likely to be safe for clinical use. The approximate LD and LD_{50} in mice after single oral dose of FRE are greater than 2000 mg/kg in female and male ICR mice. Additionally, no specific target organ or negative clinical indicator was detected in this study.

Keywords: Fermented rice extracts (FREs), acute toxicity, rat, histopathology.

INTRODUCTION

Rice (*Oryza sativa* L.) is a world-wide staple food and well known to possess no toxic side effects. In addition to its importance as a food, various rice derivatives are thought to have positive biological effects. *Makgeolli*, a Korean traditional rice wine prepared using *nuruk* (Korean traditional koji), contains a variety of microorganisms such as *Acetobacter aceti*, *Aspergillus niger*, *Aspergillus oryzae*, *Lactobacillus casei*, *Lactobacillus sakei subsp. sakei*, *Pediococcus pentosaceus*, *Saccharomyces cerevisiae*, *Weissella paramesenteroides*, among others (Yang & Lee 1996; Kwon & Sohn 2012). In contrast to raw rice, makgeolli is reported to have antioxidant (Wang *et al*., 2012), anti-hypertensive (Lee *et al*., (a) 2009) anti-diabetic (Lee *et al*., (b) 2009), and anti-cancer (Shin *et al*., 2008) effects. In particular, the fermented rice extracts (FREs) found in makgeolli have been demonstrated to show various pharmacological benefits, including antiosteoporotic (Cho *et al*., 2010), hypoglycaemic (Lu *et al*., 2010), antitumor (Ho *et al*., 2010), neuroprotective (Lee *et al*., 2010), antiatherosclerotic (Setnikar *et al*., 2005), and anti-stress and anti-fatigue (Kim *et al*., 2002) effects. These benefits are thought to be related to their probiotic effects against enterobacteria, perhaps decreasing the incidence of digestive disorders (Sindhu & Khetarpaul, 2002; Ghoneum & Gollapudi, 2005; Kataoka *et al*., 2008; Ogué-Bon *et al*., 2011).

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However, no information exists regarding the toxicity of FREs in mammals. For this reason, we first produced FREs using two microorganisms isolated from makgeolli, *Saccharomyces cerevisae* (strain No. ATCC 9804) and lactic acid bacteria *Weissella paramesenteroides* (strain No. KACC 91704). We then evaluated the toxicity of FREs in male and female ICR mice. To investigate toxicity and identify target organs, FREs were administered orally in a single dose to male and female mice and then monitored for 14 days; mortality, BW, and clinical signs were grossly observed during this period, whereas changes in organ weights and histopathological features were examined post-mortem, as per the KFDA Guidelines (2009-116, 2009).

MATERIALS AND METHODS

Animals

Forty ICR mice of both sexes, aged 6 weeks old, (SLC, Shizuoka, Japan) were treated after acclimatizing to laboratory conditions for 8 days. Animals were distributed 5 mice per a transparent plastic cage in a humidity (40-45%) and temperature (20-25°C) controlled room. Water and food (Samyang, Wonju, Korea) were supplied *ad libitum* and the light: dark cycle was 12: 12 h. Before the initial treatment and the final necroscopy, all animals were fasted overnight.

Preparation of fermented rice extracts (FREs)

The FRE used in this study was supplied by Glucan Corp. (Busan, Korea) as a brown powder. Briefly, FRE production was carried out in three fermentation steps. In the first fermentation step (saccharification), washed, non-

glutinous rice (1 kg) was soaked for 6 h, drained for 30 min, and then steamed for 15 min at 121°C. After cooling quickly, 10 g of malt powder and 4 L of water were added and the mixture was fermented in a 20-L sterile glass container at 55°C for 12 h. In the second fermentation step, 20 ml of *Saccharomyces cerevisae* (ATCC 9804) suspension was added to the first fermentate, mixed well, and incubated at 30°C for 48 h. Finally, in the third fermentation step, 20 ml of lactic acid bacteria, *Weissella paramesenteroides* (KACC 91704), was added to the second fermentate, mixed well, and incubated at 30°C for 48 h. This final fermentate was autoclaved at 150°C for 15 min, and then filtered through 40-mesh sieve to obtain the final filtrate, which was freeze-dried for 2 days until a moisture content of approximately 0.8% was obtained. The freeze-dried FREs were ground in a mill and passed through a 500-mesh sieve. The sieved material was stored at -20°C until use.

Yeast were cultured in YD medium supplemented with 2% yeast extract (Difco, NJ, USA) and 1% dextrose (Difco, USA) at 30°C for 48 h. Lactic acid bacteria were cultured in SYD medium supplemented 10% enzyme-degrading skim milk solution (skim milk degraded by 0.006% protease N [Sigma P7026; EC No. 232-642-4]), 2% yeast extract (Difco, USA), and 1% dextrose (Difco, USA) at 30°C for 48 h.

