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Original Article

28-Day dietary toxicity study of L-phenylalanine in rats

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ABSTRACT — The purpose of this study was to evaluate the toxicity of L-phenylalanine when administered daily in the diet to rats for at least 28 days. Male and female Crl:CD®(SD)IGS BR rats were assigned to four groups. Each group received diets containing basal diet or 0.5, 1.5, or 5.0% (w/w) L-phenylalanine. There were no clinical or ophthalmic observations that were considered to be related to L-phenylalanine. Effects of L-phenylalanine administration were noted in mean body weights and mean body weight gains in females fed 0.5% and in males and females fed 5.0% (w/w) L-phenylalanine diets. Effects were also noted in mean food consumption in males and females fed the 5.0% (w/w) L-phenylalanine diet. The lower food consumption and body weights of the males and females fed the 5.0% L-phenylalanine diet were considered to be signs of mild toxicity. Administration of L-phenylalanine at a dose of 5.0% of the diet was associated with mildly increased red blood cell count and mildly decreased mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and glucose in females. There were no L-phenylalanine-related toxic changes for organ weight, or macroscopic or microscopic findings. In conclusion, the no-observed-effect level (NOEL) of dietary exposure of male rats to L-phenylalanine is 1.5% (w/w) L-phenylalanine. The NOEL of dietary exposure of female rats to L-phenylalanine is less than 0.5% (w/w) L-phenylalanine. However, the no-observed-adverse-effect level (NOAEL) for males and females is 1.5% (w/w) L-phenylalanine.

Key words: L-phenylalanine, Amino acid, Food additive, Toxicity, Rat

INTRODUCTION

L-phenylalanine is a nutritionally indispensable amino acid. Of the 20 genetically coded amino acids, about half of them are nonessential, which means that they are produced naturally within the human body. However, for healthy survival humans must consume those that are essential. L-phenylalanine is found naturally in the breast milk of mammals, but has been isolated and artificially synthesized for use in health supplements and as a food additive. As an additive in foods, L-phenylalanine is used in the production of aspartame, an artificial sweetener.

L-phenylalanine is consumed in health supplements because of its potential analgesic and antidepressant properties (Sabelli, 2002). Phenylalanine may stimulate the release of endorphins in the brain, which produce a happy, carefree mood in humans. Although L-phenylalanine may not have any negative health effects on the human body, its association with the production of aspartame has caused it to receive undue scrutiny in recent years (Butchko *et al.*, 2002).

Because it can improve the flavor of the food, L-phenylalanine was authorized as a food additive in 1996. L-phenylalanine is also an ingredient listed in the pharmacopoeia in US, Europe, and Japan, and its maximum precedent dose is 10 mg for intravenous or intramuscular administration in Japan (IPEC JAPAN, 2007).

Recently, we have reported a toxicology study of glycine, because there have been a relatively small number of toxicity studies of glycine (Shibui *et al*., 2013). As for L-phenylalanine, there is some partial toxicological information reported in rats (Muramatsu *et al.*, 1971; Peng *et al.*, 1973) and monkeys (Gibbs *et al.*, 1960). However, its toxicity profile has not yet been comprehensively established, and there are no recent reports regarding orally repeated-dose (dietary) general toxicity of L-phe-

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nylalanine. Therefore, we conducted a 28-day repeated-dose oral toxicity study in approximately 6-weeks old rats administered dietary L-phenylalanine in order to evaluate the toxicity profile of L-phenylalanine.

MATERIALS AND METHODS

Animals and animal husbandry

Male and female Crl:CD®(SD)IGS BR rats were obtained from Charles River Laboratories (Portage, MI, USA) and allowed *ad libitum* access to tap water and a certified rodent diet (#8728CM meal, Harlan Teklad). The animals were housed individually in suspended, stainless-steel cages in an animal room maintained at a temperature of 18 to 26°C, a relative humidity of 30% to 70%, and a 12-hr light/12-hr dark cycle and were acclimatized for 9 days prior to random allocation. At initiation of treatment, the animals were 33 to 39 days old; the males weighed between 132 to 179 g, and the females weighed from 113 to 153 g. All animals were treated humanely according to institutional guidelines, and the experimental procedure was approved by the institutional ethics committee.

