The Japanese Society of Toxicology

Fundamental Toxicological Sciences

URL : http://www.fundtoxicolsci.org/index_e.html

Original Article

Genotoxicity and subchronic toxicity of a kaempferol aglycone-rich product produced from horseradish leaves

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(Received April 15, 2022; Accepted April 20, 2022)

ABSTRACT — Kaempferol is a kind of natural flavonoid in many edible plants and reportedly has various physiological effects. In the present study, we conducted the genotoxicity (*in vitro* and *in vivo*) and 13-week subchronic toxicity studies of a new product, a kaempferol aglycone-rich food produced from enzyme-treated horseradish leaves, to evaluate its safety. In the bacterial reverse mutation test, the kaempferol aglycone-rich product showed positive results in some *Salmonella typhimurium* strains in the presence or absence of metabolic activation as well as other flavonoids. However, it did not increase micronucleated polychromatic erythrocytes taken from male Sprague−Dawley (SD) rats administered orally by gavage up to 4000 mg/kg for 2 consecutive days. In the 13-week subchronic toxicity study in SD rats, the kaempferol aglycone-rich product was orally administered by gavage once daily to SD rats for 13 weeks (91 days) at a dose of 500, 1000, or 2000 mg/kg/day. No toxic changes were observed at up to 2000 mg/kg/day. In conclusion, these findings indicated that the kaempferol aglycone-rich product was not genotoxic *in vivo*. The no-observed-adverse-effect level for both male and female rats was 2000 mg/kg/day, the highest dose tested, in the 13-week subchronic toxicity study in rats, suggesting it is safe for use as a food.

Key words: Kaempferol aglycone-rich product, Food, Horseradish leaves, Genotoxicity, Subchronic toxicity

INTRODUCTION

Flavonoids are a family of polyphenolic compounds and are contained in many fruits, vegetables, and beverages (Yoshida *et al*., 2008), with over 8000 compounds known (Pietta, 2000). Kaempferol is a natural flavonoid in many edible plants such as tea, grapefruit, broccoli, cabbage, kale, tomato, strawberry, mustard, quinoa, and horseradish (Calderón-Montaño *et al*., 2011). This flavonoid has various physiological effects such as involvement in mitochondrial function (Montero *et al*., 2004), thyroid hormone activation (da-Silva *et al*., 2007), and various anticancer potentials (Sharma *et al*., 2007; Bestwick *et al*., 2007; Li *et al*., 2017; Mylonis *et al*., 2010). Kaempferol could potentially counteract hypoxia in the body by increasing intracellular ATP content, suggesting that dietary intake might improve muscular performance declines (Mizokami *et al*., 2021). Therefore, kaempferol should benefit human health as a functional food ingredient.

Recently, a new product, a kaempferol aglycone-rich food produced from enzyme-treated horseradish leaves, was developed by Otsuka Pharmaceutical Co., Ltd. Kaempferol aglycone, an active ingredient in the new product, is in many edible plants, and people have probably been consuming it from edible plants, for many years. However, there are limited data regarding its safety to the best

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of our knowledge. In the present study, we conducted the new product's genotoxicity and 13-week subchronic toxicity studies to evaluate its safety.

MATERIALS AND METHODS

Genotoxicity and subchronic toxicity studies complied with Good Laboratory Practice (GLP) Standards for Nonclinical Safety Studies on Drugs (Ordinance No. 21 issued by the Ministry of Health and Welfare [MHW], Japan, on 26 March 1997). The genotoxicity studies were carried out under the following guidelines: Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use (Notification 0920, No. 2) issued by the Ministry of Health, Labour and Welfare, Japan, 20 September, 2012; Organisation for Economic Co-operation and Development (OECD) Guideline for the Testing of Chemicals Test No. 474 (OECD, 2016); OECD Guideline for Testing of Chemicals Test No. 471 (OECD, 1997). The subchronic toxicity study was carried out under the revisions of the Guidelines for Toxicity Studies of Drugs: Partial Revision of the Guidelines for Repeated Dose Toxicity Studies (Notification No. 655) issued by the MHW, Japan, on 05 April 1999. The animal experiment committee of Otsuka Pharmaceutical Co., Ltd. or contract vendor approved all animal studies.

Test article

The kaempferol aglycone-rich product was supplied for each study by the Saga Nutraceuticals Research Institute of Otsuka Pharmaceutical Co., Ltd. (Saga, Japan). The test article was produced from enzyme-treated horseradish leaves and contained approximately 16.8% kaempferol aglycone as an active ingredient. The test article was suspended in 5% gum arabic solution (gum arabic: FUJIFILM Wako Pure Chemical Corp., Osaka, Japan, water for injection: Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) for subchronic toxicity and *in vivo* genotoxicity studies. It was dissolved in dimethyl sulfoxide (DMSO, FUJIFILM Wako Pure Chemical Corp.) for *in vitro* genotoxicity study.

Bacterial reverse mutation test

The *Salmonella typhimurium* strains (TA98, TA100, TA1535, and TA1537) and the *Escherichia coli* strain (WP2 *uvrA*) were obtained from the Division of Genetics and Mutagenesis, National Institute of Health Sciences (Kanagawa, Japan). The test article solution was prepared before use for each assay. The negative control article was DMSO. The positive control articles were as follows: furylfuramide (AF-2, FUJIFILM Wako Pure Chemical Corp.), sodium azide (SAZ, FUJIFILM Wako Pure Chemical Corp.), acridine mutagen ICR-191 (ICR-191, Polysciences, Inc., Warrington, USA), 2-aminoanthracene (2AA, FUJIFILM Wako Pure Chemical Corp.), and benzo[a]pyrene (B[*a*]P, FUJIFILM Wako Pure Chemical Corp.). The vehicle for the positive control articles was DMSO except for SAZ (water for injection).

Two independent assays were performed: the dose-finding and the main tests. The pre-incubation method conducted the assay in the presence and absence of metabolic activation (+S9 or −S9). Table 1 shows the doses of the test and positive control articles for each bacterial strain.

Frozen bacterial suspensions were thawed, grown for 9 hr at 37°C in a nutrient medium (Nutrient Broth No. 2, OXOID Ltd., Hampshire, UK), and used at a concentration of 1.0×10^9 cells/mL or more. The test formulations (0.1 mL each) were placed in a test tube, followed by either 0.5 mL of 0.1 M sodium phosphate buffer (pH 7.4, FUJIFILM Wako Pure Chemical Corp.) or 0.5 mL of the S9 mix for metabolic activation. After 0.1 mL of bacterial suspension was added to each tube, the tubes were incubated at 37°C for 20 min. Two mL of warmed top agar containing 0.5 mM L-histidine, D-biotin, and L-tryptophan (FUJIFILM Wako Pure Chemical Corp.) were then added to each tube and were poured immediately onto glucose agar plates. Each condition was tested in triplicate for the test articles and negative control articles or duplicate for the positive control articles. All plates were incubated at 37°C for 48 hr (the dose-finding test) or 48.5 hr (the main test) before counting.