Treatment groups and FRE administration

Upon receipt, male and female mice were allocated into eight groups with five mice per group. Because no oral toxicological data currently exist, the highest dose was set at 2,000 mg/kg in a 20-ml volume of distilled water (vehicle), which is the recommended oral dose volume in mice (Flecknell, 1996; Organisation for Economic Co-operation and Development [OECD] Guidelines #423, 2001; KFDA Guidelines 2009-116, 2009). In addition, doses of 500 and 1000 mg/kg were selected as low and intermediate doses, as per the Korea Food and Drug Administration (KFDA) Guidelines (Notification No. 2009-116, 2009). Male and female control groups (vehicle only) were also observed. The test substance was single administered using a gastric feeding tube attached to a 5-ml syringe.

Observation of clinical signs

Clinical signs (general condition, motility, autonomic nervous response, and excretion) were assessed before and after dosing, as described in the functional observational battery test (Dourish, 1987; Irwin, 1968).

Body weight (BW) change

BWs were measured on day 0 immediately before treatment, and then on days 1, 2, 7, 13, and 14 days post-treatment. In addition, to reduce the influence of individual BW differences upon treatment, BW changes from days 0-7, 7-13 and 0-14 were also calculated.

Necropsy

All animals that survived to the end of the observation period were subjected to terminal necropsy. Animals were euthanized via carbon dioxide asphyxiation after overnight fasting (approximately 18 h with water *ad libitum*).

Target organs grossly observed included the heart, lung, kidney, thymus, adrenal glands, brain, liver, gonad (ovary/testis), pancreas, spleen, epididymis/uterus, urinary bladder, gastrointestinal tract, skin, prostate, urinary bladder, and submandibular lymph nodes.

Organ weight (OW)

The absolute OW was measured and then the relative OW (% of BW) was calculated for each of the following 14 organs: lung, heart, thymus, left kidney, liver, left adrenal gland, spleen, left testis/ovary, splenic lobe of the pancreas, brain, left epididymis/total uterus, and left submandibular lymph node.

Histopathological observation

The target organs listed below were sampled at terminal necropsy, and fixed in 10% neutral-buffered formalin. Organs were embedded in paraffin and 3-4 μm sections were prepared using routine histological methods, after 18 h of fixation. Representative sections of each organ were stained with hematoxylin and eosin and examined under a light microscope. The 14 target organs included the lung (left lateral lobe), heart, liver (left lateral lobe), thymus, left kidney, left adrenal gland, left testis/ovary, spleen, left gonad, splenic lobe of the pancreas, brain, epididymis (head of left side)/total uterus, and the left submandibular lymph node.

Statistics

Multiple comparison tests among the treatment groups were performed. Variance homogeneity was evaluated using the Levene test. If the Levene test indicated no significant deviation from variance homogeneity, the data were analyzed using one-way analysis of variance (ANOVA) followed by the Scheffe test to determine whether pair-wise comparisons were significantly different. In case of a significant deviation from variance homogeneity, the non-parametric Kruskal-Wallis *H*-test was performed, followed by a Mann-Whitney *U*-test for pair-wise comparison. The LD_{50} and 95% confidence limits were calculated using the probit method.

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS for Windows (Release 14.0K, SPSS Inc., USA). In addition, the degree change in clinical signs as well as gross and histopathological findings were scored as follows: 1, slight; 2, moderate; 3, severe.

RESULTS

Mortality

No FRE-related mortality was observed in any treatment group in either gender; all animals (5/5; 100%) in the FRE treatment groups survived the 14-day observation period and all mice were subjected to the terminal necropsy.

Clinical signs

In any treatment group, no FRE-related clinical signs in either gender were observed.

Changes in BW

No significant change in BW was observed in any treatment group compared to the gender-matched control (fig. 1).

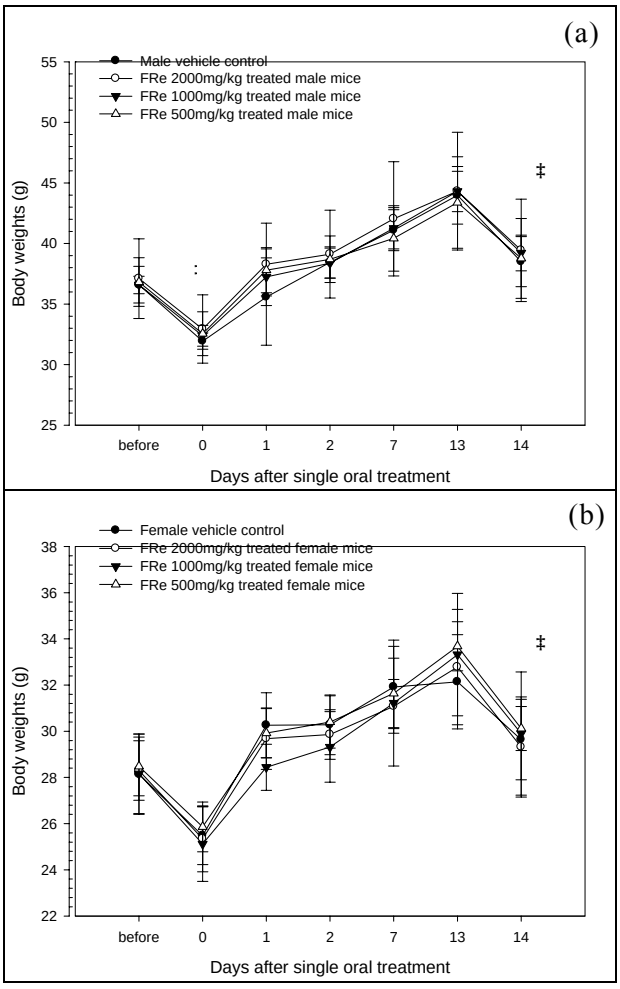


Fig. 1: Changes in BW over 14 days in male (a) and female (b) mice after a single oral FRE treatment.