L-phenylalanine and carrier

L-phenylalanine (lot 002NE14; purity 99.9%) was supplied by Ajinomoto Co., Inc. (Kanagawa, Japan) and stored in a refrigerator maintained between 2 to 8°C. Certified rodent diet (#8728CM meal, Harlan Teklad) was used as carrier.

Group designations and dose levels

Animals were assigned to treatment groups using a computerized blocking procedure designed to achieve body weight balance with respect to the treatment group. At the time of randomization, the weight variation of the animals did not exceed ± 2 standard deviations of the mean body weight for each sex. Ten male and female rats were assigned to the study groups at target dose levels of 0 (Group 1; control), 0.5 (Group 2), 1.5 (Group 3), and 5.0% (w/w) L-phenylalanine (Group 4). The control animals were fed the carrier (basal diet) only.

Dose preparation

Diets were prepared approximately weekly. Each dose level was prepared independently in sequential order of increasing concentration. The diets were stored at room temperature in covered containers until dispensed into feeding jars.

Method of administration

Dietary admixture was used because the main intake route in humans is oral. The dose preparations were administered *ad libitum* for at least 28 days. All animals were fasted the day before clinical pathology tests and necropsy. The duration of administration was set at four weeks in order to obtain information on the possible health hazards likely to arise from repeated exposure over a relatively limited period of time.

Dose analysis

Analyses for the concentration of L-phenylalanine in the diets were performed. Homogeneity was determined for the low-, mid-, and high-dose levels from a separate pre-study mix. Triplicate samples (approximately 50 g each) from all dose preparations were analyzed for weeks 1 and 4. All samples were stored at room temperature and analyzed within 10 days of preparation.

Clinical observations

The animals were observed twice daily (a.m. and p.m.) for mortality and moribundity. Once daily, cageside observations were made for each animal. Once prior to treatment and weekly during treatment, each animal was removed from its cage and examined. An indication that the animal appeared normal or abnormal was recorded.

Ophthalmology

Ophthalmic examinations were done before initiation of treatment and during week 4. The pupils were dilated with a mydriatic agent, and a veterinarian examined the eyes with an indirect ophthalmoscope.

Body weights

Individual body weight data were recorded once prior to treatment, on the first day of treatment, and weekly thereafter.

Food consumption

Individual food consumption data were recorded weekly.

Clinical pathology

Blood and urine samples were collected from each animal on the day of scheduled necropsy. Animals were fasted overnight (approximately 16 hr), and urine was collected on wet ice before blood sampling; water was provided *ad libitum*. The animals were anesthetized with sodium pentobarbital, and then blood was collected from the jugular vein. Blood samples collected into potassium EDTA anticoagulant were analyzed for red blood cell (eryth-

rocyte) count (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), reticulocyte count (RETIC), and white blood cell (leukocyte) count (WBC), white blood cell differential included neutrophil count and percentage (N-SEG), lymphocyte count and percentage (LYMPH), monocytes count and percentage (MONO), eosinophil cells count and percentage (EOSIN), and basophils count and percentage (BASO). The blood samples collected into sodium citrate anticoagulant were measured for prothrombin time (PT), activated partial thromboplastin time (PTT), and fibrinogen (FBR). Serum samples were evaluated for glucose (GLU), urea nitrogen (UN), creatinine (CRET), total protein (T PRO), albumin (ALB), globulin (GLOB), albumin/globulin ratio (A/G), total bilirubin (T BILI), aspartate aminotransferase (AST/SGOT), alanine aminotransferase (ALT/SGPT), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), calcium (Ca), inorganic phosphorus (IP), sodium ion (Na), potassium ion (K), chlorite ion (CI), and fractionation of protein including albumin ratio (E ALB), α1 globulin ratio (E A-1), α2 globulin ratio (E A-2), beta globulin ratio (E BETA), and gamma globulin ratio (E GAMMA). Urine samples were examined for appearance, volume (U VOL), specific gravity (SP GR), pH (U PH), protein, glucose, ketones, bilirubin, urobilinogen, and blood microscopic examination of sediment.