Each test plate was observed for growth inhibition and checking the presence or absence of a precipitate, and revertant colonies were counted visually or using a colony counter. The test article was considered positive for mutagenicity when the numbers of revertant colonies in the test article groups were greater than twice the values for the negative control group, and the values increased in a dose-dependent manner. Data were not analyzed statistically.

Rat bone marrow micronucleus test

Male Sprague−Dawley (SD) rats, supplied by the Atsugi breeding center of Charles River Laboratories Japan, Inc., were used for *in vivo* genotoxicity study. All animals were clinically monitored at the time of delivery to the testing facility and during the acclimation period of 7 days or more. Two or three animals in each group were housed in plastic cages with bedding (ConfiNest, Falma Co., Ltd., Tokyo, Japan) in an animal room that was controlled to maintain the temperature at 22°C–23°C with relative humidity at 45%–52%, air ventilation at 10–15 times/hr, and a 12-hr light period (7:00 a.m. to 7:00 p.m.). Each

Table 1. The experimental condition of bacterial reverse mutation test.

Dose-finding test				
Strain	Test article		Positive control	
	Dose (μg/plate)			
	−S9	+S9	−S9	+S9
TA100	5000, 1500, 500, 150, 50, 15, 5, 1.5, and 0 (DMSO)	5000, 1500, 500, 150, 50, 15, 5, 1.5, and 0 (DMSO)	AF-2: 0.01	B[*a*]P: 5.0
TA1535			SAZ: 0.5	2AA: 2.0
WP2 *uvrA*			AF-2: 0.01	2AA: 10.0
TA98			AF-2: 0.1	B[*a*]P: 5.0
TA1537			ICR-191: 1.0	B[*a*]P: 5.0
Main test				
Strain	Test article		Positive control	
	Dose Level (μg/plate)			
	−S9	+S9	−S9	+S9
TA100	1500, 750, 375, 188, 93.8, 46.9, and 0 (DMSO)	1500, 750, 375, 188, 93.8, 46.9, 23.4, 11.7, 5.86, 2.93, and 0 (DMSO)	AF-2: 0.01	B[*a*]P: 5.0
TA1535		1500, 750, 375, 188, 93.8, 46.9, and 0 (DMSO)	SAZ: 0.5	2AA: 2.0
WP2 *uvrA*			AF-2: 0.01	2AA: 10.0
TA98		1500, 750, 375, 188, 93.8, 46.9, 23.4, 11.7, 5.86, 2.93, and 0 (DMSO)	AF-2: 0.1	B[*a*]P: 5.0
TA1537			ICR-191: 1.0	B[*a*]P: 5.0

animal was allowed free access to a pelleted diet (CR-LPF, sterilized by radiation, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water via water bottles. The animals were assigned to 5 groups of 5 animals by computer block randomization based on body weight measured on allocation day. Eight-week-old rats at the start of dosing were used.

The test article was orally administered by gavage to rats for 2 consecutive days (at a 24-hr interval) at a dose of 1000, 2000, or 4000 mg/kg/day. The negative control group was administered vehicle (5% gum arabic solution) in the same manner. The positive control group was administered a single intraperitoneal dose of mitomycin C (MMC, Kyowa Kirin Co., Ltd., Tokyo, Japan) at 2 mg/kg.

The animals were sacrificed 24 hr after the final dosing, and their femurs were removed. Bone marrow was isolated from the femur, and bone marrow smear slides were prepared, dried, and fixed in methanol (2 slides per animal). From each animal, 1 slide exhibiting the better smear condition out of 2 slides prepared was selected and stained with acridine orange. The incidence of polychromatic erythrocyte (PCE) per 500 total erythrocytes (PCE + normochromatic erythrocyte [NCE]) and that of micronucleated polychromatic erythrocyte (MNPCE) per 4000 PCEs were examined.

The significance of the incidence of MNPCE was assessed using the exact probability test of Fisher (level of significance: 5%, one-tailed). Bartlett's test was conducted to examine the homogeneity of variance (level of significance: 1%) for the incidence of PCE. Since the variance was homogeneous, Dunnett's test was conducted (levels of significance: 1% and 5%, two-tailed).

Subchronic toxicity study

Male and female SD rats, supplied by the Atsugi breeding center of Charles River Laboratories Japan, Inc., were used for the 13-week subchronic oral toxicity study. All animals were clinically monitored at the time of delivery to the testing facility and during the acclimation period of 18 days. Two or three animals in each group were housed in plastic cages with bedding (ALPHA-dri, Shepherd Specialty Papers, Inc., MI, USA) in an animal room that was controlled to maintain the temperature at 22°C–24°C with relative humidity at 39%–53%, air ventilation at 10–15 times/hr, and a 12-hr light period (7:00 a.m. to 7:00 p.m.). Each animal was allowed free access to a pelleted diet (CR-LPF, sterilized by radiation, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water via an automatic watering device. Six-week-old rats at the start of dosing were used. The animals were assigned to

4 groups of 10 animals per sex by computer block randomization based on weight measured on allocation day.

The test article was orally administered by gavage once daily to rats for 13 weeks (91 days) at a dose of 500, 1000, or 2000 mg/kg/day. The vehicle control group was administered vehicle (5% gum arabic solution) in the same manner.

Animals were observed twice daily for clinical signs before dosing and 1−2 hr after dosing during the dosing period, except on weekends. On weekends, the animals were observed once daily before dosing. Body weights and food consumption were measured weekly. At the final week (Week 13), 5 males and 5 females from each group were selected and underwent an ophthalmologic examination, which included gross examinations of the anterior portion and ocular fundus. At Weeks 7 and 13, 5 males and 5 females of each group were selected, and urinalysis was performed. Urine was collected from fasted rats for 4 hr, followed by 20 hr from the same rats (not fasted). On the day after the final dosing, blood was collected from the posterior vena cava of all animals fasted under isoflurane anesthesia and examined for hematological and clinical biochemistry parameters. After blood sampling, all animals were euthanized, and necropsy consisted of macroscopic observations of the external morphology and all major internal organs for all animals. Certain organs were removed, weighed and examined microscopically for histopathological abnormalities. Table 2 shows the details of each examination.

The homogeneity of variance was analyzed using Bartlett's test (level of significance: 1%) for the data shown as follows: body weight, food consumption, quantitative urinalysis parameters, hematology, clinical biochemistry, and organ weight. Homogeneous data were assessed using Dunnett's test to compare the difference of group mean values between the vehicle control group and test article groups (levels of significance: 5% and 1%, two-tailed). Heterogeneous data were assessed using Steel's test (levels of significance: 5% and 1%, two-tailed).