No meaningful change in BW was detected in any treatment group in either gender compared to the vehicle control. Values are expressed as the means ± standard deviations (SD) of data from five rats. All mice were fasted overnight. (‡) indicates 1 day before treatment; 0 indicates just before treatment; FRE, fermented rice extracts. Vehicle control (●), FRE 2000 mg/kg (○), FRE 1000 mg/kg (▼) and FRE 500 mg/kg (△).

Changes in OW

No significant change in the weight of any organ was observed in any treatment group compared to the gender-matched control (tables 1 and 2).

Necropsy findings

Varying (slight to severe) degrees of thymic atrophy, lung congestion, splenic atrophy or hypertrophy, edematous changes of the uterus, and hypertrophy of submandibular lymph nodes were sporadically detected in all tested groups, including the vehicle controls (tables 3 and 4).

Histopathological findings

Varying (slight to severe) degrees of organ damage were sporadically detected in all tested groups, including the vehicle controls. Among these were incidences of lung congestion (thickening of alveoli and lung inflammatory cell infiltration with focal hemorrhages, fig. 2), focal decreases of lymphoid cells in the thymic cortex (fig. 3), hyperplasia of lymphoid cells in the red pulp of the spleen (fig. 4), focal inflammatory cell infiltration in the liver parenchyma (fig. 5), and diffuse hyperplasia of lymphoid cells in the submandibular lymph nodes (fig. 6). In addition, slight hyperplasia of lymphoid cells in the white pulp of the spleen (fig 4) was detected in one male (1/5; 20%) within the 500mg/kg treatment group (tables 3 and 4).

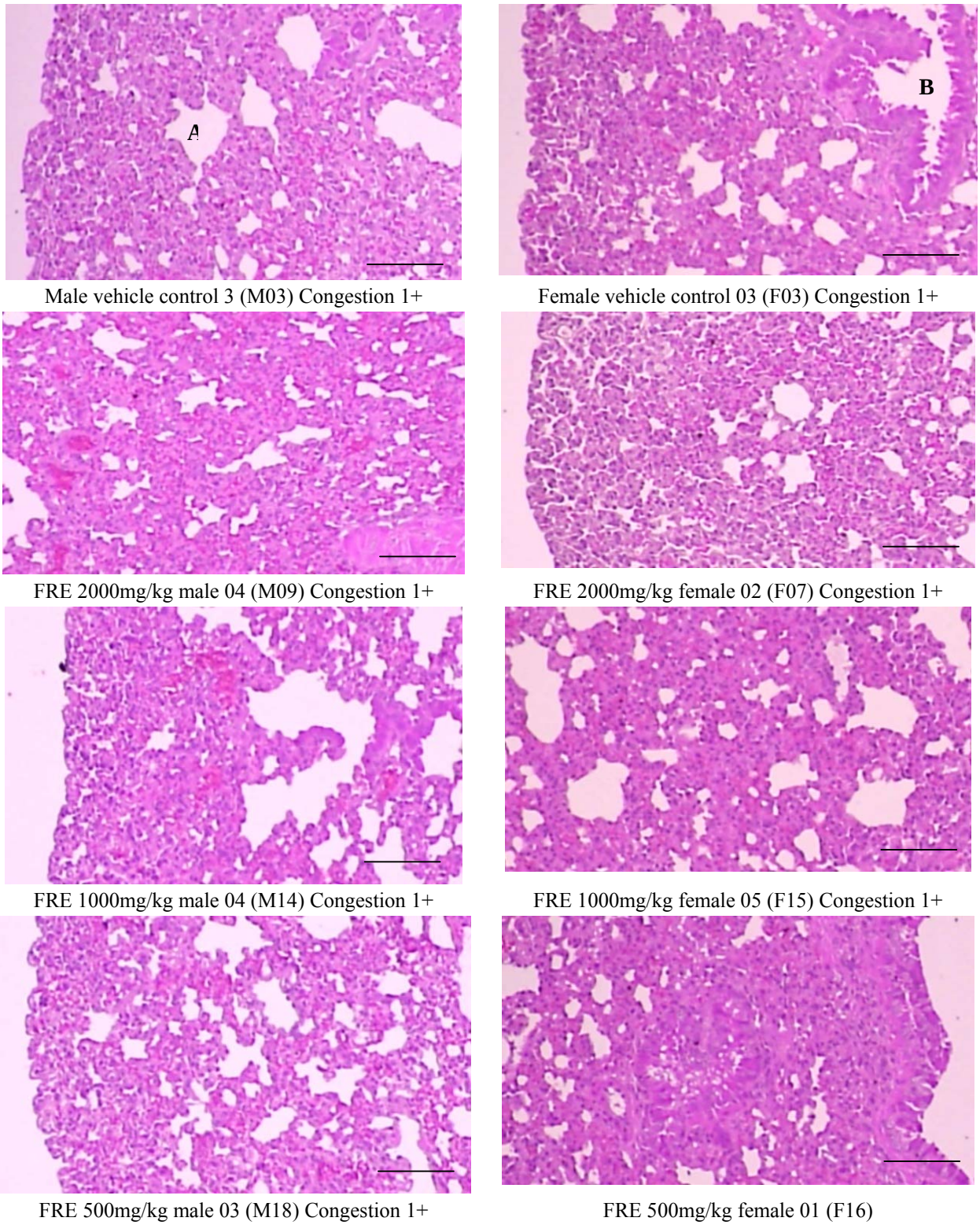
LD_{50}, approximate LD, and target organs