Postmortem evaluations

All animals that were fasted overnight were bled for clinical pathology tests, anesthetized with sodium pentobarbital, weighed, exsanguinated, and necropsied. Animals were necropsied in random order. The necropsy included a macroscopic examination of the external features of the carcass; external body orifices; the abdominal, thoracic, and cranial cavities; organs; and tissues. At sacrifice, the following organs were weighed, with paired organs weighed together: adrenal, brain (with brainstem), heart, kidney, liver, ovary and fallopian tubes, pituitary, salivary gland (mandibular), spleen, testis, thyroid with parathyroid, and thymus. Organ-to-body weight percentages were calculated. The following tissues, or representative samples, were collected and preserved in 10% neutral-buffered formalin: adrenal, aorta, brain, cecum, colon, corpus and cervix uteri, duodenum, epididymis, esophagus, eyes, femur with bone marrow (articular surface of the distal end), Harderian gland, heart, ileum, jejunum, kidney, lacrimal gland (exorbital), lesions, liver, lung, lymph node (mesenteric and mandibular), gut associated lymphoid tissues (Peyer's patches), mammary glands (females only), nasal turbinates, optic nerve, ovary and fallopian tubes, pancreas, pituitary, prostate, rectum, salivary gland (mandibular and sublingual), sciatic nerve, seminal vesicle, skeletal muscle (thigh), skin, spinal cord (cervical, thoracic, and lumbar), spleen, stomach, testis, thymus, thyroid with parathyroid, tongue, trachea, urinary bladder, uterus, vagina, and Zymbal's gland. Tissues from each animal (as appropriate) were embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Tissues from the control and high-dose groups and of all macroscopic lesions were examined microscopically.

Data analysis

Levene's test was done to test for variance homogeneity (Levene, 1960). In cases of heterogeneity of variance at $p < 0.05$, transformations were used to stabilize the variance (Conover and Iman, 1981; Dixon and Massey, 1969). Comparison tests took variance heterogeneity into consideration. One-way analysis of variance (ANOVA) was used to analyze body weights, body weight changes, food consumption, continuous clinical pathology values, and organ weight data (Winer, 1971). If the ANOVA was significant, Dunnett's *t*-test was used for control versus treated group comparisons (Dunnett, 1964). Group comparisons (Groups 2 through 4 versus Group 1) were evaluated at the 5.0% two-tailed probability level.

RESULTS

Dose analysis

Mean values of the homogeneity analyses ranged from 96.0 to 102%, 101 to 105%, and 98.6 to 102% of the theoretical concentrations of 0.5, 1.5, and 5.0% (w/w) L-phenylalanine, respectively. These results indicate that the mixing procedure produced a homogeneous distribution of L-phenylalanine in the dose preparations. The mean concentrations of the dose preparation analyses for all levels ranged from 101 to 105% and from 102 to 112% of the theoretical concentrations for weeks 1 and 4, respectively. These data indicate that the levels of L-phenylalanine in the dose preparations were acceptable.

Survival and clinical observations

All animals survived to the scheduled sacrifice. No clinical observations were considered to be related to administration of L-phenylalanine. Incidental observations included sores or scabs on the head, shoulder, abdomen, or side. These observations were noted for two males fed the basal diet, one male fed the 5.0% diet, one female fed the 1.5% diet, and one female fed the 5.0% diet.

Ophthalmology

No L-phenylalanine-related ophthalmic observations were noted at the week 4 examination.

Body weights

Mean body weights for males and females fed 5.0% (w/w) L-phenylalanine diets were significantly lower during weeks 2 through 5 than animals fed the basal diet (Tables 1 and 2). These lower mean body weights were considered to be signs of mild toxicity of L-phenylalanine. The mean body weight for females fed the 0.5% diet was also significantly lower during week 5. However, the difference was not dose-related but rather was consistent with lower body weight gains and lower food consumption for animals in this group. Overall mean body weight gains paralleled the absolute body weights. Overall mean body weight gains for females fed 0.5% and males and females fed 5.0% (w/w) L-phenylalanine diets were significantly lower than those of animals fed the basal diet. These decreases in body weight parameters correlated