RESULTS

Bacterial reverse mutation test

In the dose-finding test, precipitation was observed on the agar plates at the dose of 5000 μg/plate in the presence or absence of the S9 mix. Growth inhibitory effect was observed in any test strain at 1500 μg/plate or more regardless of the presence or absence of the S9 mix. The numbers of revertant colonies increased in the TA98, TA100, and TA1537 with or without the S9 mix. The numbers of revertant colonies in the TA100 with the S9 mix and the TA98 and TA1537 with or without the S9 mix increased more than twice as much as the control group.

The main test was conducted up to 1500 μg/plate with the presence or absence of the S9 mix. The main bacterial reverse mutation test results are presented in Table 3. Growth inhibitory effect was observed in any test strain regardless of 1500 μg/plate in the presence or absence of the S9 mix. The numbers of revertant colonies increased in the TA98, TA100, and TA1537 with or without the S9 mix. The numbers of revertant colonies were more than twice that in the TA100 with the S9 mix and the TA98 and TA1537 with or without the S9 mix compared to the control group. The results described above concluded that the kaempferol aglycone-rich product was mutagenic (positive) under the conditions employed in the present study.

Rat bone marrow micronucleus test

Table 4 presents the rat bone marrow micronucleus test results.

There were no statistically significant treatment-related differences in incidences of MNPCE or PCE, whereas the positive control induced significantly increased incidence of MNPCE. Thus, the results demonstrated that the kaempferol aglycone-rich product did not induce chromosomal damage in rat bone marrow.

Subchronic toxicity study

There were no dead animals as well as abnormal clinical signs in any group throughout the study period. As demonstrated in Figs. 1 and 2, no significant changes in the body weight and food consumption were noted for any treated group as compared to the control group throughout the study period. No treatment-related ophthalmological findings were identified in any animal at Week 13.

There were no treatment-related changes in the semiquantitative urinalysis for any treated group compared to the control group. Table 5 shows the quantitative urinalysis results. Statistically significant decreased urine volume was observed in males at 2000 mg/kg/day at Week 7. This value was within the background data and was therefore considered not to be treatment-related. At 500 mg/kg/day, decreased urine volume at Weeks 7 and 13 and increased urinary osmolality at Week 7 were observed in males. These changes were not dose-related and were therefore considered not to be treatment-related.

No statistically significant changes were identified for any hematological parameter in any treatment group at Week 13 as compared to the control group (Table 6).

Clinical biochemistry at Week 13 (Table 7) revealed statistically significant increases in T-BIL, measured by

Table 2. Details of examinations in the subchronic toxicity study.

Examination	Parameters / Organs
Urinalysis	1. Semiquantitative paper test: pH, protein, glucose, ketones, bilirubin, occult blood, and urobilinogen (an automated urine analyzer, CLINTEK 500, Siemens Healthcare Diagnostics Inc., Tokyo, Japan) 2. Urine sediments 3. Urine volume and color tone 4. Osmolality (an automated osmotic pressure analyzer, OSMO STATION OM-6060, ARKRAY, Inc., Kyoto, Japan) 5. Sodium (Na), potassium (K), and chloride (Cl) excretion (an automated clinical chemistry analyzer, TBA-120FR, CANON MEDICAL SYSTEMS CORP., Tochigi, Japan)
Hematology	1. Red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit value (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), reticulocyte count (Retic), platelet count (PLT), white blood cell count (WBC), and differential white blood cell count (lymphocyte [LYMP], neutrophil [NEUT], eosinophil [EOS], basophil [BASO], monocyte [MONO], and large unstained cells [LUC]) (a hematology analyzer, ADVIA 2120i, Siemens Healthcare Diagnostics Inc., Tokyo, Japan) 2. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen (FIB) (an auto coagulation analyzer, ACL Elite Pro, Instrumentation Laboratory Corp., Massachusetts, USA)
Clinical biochemistry	Aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total cholesterol (T-CHO), triglycerides (TG), phospholipids (PL), total bilirubin (T-BIL*), glucose (GLU), blood urea nitrogen (BUN), creatinine (CRNN), sodium (Na), potassium (K), chloride (Cl), calcium (Ca), inorganic phosphate (P), total protein (TP), albumin (ALB), and A/G ratio (an automated clinical chemistry analyzer, TBA-120FR, CANON MEDICAL SYSTEMS CORP., Tochigi, Japan) *: T-BIL was first measured by the bilirubin oxidase (BOD) method. Then, T-BIL was also measured by the vanadate oxidation (VO) method using the same samples as a non-GLP additional experiment (an automated clinical chemistry analyzer, Hitachi-7180, Hitachi High-Technologies Corp., Tokyo, Japan).
Organ weight	Brain, pituitary gland, salivary glands (submandibular glands and sublingual glands), thyroid glands (including the parathyroid glands), thymus, heart, lungs with bronchi, liver, spleen, adrenal glands, kidneys, testes, seminal vesicles with coagulating glands, prostate, ovaries, and uterus
Histopathology	Cerebrum, cerebellum, spinal cords at thoracic region, sciatic nerve, eyes, optic nerves, harderian glands, pituitary gland, thyroid glands, parathyroid glands, adrenal glands, thymus, spleen, submandibular lymph nodes, mesenteric lymph nodes, heart, aorta, trachea, lungs with bronchi, tongue, esophagus, stomach, duodenum, jejunum, ileum with Peyer's patch, cecum, colon, rectum, submandibular glands, sublingual glands, liver, pancreas, kidneys, urinary bladder, testes, ovaries, epididymides, uterus, prostate, vagina, seminal vesicles with coagulating glands, mammary glands, sternum with bone marrow, femur with stifle joint and bone marrow, femoral skeletal muscle, and skin

BOD method, in males at 500 mg/kg/day or more and females at 1000 mg/kg or more as compared to the control group. When T-BIL was measured by the VO method using the same samples as an additional experiment, no increase in T-BIL was observed.

Statistically significant decreases in BUN and CRNN in females at 2000 mg/kg/day. Because these were decreased responses, the changes were of no toxicological significance.

Measurement of the organ weights at Week 13 (Table 8) revealed statistically significant increases in the relative weights of the lungs in males at 2000 mg/kg/day and the kidneys in males at 1000 mg/kg or more than the control group. However, since no absolute weight increases were noted, the changes were of no toxicological significance. Although statistically significant increases in the absolute weights of the adrenal glands in females at 500 mg/kg/day and the absolute and relative weights of the prostate in males at 1000 mg/kg/day were observed as compared to the control group, these were not dose-related changes.