The LD_{50} and the approximate LD of FREs after a single oral treatment were each >2000 mg/kg in both female and male mice, as no FRE-related mortality was observed after administration of this maximal dose. In addition, the administration of a single dose resulted in no adverse effects in terms of clinical signs or damage to the indicated target organs.

DISCUSSION

In this study, we examined the single-dose oral toxicity of FREs in male and female ICR mice at doses of 0 (vehicle control), 500, 1000 and 2000 mg/kg. Mortality and changes in BW and clinical signs were monitored for 14 days after treatment.

According to the KFDA (Notification 2009-116, 2009) and OECD (#423, 2001) Guidelines, the recommended maximum dose for an unknown substance is 2000 mg/kg. It is also recommended to maintain the maximum dose volume below 20 ml/kg in mice undergoing acute toxicity tests (Flecknell, 1996). Accordingly, we examined several doses up to a maximum dose of 2000 mg/kg delivered in



Male vehicle control 3 (M03) Congestion 1+ | Female vehicle control 03 (F03) Congestion 1+

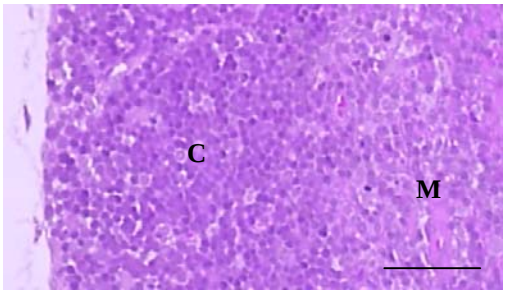
FRE 2000mg/kg male 04 (M09) Congestion 1+ | FRE 2000mg/kg female 02 (F07) Congestion 1+

FRE 1000mg/kg male 04 (M14) Congestion 1+ | FRE 1000mg/kg female 05 (F15) Congestion 1+

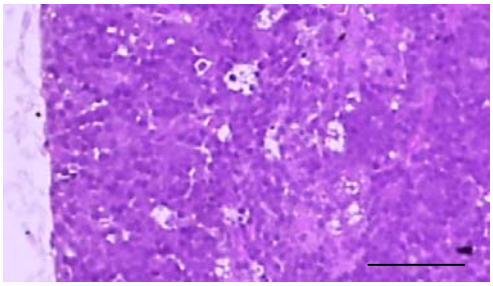
FRE 500mg/kg male 03 (M18) Congestion 1+ | FRE 500mg/kg female 01 (F16)

Fig. 2: Histopathological changes in the lung.

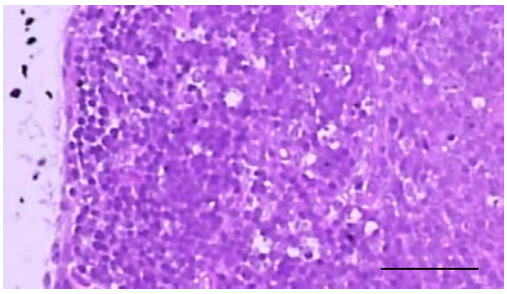
Note that slight (1) focal lung congestion (indicated by thickening of alveoli and inflammatory cell infiltration with/without focal hemorrhages) was detected randomly in all groups, including the vehicle control.
A, alveolar sac-respiratory bronchiole; B, primary bronchiole; FRE, fermented rice extracts. Hematoxylin and eosin stain; Scale bar = 160 µm.



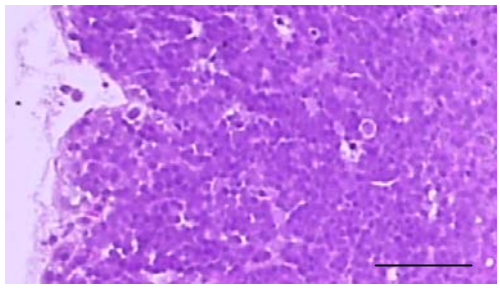
Male vehicle control 03 (M03) Congestion 1+



Female vehicle control 04 (F04) Congestion 1+



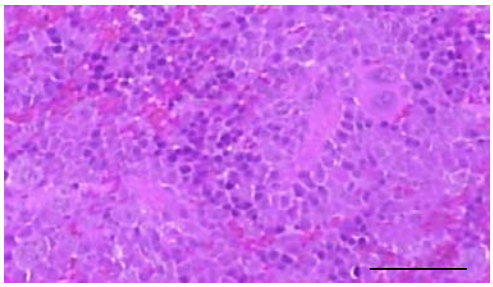
FRE 2000mg/kg female 05 (F10) Congestion 1+



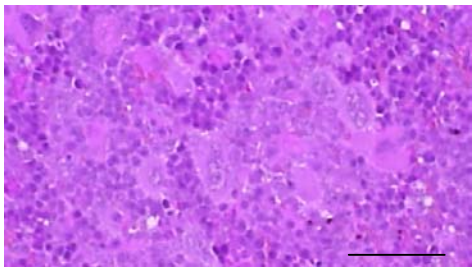
FRE 500mg/kg male 04 (M19) Congestion 1+

Fig. 3: Histopathological changes in the thymus.