Table 1. Summary of body weight data (g) in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
Week 1	154 ± 10.8	151 ± 8.9	151 ± 8.4	153 ± 11.2	135 ± 10.1	135 ± 10.3	134 ± 10.4	132 ± 9.2
Week 2	216 ± 14.6	211 ± 10.7	210 ± 11.5	192 ± 22.3*	174 ± 8.4	165 ± 12.0	166 ± 12.1	143 ± 11.4*
Week 3	269 ± 18.5	263 ± 14.9	261 ± 13.9	241 ± 26.4*	194 ± 13.0	187 ± 17.4	188 ± 12.3	160 ± 13.2*
Week 4	318 ± 20.5	311 ± 18.9	308 ± 16.0	289 ± 26.0*	221 ± 13.8	205 ± 17.0	210 ± 13.5	179 ± 16.0*
Week 5	356 ± 23.3	347 ± 22.4	342 ± 20.0	320 ± 28.8*	238 ± 17.5	218 ± 16.5*	225 ± 13.0	196 ± 16.7*

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$.

Table 2. Summary of body weight gain data (g) in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
Week 1-2	62 ± 7.3	60 ± 5.1	60 ± 4.4	39 ± 16.0*	38 ± 5.1	30 ± 6.3*	32 ± 5.9	11 ± 7.5*
Week 2-3	53 ± 5.4	53 ± 5.6	51 ± 3.4	49 ± 10.1	20 ± 6.1	22 ± 7.6	22 ± 5.5	17 ± 7.1
Week 3-4	49 ± 5.0	48 ± 6.6	47 ± 3.5	48 ± 5.8	27 ± 4.9	18 ± 3.3*	22 ± 7.5	19 ± 4.2*
Week 4-5	39 ± 6.2	35 ± 6.1	35 ± 5.3	31 ± 13.2	17 ± 7.2	12 ± 5.1	15 ± 6.7	17 ± 6.1
Week 1-5	203 ± 18.4	196 ± 17.2	191 ± 12.9	167 ± 24.3*	103 ± 15.1	83 ± 11.1*	91 ± 7.9	64 ± 14.9*

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$.

Table 3. Summary of food consumption data (g) in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
Week 1	168 ± 12.1	164 ± 12.3	160 ± 6.3	139 ± 19.3*	141 ± 8.7	128 ± 8.2*	131 ± 8.2	95 ± 8.5*
Week 2	183 ± 15.1	177 ± 14.3	177 ± 7.6	168 ± 12.6	139 ± 10.4	127 ± 11.2	129 ± 5.9	101 ± 13.3*
Week 3	191 ± 11.3	184 ± 13.6	186 ± 8.0	174 ± 11.9*	150 ± 13.4	125 ± 7.3*	130 ± 7.8*	113 ± 10.9*
Week 4	197 ± 15.3	190 ± 18.9	193 ± 7.6	172 ± 20.0*	154 ± 18.5	131 ± 8.6*	139 ± 8.2	116 ± 12.9*

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$.

Table 4. Summary of test material consumption data (mg/kg/day) in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male			Female		
Dose (% w/w)	0.5	1.5	5.0	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10
Week 1	649 ± 30.9	1901 ± 95.2	5658 ± 498	607 ± 42.7	1874 ± 112	4987 ± 486
Week 2	534 ± 30.8	1601 ± 74.4	5472 ± 434	512 ± 43.1	1560 ± 93.7	4752 ± 596
Week 3	457 ± 15.7	1392 ± 59.3	4630 ± 266	462 ± 41.9	1417 ± 76.8	4752 ± 381
Week 4	407 ± 14.8	1261 ± 58.5	4033 ± 313	444 ± 30.0	1366 ± 70.5	4378 ± 396
Week 1-4	523	1548	4903	509	1555	4701

Values are means ± S.D.

with decreased food consumption in these groups and are considered to be L-phenylalanine-related.

Food consumption

The mean food consumption of males and females fed the 5.0% (w/w) L-phenylalanine diet was significantly lower throughout the study than the animals fed the basal diet (Table 3). The mean food consumption of females fed 1.5 or 0.5% (w/w) L-phenylalanine diets was also lower during week 3 and weeks 1, 3, and 4, respectively, than the females fed the basal diet. The lower food consumption suggests that the diets containing L-phenylalanine were less palatable. The differences in food consumption of the females fed the 1.5% diet were not dose-related and were relatively small compared with the more notable decreased food consumption of females fed the 5.0% diet. Decreased mean food consumption correlated with decreased mean body weight parameters in these groups and are considered to be L-phenylalanine-related.