The results of the gross pathology are shown in Table 9. Although some findings occurred in treated groups, these were spontaneous and not dose-related and therefore not considered treatment-related.

No treatment-related histopathological findings were evident in any animal at the termination of the dosing period (Table 10). Although some findings occurred in treated groups, these were spontaneous and not dose-related and therefore not considered treatment-related.

Table 3. Results of the main bacterial reverse mutation test.

metabolic activation	Dose (μg/plate)	Number of revertant colonies/plate				
		TA100	TA1535	WP2 *uvrA*	TA98	TA1537
−S9	0[a]	119 ± 1.5	9 ± 1.0	20 ± 4.0	18 ± 1.0	9 ± 0.6
	46.9	134 ± 2.5	10 ± 1.7	21 ± 5.9	19 ± 2.9	9 ± 1.0
	93.8	138 ± 13.2	9 ± 2.0	24 ± 11.2	17 ± 1.7	8 ± 1.5
	188	138 ± 16.3	10 ± 3.1	21 ± 6.9	21 ± 6.0	12 ± 1.2
	375	139 ± 4.0	7 ± 2.6	23 ± 5.3	22 ± 1.5	26 ± 4.7
	750	174 ± 11.0	7 ± 2.1	19 ± 3.5	37 ± 4.5	35 ± 4.6
	1500	75 ± 20.5*	1 ± 0.6*	21 ± 1.5*	49 ± 5.1*	4 ± 2.5*
	Positive control (Dose [μg/plate])	AF-2 (0.01) 704	SAZ (0.5) 308	AF-2 (0.01) 92	AF-2 (0.1) 302	ICR-191 (1.0) 1782
+S9	0[a]	115 ± 14.6	11 ± 2.5	26 ± 3.6	25 ± 4.2	11 ± 1.5
	2.93	131 ± 7.6	NA	NA	37 ± 1.2	11 ± 3.5
	5.86	179 ± 10.5	NA	NA	57 ± 1.5	13 ± 2.5
	11.7	229 ± 9.8	NA	NA	87 ± 11.8	14 ± 3.1
	23.4	288 ± 19.3	NA	NA	200 ± 4.2	29 ± 6.5
	46.9	327 ± 22.5	12 ± 1.7	25 ± 6.2	235 ± 17.8	33 ± 6.8
	93.8	404 ± 15.0	12 ± 1.5	30 ± 0.6	286 ± 14.6	56 ± 9.0
	188	472 ± 15.4	16 ± 1.7	29 ± 7.8	363 ± 26.1	68 ± 13.7
	375	494 ± 52.8	14 ± 1.5	30 ± 5.8	450 ± 66.0	81 ± 10.4
	750	446 ± 33.3	11 ± 7.2	34 ± 5.8	446 ± 27.2	92 ± 12.1
	1500	210 ± 18.6*	6 ± 1.5*	30 ± 2.1*	431 ± 20.2*	80 ± 10.8*
	Positive control (Dose [μg/plate])	B[*a*]P (5.0) 1178	2AA (2.0) 228	2AA (10.0) 559	B[*a*]P (5.0) 285	B[*a*]P (5.0) 82

All data are presented as mean ± S.D. (n = 3), except for the positive control articles (mean, n = 2).
[a]: Negative control (DMSO), NA: Not applicable.
* Growth inhibitory effect was observed.
AF-2: furylfuramide, SAZ: sodium azide, ICR-191: acridine mutagen ICR-191, B[*a*]P: benzo[*a*]pyrene, 2AA: 2-aminoanthracene.

Table 4. Results of the rat bone marrow micronucleus test.

Dose (mg/kg/day)	MNPCE		PCE	
	Number of MNPCE in 4000 PCE	Incidence of MNPCE (%)	Number of PCE in 500 Erythrocytes	Incidence of PCE (%)
0[a]	6 ± 2	0.15 ± 0.06	284 ± 20	56.9 ± 4.1
1000	6 ± 1	0.15 ± 0.02	300 ± 26	60.0 ± 5.1
2000	4 ± 2	0.10 ± 0.05	285 ± 25	56.9 ± 5.0
4000	5 ± 3	0.12 ± 0.06	280 ± 29	56.1 ± 5.7
2 (Positive control)	115 ± 25*	2.88 ± 0.63	258 ± 28	51.7 ± 5.7

All data are presented as mean ± S.D. (n = 5).
[a]: Negative control: 5% gum arabic solution; Positive control: mitomycin C.
* $P < 0.05$, compared to negative control.

DISCUSSION

To evaluate the safety of a new product, a kaempferol aglycone-rich food produced from enzyme-treated horseradish leaves, the following studies were conducted: bacterial reverse mutation test, rat bone marrow micronucleus test, and 13-week subchronic toxicity study in rats.

In the bacterial reverse mutation test, the numbers of revertant colonies increased in the TA98, TA100, and TA1537 with or without the S9 mix. The numbers of revertant colonies were more than twice that in the TA100 with the S9 mix and the TA98 and TA1537 with or with-

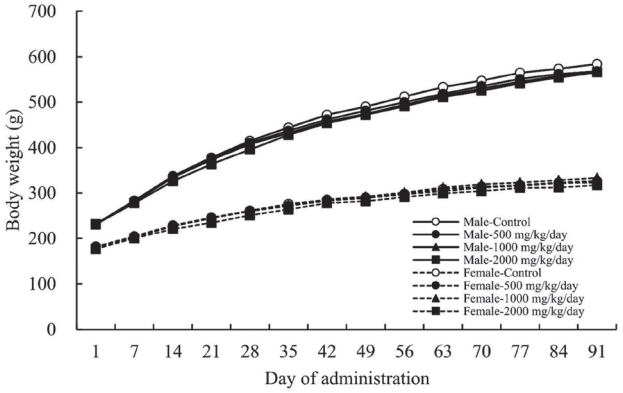

Fig. 1. Changes in body weights of rats in the subchronic toxicity study. All data are presented as mean (n = 10, male: solid lines, female: dashed lines). No significant difference in any treated groups from control group.

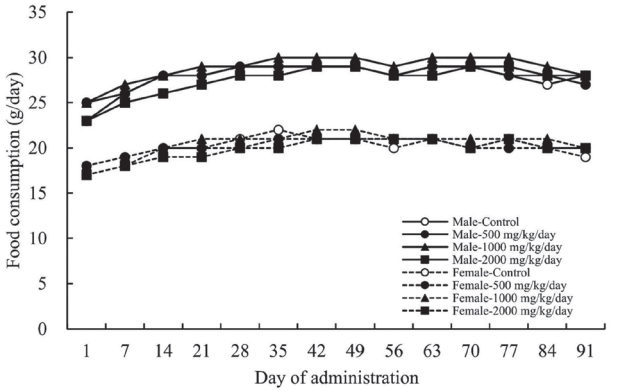

Fig. 2. Changes in food consumptions of rats in the subchronic toxicity study. All data are presented as mean (n = 10, male: solid lines, female: dashed lines). No significant difference in any treated groups from control group.