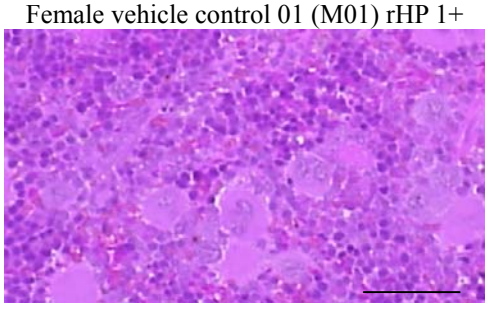
Note that slight (1) focal decreases in lymphoid cells in the thymic cortex (cDE, as indicated by empty vacuoles or loosening between the thymic cortex lymphoid cells) were detected in only one female mouse in the 2000 mg/kg treatment group and one male in the 500 mg/kg group. The lack of dose-dependency in these effects indicates that they were not related to FRE treatment.
C, cortex; M, medulla; FRE, fermented rice extracts. Hematoxylin and eosin stain; Scale bar = 160 µm.



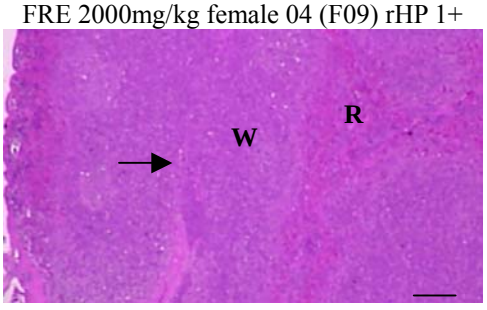
Female vehicle control 01 (M01) rHP 1+



FRE 2000mg/kg female 04 (F09) rHP 1+



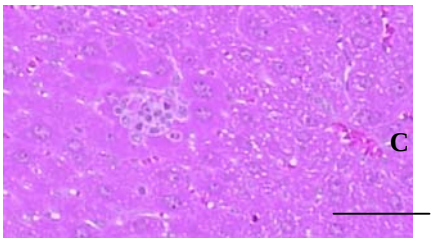
FRE 1000mg/kg female 02 (F12) rHP 1+



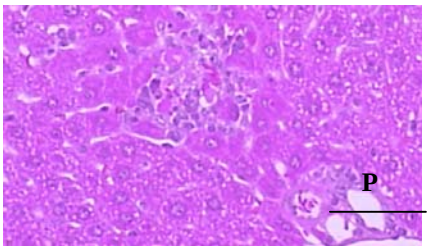
FRE 500mg/kg male 05 (M20) wDE 1+

Fig. 4: Histopathological changes in the spleen.

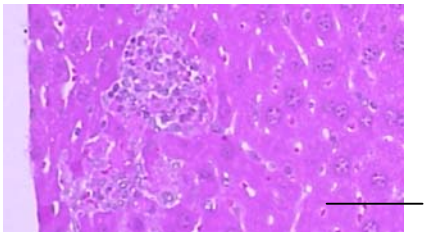
Note that slight (1) hyperplasia of lymphoid cells in the red pulp of the spleen (rHP) were randomly detected throughout the female groups, including the vehicle control. In addition, slight hyperplasia of lymphoid cells in the white pulp of the spleen (wDE) was detected in only one male (1/5; 20%) mouse in the 500 mg/kg treated group. The lack of dose-dependency in these effects indicates that they were not related to FRE treatment.
Arrow, central arteriole; W, white pulp; R, red pulp; FRE, fermented rice extracts. Hematoxylin and eosin stain; Scale bar = 160 µm.



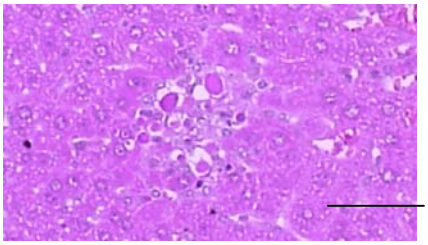
Male vehicle control 03 (M03) Focal inflammatory cell infiltration 1+



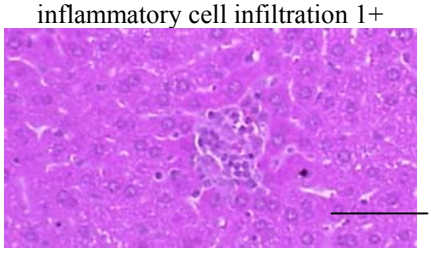
Female vehicle control 03 (F03) Focal inflammatory cell infiltration 1+