L-phenylalanine Consumption

Animals were fed diets containing 0.5, 1.5, or 5% (w/w) L-phenylalanine. Assuming the ingestion of 20 g of feed by a 200 g rat each day, these diet concentrations of 0.5, 1.5, or 5% (w/w) L-phenylalanine translate into calculated theoretical dose levels of 500, 1,500, or 5,000 mg/kg body weight/day. The weekly average amounts of L-phenylalanine consumed by males and females on a mg/kg of body weight/day basis are summarized in Table 4.

Clinical pathology

Administration of L-phenylalanine at a dose of 5.0% of the diet was associated with mildly increased red blood cell count and mildly decreased mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, and glucose for females (Tables 5-7). There were no similar changes in males. Moreover, these changes were not considered to be toxic since the magnitude of the changes was relatively small.

Table 5. Summary of clinical hematology data in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
RBC ($\times 10^6$/mm^3)	7.34 ± 0.29	7.50 ± 0.29	7.50 ± 0.25	7.57 ± 0.30	7.50 ± 0.37	7.43 ± 0.25	7.31 ± 0.32	8.00 ± 0.64*
HGB (g/dL)	14.8 ± 0.56	15.2 ± 0.59	15.2 ± 0.37	15.2 ± 0.53	14.9 ± 0.58	15.1 ± 0.34	14.9 ± 0.33	14.9 ± 0.74
HCT (%)	43.6 ± 1.47	44.8 ± 1.60	44.9 ± 1.00	44.8 ± 1.68	43.7 ± 1.74	44.2 ± 1.28	43.5 ± 0.97	44.5 ± 2.35
MCV (fL)	59.4 ± 1.10	59.8 ± 1.58	59.9 ± 0.91	59.3 ± 1.87	58.2 ± 1.70	59.5 ± 1.30	59.5 ± 1.75	55.7 ± 2.57*
MCH (pg)	20.2 ± 0.61	20.4 ± 0.67	20.3 ± 0.30	20.0 ± 0.63	19.9 ± 0.54	20.3 ± 0.47	20.4 ± 0.80	18.7 ± 1.02*
MCHC (%)	33.9 ± 0.49	34.0 ± 0.44	33.9 ± 0.35	33.8 ± 0.34	34.1 ± 0.40	34.1 ± 0.46	34.2 ± 0.47	33.6 ± 0.37*
PLT ($\times 10^3$/mm^3)	1017 ± 107.0	1069 ± 79.1	1036 ± 106.7	1020 ± 78.1	1053 ± 105.0	1108 ± 127.2	1074 ± 97.0	1108 ± 132.0
RETIC (%)	2.2 ± 0.65	2.6 ± 1.23	2.5 ± 0.77	2.3 ± 0.45	1.9 ± 0.45	1.3 ± 0.51	1.4 ± 0.59	1.8 ± 0.60
RETIC ($\times 10^3$/mm^3)	159 ± 48.2	190 ± 87.0	186 ± 56.9	175 ± 32.2	140 ± 35.7	95 ± 37.4	105 ± 39.9	142 ± 49.3
WBC ($\times 10^3$/mm^3)	9.1 ± 1.43	9.9 ± 2.55	8.2 ± 1.23	8.5 ± 2.09	7.1 ± 2.07	6.0 ± 2.04	6.1 ± 1.79	7.4 ± 2.06
N-SEG ($\times 10^3$/mm^3)	1.2 ± 0.72	1.0 ± 0.65	0.8 ± 0.39	1.0 ± 0.66	1.0 ± 0.87	0.5 ± 0.09	0.4 ± 0.17	0.7 ± 0.53
LYMPH ($\times 10^3$/mm^3)	7.3 ± 1.66	8.2 ± 2.73	7.0 ± 1.31	7.0 ± 1.83	5.6 ± 1.50	5.1 ± 1.83	5.4 ± 1.73	6.3 ± 1.64
MONO ($\times 10^3$/mm^3)	0.5 ± 0.24	0.5 ± 0.26	0.3 ± 0.15	0.4 ± 0.20	0.3 ± 0.17	0.3 ± 0.13	0.2 ± 0.13	0.3 ± 0.18
EOSIN ($\times 10^3$/mm^3)	0.1 ± 0.00	0.1 ± 0.04	0.1 ± 0.05	0.1 ± 0.00	0.1 ± 0.05	0.1 ± 0.20	0.1 ± 0.05	0.1 ± 0.06
BASO ($\times 10^3$/mm^3)	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
N-SEG (%)	13 ± 9.1	12 ± 9.7	10 ± 5.9	12 ± 5.8	13 ± 8.3	9 ± 3.3	7 ± 2.8	9 ± 4.8
LYMPH (%)	80 ± 10.3	81 ± 10.7	85 ± 7.4	82 ± 6.9	81 ± 9.3	84 ± 3.4	88 ± 4.3	86 ± 5.6
MONO (%)	6 ± 2.7	6 ± 2.4	4 ± 1.8	5 ± 2.4	5 ± 1.8	5 ± 2.5	4 ± 1.7	3 ± 2.1
EOSIN (%)	1 ± 0.3	1 ± 0.5	1 ± 0.6	1 ± 0.5	1 ± 0.4	2 ± 1.9	1 ± 0.6	1 ± 0.4
BASO (%)	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0	0 ± 0.0
PT (sec)	12.8 ± 0.41	13.0 ± 0.46	12.8 ± 0.44	12.7 ± 0.50	12.2 ± 0.42	12.5 ± 0.56	12.4 ± 0.36	12.3 ± 0.38
PTT (sec)	17.9 ± 2.63	17.1 ± 1.00	17.4 ± 0.92	16.6 ± 0.94	15.4 ± 0.99	16.0 ± 0.61	15.5 ± 0.57	15.2 ± 0.92
FBR (mg/dL)	250 ± 41.8	271 ± 22.5	258 ± 16.7	254 ± 32.1	228 ± 25.9	209 ± 26.6	215 ± 18.5	208 ± 31.0