Table 5. Results of quantitative parameters of urinalysis in the subchronic toxicity study.

Parameter		Dose (mg/kg/day)			
		0 (Control)	500	1000	2000
Males					
Urine Volume (mL/24 hr)	Week 7	20.2 ± 6.3	10.3 ± 3.4*	14.6 ± 3.8	11.5 ± 5.9*
	Week 13	20.0 ± 2.9	13.2 ± 1.1*	22.2 ± 9.8	15.8 ± 4.9
Osmotic Pressure (mOsm/kg)	Week 7	1444 ± 478	2218 ± 222*	2033 ± 328	1942 ± 424
	Week 13	1788 ± 327	1910 ± 121	1654 ± 533	1653 ± 119
Na excretion (mmol/24 hr)	Week 7	2.3 ± 0.5	2.0 ± 0.6	2.4 ± 0.5	1.8 ± 0.6
	Week 13	3.2 ± 1.1	2.1 ± 0.4	3.2 ± 1.2	2.1 ± 0.7
K excretion (mmol/24 hr)	Week 7	5.0 ± 0.7	4.3 ± 1.2	5.3 ± 0.6	3.8 ± 1.0
	Week 13	6.6 ± 1.3	4.6 ± 0.4	6.2 ± 2.0	4.7 ± 1.5
Cl excretion (mmol/24 hr)	Week 7	3.6 ± 0.6	3.0 ± 0.8	3.6 ± 0.4	2.6 ± 0.8
	Week 13	4.5 ± 1.4	2.9 ± 0.3	4.3 ± 1.6	3.1 ± 1.2
Females					
Urine Volume (mL/24 hr)	Week 7	10.0 ± 5.4	6.3 ± 4.9	7.7 ± 2.4	8.7 ± 2.9
	Week 13	7.9 ± 2.6	4.9 ± 4.1	9.0 ± 3.4	11.9 ± 6.3
Osmotic Pressure (mOsm/kg)	Week 7	2065 ± 468	2479 ± 689	2168 ± 316	1931 ± 502
	Week 13	2004 ± 256	2206 ± 683	1744 ± 119	1458 ± 492
Na excretion (mmol/24 hr)	Week 7	1.6 ± 0.8	1.1 ± 0.6	1.3 ± 0.2	1.4 ± 0.2
	Week 13	1.3 ± 0.5	0.7 ± 0.3	1.4 ± 0.5	1.3 ± 0.6
K excretion (mmol/24 hr)	Week 7	3.4 ± 1.4	2.4 ± 1.0	2.9 ± 0.6	2.9 ± 0.2
	Week 13	2.9 ± 0.9	1.6 ± 0.6	2.9 ± 1.1	2.7 ± 1.2
Cl excretion (mmol/24 hr)	Week 7	2.3 ± 1.0	1.7 ± 0.8	2.0 ± 0.3	1.9 ± 0.2
	Week 13	2.0 ± 0.6	1.0 ± 0.4	2.0 ± 0.7	1.9 ± 0.8

All data are presented as mean ± S.D. (n = 5).
* $P < 0.05$, compared to control group (0 mg/kg/day).

out the S9 mix compared to the control group. Some flavonoids, such as galangin, kaempferol, and quercetin, are known to show mutagenicity in *Salmonella typhimurium* strains, probably by yielding reactive intermediates (Resende *et al*., 2012). The mutagenicity of kaempferol in the bacterial strains is largely due to its metabolic activation (Resende *et al*., 2012), and the metabolization of kaempferol by the S9 mix gives rise to quercetin, increas-

Table 6. Results of Hematological parameters in the subchronic toxicity study.

Parameter	Dose (mg/kg/day)			
	0 (Control)	500	1000	2000
Males				
RBC (10^4/μL)	849 ± 45	852 ± 21	842 ± 29	841 ± 51
HGB (g/dL)	15.2 ± 0.8	14.8 ± 0.4	15.1 ± 0.5	15.0 ± 0.8
HCT (%)	44.1 ± 2.3	43.2 ± 1.0	43.8 ± 1.4	43.4 ± 2.5
MCV (fL)	52.0 ± 1.7	50.8 ± 1.6	52.1 ± 1.6	51.6 ± 1.8
MCH (pg)	17.9 ± 0.5	17.4 ± 0.7	17.9 ± 0.5	17.8 ± 0.6
MCHC (g/dL)	34.5 ± 0.3	34.2 ± 0.5	34.4 ± 0.1	34.6 ± 0.7
RDW (%)	13.1 ± 0.9	13.6 ± 1.2	13.3 ± 0.8	13.4 ± 0.6
Retic (10^9/L)	192.9 ± 35.3	190.7 ± 49.2	175.8 ± 26.1	198.7 ± 42.1
PLT (10^4/μL)	116.0 ± 14.0	116.3 ± 30.2	121.1 ± 10.8	121.8 ± 14.2
WBC (10^2/μL)	99.6 ± 22.8	94.4 ± 25.1	88.9 ± 27.5	103.0 ± 19.9
LYMP (10^2/μL)	79.3 ± 20.9	71.9 ± 19.8	70.5 ± 28.2	78.6 ± 14.5
NEUT (10^2/μL)	14.6 ± 4.1	17.3 ± 7.3	13.7 ± 4.4	18.8 ± 7.7
EOS (10^2/μL)	1.7 ± 0.4	1.4 ± 0.7	1.3 ± 0.5	1.5 ± 0.5
BASO (10^2/μL)	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
MONO (10^2/μL)	2.8 ± 1.0	2.7 ± 0.9	2.3 ± 0.3	2.9 ± 0.7
LUC (10^2/μL)	1.2 ± 0.6	1.1 ± 0.6	1.0 ± 0.5	1.0 ± 0.5
PT (sec)	14.3 ± 0.8	13.8 ± 0.8	14.2 ± 1.1	14.3 ± 0.9
APTT (sec)	16.7 ± 2.9	16.2 ± 1.8	17.2 ± 2.2	17.1 ± 1.3
FIB (mg/dL)	297 ± 35	285 ± 27	297 ± 27	300 ± 37
Females				
RBC (10^4/μL)	782 ± 40	773 ± 31	754 ± 33	757 ± 27
HGB (g/dL)	14.7 ± 0.5	14.5 ± 0.6	14.4 ± 0.4	14.4 ± 0.3
HCT (%)	41.8 ± 1.9	41.2 ± 1.6	40.7 ± 1.4	40.8 ± 1.2
MCV (fL)	53.6 ± 3.0	53.3 ± 1.1	54.0 ± 1.4	53.9 ± 1.3
MCH (pg)	18.8 ± 0.9	18.7 ± 0.4	19.1 ± 0.5	19.0 ± 0.7
MCHC (g/dL)	35.1 ± 0.7	35.1 ± 0.4	35.3 ± 0.5	35.3 ± 0.8
RDW (%)	12.0 ± 0.6	11.6 ± 0.2	11.7 ± 0.3	12.0 ± 0.2
Retic (10^9/L)	147.1 ± 19.4	132.5 ± 29.6	151.7 ± 25.4	151.1 ± 20.9
PLT (10^4/μL)	114.4 ± 12.5	108.9 ± 8.4	112.4 ± 10.3	118.4 ± 11.7
WBC (10^2/μL)	63.1 ± 19.0	52.5 ± 11.6	54.4 ± 15.5	54.5 ± 11.7
LYMP (10^2/μL)	48.6 ± 14.5	41.4 ± 10.4	41.9 ± 14.7	42.0 ± 11.8
NEUT (10^2/μL)	10.9 ± 4.5	7.9 ± 2.2	9.6 ± 5.4	9.0 ± 3.4
EOS (10^2/μL)	0.9 ± 0.3	1.0 ± 0.5	0.8 ± 0.2	0.9 ± 0.4
BASO (10^2/μL)	0.1 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.1
MONO (10^2/μL)	1.8 ± 0.8	1.7 ± 0.7	1.6 ± 0.4	1.8± 0.7
LUC (10^2/μL)	0.7 ± 0.4	0.5 ± 0.2	0.5 ± 0.2	0.8± 0.3
PT (sec)	13.3 ± 0.8	13.2 ± 0.8	13.2 ± 1.0	13.3± 1.1
APTT (sec)	14.1 ± 1.1	14.0 ± 1.3	14.0 ± 1.7	14.2± 2.0
FIB (mg/dL)	222 ± 42	221 ± 22	213 ± 20	207± 19