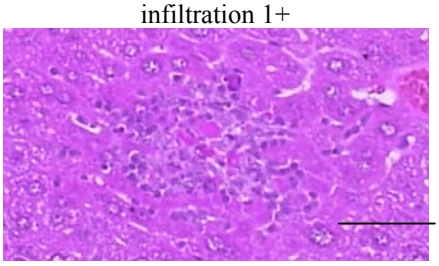
FRE 2000mg/kg Male 02 (M07) Focal inflammatory cell infiltration 1+



FRE 2000mg/kg female 01 (F06) Focal inflammatory cell infiltration 1+



FRE 1000mg/kg female 01 (M11) Focal inflammatory cell infiltration 1+

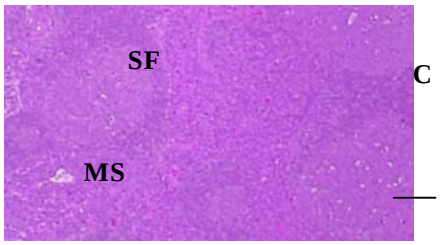


FRE 500mg/kg female 05 (F20) Focal inflammatory cell infiltration 1+

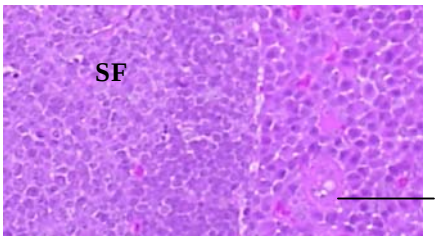
Fig. 5: Histopathological changes in the liver.

Note that slight (1) focal inflammatory cell infiltration in the liver parenchyma was randomly detected throughout most groups, including the vehicle control. This indicates that these effects were not FRE-related toxicological signs.

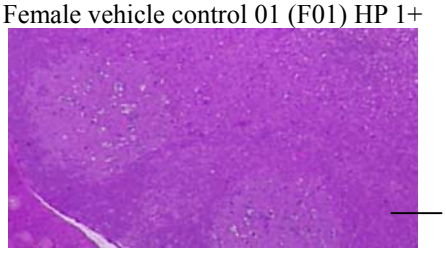
C, central vein; P, portal triad; FRE, fermented rice extracts. Hematoxylin and eosin stain; Scale bar = 160 µm.



Female vehicle control 01 (F01) HP 1+



Enlarged images of left column



Female vehicle control 01 (F01) HP 1+

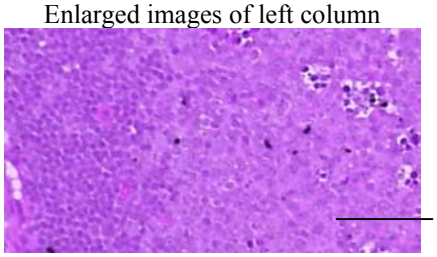


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Fig. 6: Histopathological changes in the submandibular lymph nodes.

Note that varyingly (slight to severe, 1–3) diffuse hyperplasia of lymphoid cells in the submandibular lymph nodes (HP) was observed in two female mice (2/5; 40%) in the vehicle control, as well as in the 2000 mg/kg treated group, making it unlikely that this effect was the result of FRE treatment.

C, cortex; MS, medullary sinus; SF, secondary lymphatic follicle; FRE, fermented rice extracts. Hematoxylin and eosin stain; Scale bar = 160 µm.

Table 1: Changes in absolute and relative organ weights in male mice after a single oral FRE treatment

Groups	Changes on the Absolute Organ Weights											
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Testis L	Liver	Pancreas L	Brain	Epididymis L	LN L
Control	0.201± 0.016	0.185± 0.017	0.058± 0.008	0.293± 0.018	0.003± 0.002	0.120± 0.017	0.105± 0.019	1.564± 0.111	0.177± 0.019	0.463 ±0.011	0.045 ±0.004	0.007 ±0.004
FRE-treated group												
2000 mg/kg	0.199± 0.021	0.191± 0.011	0.056 ±0.015	0.333± 0.054	0.004± 0.002	0.154 ±0.041	0.109± 0.012	1.659± 0.171	0.188± 0.028	0.475 ±0.012	0.047 ±0.005	0.007 ±0.003
1000 mg/kg	0.204± 0.012	0.185± 0.019	0.065± 0.014	0.298± 0.030	0.006± 0.003	0.140± 0.026	0.125± 0.030	1.608± 0.053	0.167 ±0.018	0.478 ±0.030	0.050 ±0.004	0.005 ±0.001
500 mg/kg	0.191± 0.011	0.186 ±0.013	0.066± 0.026	0.318± 0.023	0.004± 0.002	0.121 ±0.021	0.116± 0.009	1.631± 0.140	0.182 ±0.015	0.471 ±0.015	0.045 ±0.005	0.005 ±0.003
Groups	Changes on the Relative Organ Weights											
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Testis L	Liver	Pancreas L	Brain	Epididymis L	LN L
Control	0.522 ±0.033	0.483 ±0.055	0.151 ±0.026	0.762 ±0.071	0.009 ±0.004	0.312 ±0.054	0.272 ±0.048	4.059 ±0.087	0.462 ±0.063	1.206 ±0.078	0.116 ±0.012	0.019 ±0.011
FRE-treated group												
2000mg/kg	0.507 ±0.049	0.487 ±0.034	0.142 ±0.041	0.846 ±0.117	0.010 ±0.004	0.392 ±0.109	0.278 ±0.043	4.213 ±0.234	0.476 ±0.046	1.214 ±0.107	0.121 ±0.020	0.017 ±0.006
1000mg/kg	0.520 ±0.029	0.471 ±0.033	0.166 ±0.032	0.759 ±0.065	0.015 ±0.007	0.356 ±0.068	0.319 ±0.074	4.105 ±0.239	0.425 ±0.036	1.220 ±0.079	0.128 ±0.014	0.013 ±0.003
500mg/kg	0.495 ±0.029	0.482 ±0.050	0.169 ±0.060	0.825 ±0.085	0.009 ±0.004	0.312 ±0.035	0.301 ±0.029	4.216 ±0.294	0.472 ±0.051	1.223 ±0.115	0.115 ±0.010	0.012 ±0.009

Values are expressed as the means ± standard deviations (SD) of data from five rats. L, left side; S, splenic lobes; G, gland; LN, submandibular lymph node; FRE, fermented rice extracts.

Table 2: Changes in absolute and relative organ weights observed in female mice after a single oral FRE treatment

Groups	Changes on the Absolute Organ Weights											
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Ovary L	Liver	Pancreas S	Brain	Uterus	LN L
Control	0.171 ±0.014	0.137 ±0.011	0.076 ±0.016	0.169 ±0.022	0.006 ±0.002	0.172 ±0.075	0.018 ±0.007	1.200 ±0.086	0.157 ±0.020	0.461 ±0.027	0.161 ±0.041	0.026 ±0.040
FRE-treated group												
2000mg/kg	0.179 ±0.018	0.138 ±0.011	0.077 ±0.024	0.170 ±0.024	0.006 ±0.001	0.170 ±0.031	0.017 ±0.005	1.220 ±0.065	0.147 ±0.018	0.461 ±0.015	0.166 ±0.036	0.015 ±0.008
1000mg/kg	0.181 ±0.012	0.131 ±0.011	0.073 ±0.009	0.176 ±0.023	0.008 ±0.003	0.143 ±0.021	0.016 ±0.002	1.266 ±0.205	0.159 ±0.011	0.453 ±0.016	0.184 ±0.033	0.008 ±0.005
500mg/ kg	0.169 ±0.011	0.134 ±0.013	0.067 ±0.007	0.175 ±0.012	0.006 ±0.003	0.129 ±0.016	0.020 ±0.005	1.258 ±0.042	0.155 ±0.009	0.480 ±0.024	0.116 ±0.034	0.006 ±0.003
Groups	Changes on the Relative Organ Weights											
	Lung	Heart	Thymus	Kidney L	Adrenal G L	Spleen	Ovary L	Liver	Pancreas S	Brain	Uterus	LN L
Control	0.578 ±0.049	0.461 ±0.029	0.257 ±0.046	0.570 ±0.068	0.020 ±0.006	0.579 ±0.246	0.061 ±0.026	4.049 ±0.135	0.530 ±0.075	1.557 ±0.053	0.547 ±0.149	0.086 ±0.135
FRE-treated group												
2000mg/kg	0.611 ±0.034	0.469 ±0.022	0.261 ±0.080	0.578 ±0.052	0.019 ±0.003	0.585 ±0.125	0.059 ±0.018	4.171 ±0.274	0.500 ±0.030	1.578 ±0.110	0.566 ±0.116	0.054 ±0.028
1000mg/kg	0.609 ±0.041	0.440 ±0.029	0.246 ±0.044	0.588 ±0.029	0.028 ±0.010	0.480 ±0.063	0.054 ±0.006	4.221 ±0.409	0.532 ±0.032	1.525 ±0.137	0.618 ±0.110	0.028 ±0.016
500mg/kg	0.562 ±0.034	0.446 ±0.035	0.224 ±0.030	0.580 ±0.045	0.019 ±0.008	0.427 ±0.054	0.066 ±0.019	4.182 ±0.245	0.516 ±0.045	1.594 ±0.080	0.384 ±0.106	0.020 ±0.011

Values are expressed as the means ± standard deviations (SD) of data from five rats. L, left side; S, splenic lobes; G, gland; LN, submandibular lymph node; FRE, fermented rice extracts

a volume of 20 ml of pure water, with low and intermediate doses of 500 and 1000 mg/kg, as also recommended by the KFDA Guidelines (Notification No. 2009-116, 2009). No treatment-related mortality was observed within 14 days. In addition, no FRE-related changes in body or organ weight, clinical signs, or histopathological findings were detected. All treated and age-matched control mice showed normal BW and OWchanges, as reported previously (Tajima, 1989; Fox *et al*., 1984).