All data are presented as mean ± S.D. (n = 10).
No significant difference in any treated groups from control group (0 mg/kg/day).

ing its genotoxicity (Cushnie and Lamb, 2005). Given that the product contains kaempferol aglycone as an active ingredient and the product-induced mutagenicity was also more marked under the metabolic activation, its mutagenicity was probably caused by kaempferol.

In the rat bone marrow micronucleus test, the kaempferol aglycone-rich product did not induce increases in micronucleated polychromatic erythrocytes in rat bone marrow. Quercetin shows the negative results of the *in vivo* genotoxicity studies (Utesch *et al*., 2008) though the compound shows strong mutagenicity in a bacterial reverse mutation test (Resende *et al*., 2012). Kaempfer-

Table 7. Results of clinical biochemistry parameters in the subchronic toxicity study.

Parameter		Dose (mg/kg/day)			
		0 (Control)	500	1000	2000
Males					
AST (IU/L)		62 ± 10	74 ± 46	67 ± 23	59 ± 7
ALT (IU/L)		36 ± 6	41 ± 30	38 ± 14	33 ± 5
LDH (IU/L)		47 ± 19	40 ± 15	47 ± 23	42 ± 14
ALP (IU/L)		329 ± 40	291 ± 78	305 ± 58	341 ± 106
T-CHO (mg/dL)		77 ± 15	69 ± 12	73 ± 14	74 ± 17
TG (mg/dL)		74 ± 39	65 ± 24	52 ± 19	63 ± 28
PL (mg/dL)		115 ± 17	107 ± 14	107 ± 19	112 ± 19
T-BIL (mg/dL)	BOD method	0.1 ± 0.0	0.4 ± 0.1**	0.4 ± 0.1**	0.5 ± 0.1**
	VO method	0.10 ± 0.00	NM	NM	0.10 ± 0.00
GLU (mg/dL)		149 ± 17	144 ± 17	136 ± 15	141 ± 11
BUN (mg/dL)		17 ± 3	18 ± 3	16 ± 2	16 ± 2
CRNN (mg/dL)		0.30 ± 0.04	0.29 ± 0.03	0.28 ± 0.03	0.27 ± 0.04
Na (mmol/L)		144 ± 1	144 ± 1	144 ± 0	144 ± 1
K (mmol/L)		3.7 ± 0.3	3.8 ± 0.3	3.7 ± 0.2	3.8 ± 0.2
Cl (mmol/L)		106 ± 2	106 ± 2	106 ± 1	106 ± 1
Ca (mg/dL)		10.4 ± 0.3	10.5 ± 0.3	10.4 ± 0.3	10.6 ± 0.3
P (mg/dL)		5.9 ± 0.8	5.8 ± 0.7	5.9 ± 0.7	5.9 ± 0.5
TP (g/dL)		6.5 ± 0.2	6.4 ± 0.2	6.5 ± 0.3	6.5 ± 0.3
ALB (g/dL)		3.4 ± 0.1	3.4 ± 0.1	3.4 ± 0.2	3.5 ± 0.2
A/G ratio		1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1
Females					
AST (IU/L)		60 ± 7	57 ± 8	59 ± 8	56 ± 8
ALT (IU/L)		32 ± 8	31 ± 9	34 ± 8	29 ± 8
LDH (IU/L)		33 ± 7	30 ± 7	36 ± 13	29 ± 8
ALP (IU/L)		146 ± 47	150 ± 46	141 ± 26	150 ± 24
T-CHO (mg/dL)		87 ± 17	89 ± 21	87 ± 24	88 ± 13
TG (mg/dL)		71 ± 63	50 ± 22	67 ± 47	38 ± 20
PL (mg/dL)		171 ± 24	167 ± 31	167 ± 30	165 ± 20
T-BIL (mg/dL)	BOD method	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1*	0.2 ± 0.1*
	VO method	0.08 ± 0.04	NM	NM	0.10 ± 0.00
GLU (mg/dL)		118 ± 7	114 ± 10	120 ± 11	120 ± 9
BUN (mg/dL)		22 ± 5	19 ± 4	18 ± 1	16 ± 2*
CRNN (mg/dL)		0.37 ± 0.06	0.35 ± 0.04	0.33 ± 0.04	0.32 ± 0.03*
Na (mmol/L)		143 ± 1	142 ± 1	142 ± 1	143 ± 1
K (mmol/L)		3.3 ± 0.2	3.4 ± 0.1	3.4 ± 0.2	3.4 ± 0.1
Cl (mmol/L)		107 ± 2	107 ± 1	107 ± 1	107 ± 2
Ca (mg/dL)		10.6 ± 0.2	10.5 ± 0.2	10.6 ± 0.4	10.7 ± 0.2
P (mg/dL)		4.8 ± 1.3	4.8 ± 1.1	4.7 ± 1.1	4.8 ± 1.0
TP (g/dL)		7.1 ± 0.4	7.0 ± 0.3	7.0 ± 0.3	7.1 ± 0.4
ALB (g/dL)		4.1 ± 0.3	4.1 ± 0.1	4.1 ± 0.2	4.2 ± 0.2
A/G ratio		1.4 ± 0.2	1.4 ± 0.1	1.5 ± 0.2	1.5 ± 0.1