Table 3: Necropsy and histopathological findings in male mice after a single oral FRE treatment

Necropsy Findings at Sacrifice (Day 14)		Vehicle control	FRE treated as		
			2000 mg/kg	1000 mg/kg	500 mg/kg
Lung	Normal	3/5	4/5	4/5	4/5
	Congestion	2/5	1/5	1/5	1/5
Thymus	Normal	3/5	5/5	4/5	4/5
	Atrophy	2/5	0/5	1/5	1/5
Spleen	Normal	4/5	4/5	5/5	4/5
	Atrophy	1/5	1/5	0/5	1/5
Liver	Normal	5/5	5/5	5/5	5/5
Lymph node [a)]	Normal	4/5	3/5	4/5	5/5
	Hypertrophy	1/5	2/5	1/5	0/5
Others	Normal	5/5	5/5	5/5	5/5
Histopathological Findings at Sacrifice (Day 14)		Vehicle control	FRE treated as		
			2000 mg/kg	1000 mg/kg	500 mg/kg
Lung	Normal	3/5	4/5	4/5	4/5
	Congestion	2/5	1/5	1/5	1/5
Thymus	Normal	4/5	5/5	5/5	4/5
	cDE*	1/5	0/5	0/5	1/5
Spleen	Normal	5/5	5/5	5/5	4/5
	wHP**	0/5	0/5	0/5	1/5
Liver	Normal	4/5	4/5	5/5	5/5
	Focal inflamma-tory cell infilteration	1/5	1/5	0/5	0/5
Lymph node [a)]	Normal	5/5	5/5	5/5	5/5

Values are expressed as the observed animals/total observed animals (five mice per group); [a)] Bilateral submandibular lymph node; *cDE, decreases in lymphoid cells in the thymic cortex; **wHP, hyperplasia of lymphoid cells in the splenic white pulp; FRE, fermented rice extracts.

Histopathological examination revealed varying degrees of thymic atrophy, lung congestion, splenic atrophy or hypertrophy, edematous changes to the uterus, and hypertrophy of submandibular lymph nodes, as well as focal decreases in lymphoid cells in the thymic cortex, hyperplasia of lymphoid cells in the white pulp of the spleen, focal inflammatory cell infiltration in the liver parenchyma, and diffuse hyperplasia of lymphoid cells in the submandibular lymph nodes. Because these findings were sporadically detected in all treatment and control groups, as well as in both genders, they were not considered to be related to FRE treatment. In fact, these signs are commonly observed in normal mice (Hasechek and Rousseaux, 1998; Greaves, 1990; Boorman *et al*., 1990). For example, edematous changes to the uterus are the result of individual progression through the estrus cycle (Banks, 1986; Pineda, 1989). Hyperplasia of lymphoid cells in the white pulp of the spleen was restricted to just one male mouse in the 500 mg/kg treatment group, and no other; the lack of dose-dependency indicates that this occurrence was not related to FRE treatment.

Table 4: Necropsy and histopathological findings in female mice after a single oral FRE treatment

Necropsy Findings at Sacrifice (Day 14)		Vehicle control	FRE treated as		
			2000 mg/kg	1000 mg/kg	500 mg/kg
Lung	Normal	3/5	4/5	4/5	4/5
	Congestion	2/5	1/5	1/5	1/5
Thymus	Normal	4/5	4/5	5/5	5/5
	Atrophy	1/5	1/5	0/5	0/5
Spleen	Normal	4/5	4/5	4/5	5/5
	Atrophy	0/5	0/5	0/5	0/5
	Hypertrophy	1/5	1/5	1/5	0/5
Liver	Normal	5/5	5/5	5/5	5/5
Uterus	Normal	2/5	2/5	2/5	4/5
	Edematous changes	3/5	3/5	3/5	1/5
Lymph node [a)]	Normal	3/5	3/5	4/5	4/5
	Hypertrophy	2/5	2/5	1/5	1/5
Others	Normal	5/5	5/5	5/5	5/5
Histopathological Findings at Sacrifice (Day 14)		Vehicle control	FRE treated as		
			2000 mg/kg	1000 mg/kg	500 mg/kg
Lung	Normal	3/5	4/5	4/5	4/5
	Congestional spot	2/5	1/5	1/5	1/5
Thymus	Normal	4/5	4/5	5/5	5/5
	cDE*	1/5	1/5	0/5	0/5
Spleen	Normal	3/5	4/5	4/5	4/5
	rHP**	2/5	1/5	1/5	1/5
Liver	Normal	3/5	3/5	3/5	3/5
	Focal infla-mmatory cell infilteration	2/5	2/5	2/5	2/5
Uterus	Normal	5/5	5/5	5/5	5/5
Lymph node [a)]	Normal	3/5	3/5	5/5	5/5
	Hyperplasia of lymphoid cells	2/5	2/5	0/5	0/5

Values are expressed as observed animals/total observed animals (five mice per group); [a)] Bilateral submandibular lymph node; *cDE, decreases in lymphoid cells in the thymic cortex; **rHP, decreases in lymphoid cells in the splenic white pulp; FRE, fermented rice extracts.

CONCLUSION

The results described here indicate that the FREs are non-toxic in miceand are therefore likely to be safe for clinical use. The LD_{50} and approximate LD in mice after a single oral dose of FRE were considered to be greater than 2000

mg/kg in both females and males. Additionally, no damage to target organs or other clinical sign was detected.

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