All data are presented as mean ± S.D. (n = 10), except for T-BIL measured by VO method (n = 5).
NM: Not measured.
* $P < 0.05$, ** $P < 0.01$, compared to control group (0 mg/kg/day).

ol is also mutagenic *in vitro* but has also been reported to be non-carcinogenic in a carcinogenicity study in rats (Takanashi *et al*., 1983). The flavonoid may show no toxic effects *in vivo* because of its low oral bioavailability (Calderón-Montaño *et al*., 2011). Therefore, the kaempferol aglycone-rich product was not considered genotox-

Table 8. Results of absolute and relative organ weights in the subchronic toxicity study.

Organ		Dose (mg/kg/day)			
		0 (control)	500	1000	2000
Males					
Terminal body weight (g)		560 ± 56	542 ± 38	541 ± 45	541 ± 48
Brain	(g)	2.23 ± 0.09	2.18 ± 0.08	2.21 ± 0.07	2.23 ± 0.10
	(g/100 g BW)	0.40 ± 0.03	0.41 ± 0.03	0.41 ± 0.03	0.41 ± 0.03
Pituitary gland	(mg)	13.2 ± 0.9	13.5 ± 1.2	13.2 ± 1.0	13.0 ± 1.3
	(mg/100 g BW)	2.4 ± 0.2	2.5 ± 0.3	2.4 ± 0.2	2.4 ± 0.3
Thyroid glands	(mg)	25.3 ± 8.6	24.3 ± 4.6	21.7 ± 3.0	23.9 ± 3.7
	(mg/100 g BW)	4.6 ± 1.7	4.5 ± 1.0	4.0 ± 0.6	4.4 ± 0.7
Salivary glands	(mg)	730 ± 78	777 ± 102	746 ± 76	744 ± 84
	(mg/100 g BW)	131 ± 12	144 ± 22	138 ± 9	138 ± 10
Thymus	(mg)	302 ± 96	307 ± 86	284 ± 50	303 ± 67
	(mg/100 g BW)	54 ± 18	57 ± 15	53 ± 9	56 ± 13
Heart	(g)	1.55 ± 0.08	1.50 ± 0.12	1.52 ± 0.09	1.56 ± 0.16
	(g/100 g BW)	0.28 ± 0.01	0.28 ± 0.01	0.28 ± 0.02	0.29 ± 0.02
Lungs	(g)	1.55 ± 0.07	1.52 ± 0.07	1.57 ± 0.12	1.62 ± 0.15
	(g/100 g BW)	0.28 ± 0.02	0.28 ± 0.02	0.29 ± 0.01	0.30 ± 0.02*
Liver	(g)	13.85 ± 1.88	13.59 ± 1.65	13.55 ± 0.63	14.24 ± 2.07
	(g/100 g BW)	2.47 ± 0.17	2.50 ± 0.19	2.52 ± 0.24	2.62 ± 0.20
Spleen	(g)	0.82 ± 0.08	0.84 ± 0.18	0.83 ± 0.12	0.81 ± 0.14
	(g/100 g BW)	0.15 ± 0.01	0.16 ± 0.03	0.15 ± 0.02	0.15 ± 0.02
Kidneys	(g)	3.19 ± 0.22	3.27 ± 0.30	3.43 ± 0.27	3.41 ± 0.51
	(g/100 g BW)	0.57 ± 0.04	0.60 ± 0.04	0.64 ± 0.04**	0.63 ± 0.05**
Adrenal glands	(mg)	54 ± 6	62 ± 16	55 ± 8	53 ± 4
	(mg/100 g BW)	10 ± 1	11 ± 3	10 ± 1	10 ± 0
Testes	(g)	3.42 ± 0.34	3.47 ± 0.24	3.56 ± 0.50	3.62 ± 0.28
	(g/100 g BW)	0.62 ± 0.07	0.64 ± 0.06	0.66 ± 0.06	0.67 ± 0.07
prostate	(g)	1.24 ± 0.15	1.25 ± 0.22	1.43 ± 0.19*	1.23 ± 0.09
	(g/100 g BW)	0.22 ± 0.03	0.23 ± 0.04	0.27 ± 0.04*	0.23 ± 0.03
Seminal vesicles	(g)	1.65 ± 0.22	1.68± 0.25	1.79± 0.31	1.84± 0.34
	(g/100 g BW)	0.30 ± 0.05	0.31± 0.04	0.33± 0.05	0.34± 0.06
Females					
Terminal body weight (g)		311 ± 46	309 ± 19	319 ± 38	302 ± 32
Brain	(g)	2.02 ± 0.09	2.01 ± 0.09	2.02 ± 0.11	2.04 ± 0.08
	(g/100 g BW)	0.66 ± 0.07	0.65 ± 0.04	0.64 ± 0.06	0.68 ± 0.06
Pituitary gland	(mg)	16.6 ± 3.3	17.4 ± 3.0	16.8 ± 3.1	17.4 ± 2.3
	(mg/100 g BW)	5.4 ± 1.1	5.6 ± 0.8	5.3 ± 1.0	5.9 ± 1.1
Thyroid glands	(mg)	16.5 ± 3.3	19.4 ± 4.1	18.1 ± 4.4	16.3 ± 3.6
	(mg/100 g BW)	5.4 ± 1.2	6.3 ± 1.1	5.7 ± 1.3	5.4 ± 0.9
Salivary glands	(mg)	445 ± 48	484 ± 39	442 ± 37	462 ± 72
	(mg/100 g BW)	144 ± 14	157 ± 19	140 ± 16	153 ± 18
Thymus	(mg)	245 ± 52	289 ± 61	245 ± 50	243 ± 77
	(mg/100 gv)	79 ± 15	94 ± 21	77 ± 13	80 ± 21
Heart	(g)	0.97 ± 0.10	0.97 ± 0.06	0.98 ± 0.06	1.01 ± 0.07
	(g/100 g BW)	0.31 ± 0.02	0.31 ± 0.02	0.31 ± 0.02	0.34 ± 0.03
Lungs	(g)	1.20 ± 0.10	1.22 ± 0.08	1.20 ± 0.12	1.20 ± 0.08
	(g/100 g BW)	0.39 ± 0.04	0.40 ± 0.02	0.38 ± 0.03	0.40 ± 0.03
Liver	(g)	7.47 ± 1.03	7.61 ± 0.74	7.88 ± 1.03	7.75 ± 0.64
	(g/100 g BW)	2.41 ± 0.18	2.46 ± 0.17	2.48 ± 0.14	2.58 ± 0.15
Spleen	(g)	0.52 ± 0.05	0.51 ± 0.11	0.55 ± 0.14	0.55 ± 0.08
	(g/100 g BW)	0.17 ± 0.02	0.17 ± 0.04	0.17 ± 0.03	0.18 ± 0.02
Kidneys	(g)	2.04 ± 0.23	2.01 ± 0.15	2.07 ± 0.24	2.04 ± 0.15
	(g/100 g BW)	0.66 ± 0.06	0.65 ± 0.03	0.65 ± 0.04	0.68 ± 0.05
Adrenal glands	(mg)	62 ± 5	68 ± 5*	64 ± 11	72 ± 14
	(mg/100 g BW)	20 ± 3	22 ± 2	20 ± 3	24 ± 6
Ovaries	(mg)	82.0 ± 10.2	81.3 ± 7.5	79.6 ± 16.8	76.8 ± 14.1
	(mg/100 g BW)	26.6 ± 3.2	26.4 ± 2.7	24.8 ± 3.0	25.4 ± 3.5
Uterus	(mg)	604 ± 78	665 ± 128	681 ± 121	743 ± 174
	(mg/100 g BW)	199 ± 42	216 ± 46	214 ± 29	250 ± 72

All data are presented as mean ± S.D. (n = 10).
* $P < 0.05$, ** $P < 0.01$, compared to control group (0 mg/kg/day).

Table 9. Results of gross pathology in the subchronic toxicity study.

Organs/Findings	Dose (mg/kg/day)			
	0 (control)	500	1000	2000
Males				
Number of animals examined	10	10	10	10
Incisor, upper jaw/				
Fracture	0	1	0	0
Intestine, ileum (Peyer's patch)/				
Diverticulum	1	0	0	1
Kidney/				
Dilation, pelvis	0	1	0	0
Stomach (glandular)/				
Focus, dark red	1	0	0	1
Testis/				
Large	0	0	1	0
Females				
Number of animals examined	10	10	10	10
Liver/				
Small, lobar	0	0	1	0
Pituitary gland/				
Cyst	0	0	0	1
Stomach (glandular)/				
Focus, dark red	2	0	2	1

Values are the number of animals with findings.

ic *in vivo*.

In rat's 13-week subchronic toxicity study, clinical biochemistry revealed increases in T-BIL, measured by BOD method, in males at 500 mg/kg/day or more and females at 1000 mg/kg or more. However, no increase in T-BIL was observed when T-BIL was measured by the VO method using the same samples. It has been reported that T-BIL value measured by the BOD method was higher due to positive interference by carotene (Ida *et al*., 2002; Kiuchi *et al*., 2019). On the other hand, the VO method is relatively free of interferences from coexisting serum substances (Tokuda and Tanimoto, 1993).

Since the kaempferol aglycone-rich product is produced from enzyme-treated horseradish leaves, it may contain various plant-derived ingredients. Given that there were no changes in other clinical biochemistry parameters or histopathological lesions related to the hepatobiliary system, the kaempferol aglycone-rich product-induced increases in T-BIL were judged to be false positive due to differences in assay methods. As a result, the oral dosing of the product at up to 2000 mg/kg/day for 13 weeks did not show any significant toxicological changes. Therefore, the no-observed-adverse-effect level (NOAEL) for both male and female rats was 2000 mg/kg/day under the conditions of this study.

In conclusion, these findings indicated that the kaempferol aglycone-rich product was not genotoxic *in vivo*. The NOAEL was 2000 mg/kg/day in a 13-week subchronic toxicity study in rats, suggesting it is safe for use as a food.

ACKNOWLEDGMENTS

The authors are grateful to the researchers and technical staff at Tokushima Research Institute of Otsuka Pharmaceutical Co., Ltd.

Conflict of interest---- All authors are employees of Otsuka Pharmaceutical Co., Ltd.

Table 10. Results of histopathology in the subchronic toxicity study.

Organs/Findings	Dose (mg/kg/day)			
	0 (control)	500	1000	2000
Males				
Number of animals examined	10	10	10	10
Epididymis/				
Infiltrate, inflammatory cell	1 (minimal)	NE	NE	0
Epididymis/				
Dysplasia, retina	0	NE	NE	1 (minimal)
Harderian gland/				
Infiltrate, inflammatory cell	0	NE	NE	1 (mild)
Heart/				
Cardiomyopathy, rodent progressive	3 (minimal)	NE	NE	2 (minimal)
Intestine, ileum (Peyer's patch)/				
Diverticulum	1 (mild)	NE	NE	1 (mild)
Kidney/				
Basophilia, tubule	3 (minimal)	NE	NE	4 (minimal)
Cast, hyaline	2 (minimal)	NE	NE	0
Lung(bronchus)/				
Aggregation, alveolar macrophage	2 (minimal)	NE	NE	0
Pancreas/				
Atrophy, acinar cell, focal	3 (minimal)	NE	NE	0
Fibrosis, islet	0	NE	NE	1 (minimal)
Prostate/				
Infiltrate, inflammatory cell	3 (minimal)	NE	NE	4 (minimal), 1 (mild)
Stomach/				
Erosion-Ulcer, glandular stomach	1 (minimal)	NE	NE	1 (minimal)
Females				
Number of animals examined	10	10	10	10
Heart/				
Cardiomyopathy, rodent progressive	0	NE	NE	1 (minimal)
Kidney/				
Basophilia, tubule	1 (minimal)	NE	NE	0
Cast, hyaline	3 (minimal)	NE	NE	1 (minimal)
Fibrosis, focal	3 (minimal)	NE	NE	0
Lung (bronchus)/				
Aggregation, alveolar macrophage	2 (minimal)	NE	NE	1 (minimal)
Ovary/				
Atrophy, age-related	2 (minimal)	NE	NE	1 (mild)
Pituitary gland/				
Cyst, pars distalis	0	NE	NE	1 (mild)
Stomach/				
Erosion-Ulcer, glandular stomach	2 (minimal)	NE	NE	1 (minimal)
Uterus/				
Infiltrate, inflammatory cell	1 (minimal)	NE	NE	0

Values are the number of animals with findings. Words in parenthesis indicate the grades of lesions.
Organs not listed in the table are those in which no findings were observed in the examination of animals at 0 and 2000 mg/kg/day.
NE: Not examined.

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