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$.

RBC, red blood cell count; HGB, hemoglobin; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; PLT, platelet count; RETIC, reticulocyte count and its percentage. WBC, white blood cell count; N-SEG, neutrophil count and its percentage; LYMPH, lymphocyte count and its percentage; MONO, monocytes count and its percentage; EOSIN, eosinophil cells count and its percentage; BASO, basophils count and its percentage; PT, prothrombin time; PTT, activated partial thromboplastin time; FBR, fibrinogen.

Table 6-1. Summary of clinical chemistry data in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
GLU (mg/dL)	108 ± 11.3	100 ± 7.3	108 ± 8.5	102 ± 9.8	114 ± 14.5	110 ± 9.2	113 ± 10.4	99 ± 10.3*
UN (mg/dL)	13 ± 1.8	14 ± 1.3	14 ± 1.9	13 ± 2.0	14 ± 2.5	15 ± 1.1	15 ± 1.5	18 ± 5.7
CRET (mg/dL)	0.6 ± 0.04	0.6 ± 0.07	0.6 ± 0.03	0.6 ± 0.03	0.7 ± 0.06	0.7 ± 0.07	0.7 ± 0.05	0.6 ± 0.07
T PRO (g/dL)	6.5 ± 0.36	6.4 ± 0.30	6.4 ± 0.22	6.7 ± 0.24	6.8 ± 0.31	6.7 ± 0.31	6.8 ± 0.23	6.6 ± 0.28
ALB (g/dL)	4.5 ± 0.20	4.5 ± 0.22	4.5 ± 0.18	4.7 ± 0.30	5.1 ± 0.24	5.0 ± 0.29	5.0 ± 0.19	4.9 ± 0.20
GLOB (g/dL)	2.0 ± 0.37	1.9 ± 0.20	1.9 ± 0.22	1.9 ± 0.24	1.7 ± 0.23	1.7 ± 0.21	1.8 ± 0.16	1.8 ± 0.14
A/G ratio	2.3 ± 0.44	2.4 ± 0.29	2.4 ± 0.35	2.5 ± 0.38	3.0 ± 0.42	3.0 ± 0.49	2.9 ± 0.32	2.8 ± 0.24
T BILI (mg/dL)	0.2 ± 0.07	0.1 ± 0.04	0.1 ± 0.04	0.1 ± 0.04	0.2 ± 0.05	0.2 ± 0.05	0.2 ± 0.05	0.1 ± 0.05
AST (IU/L)	133 ± 33.8	134 ± 22.3	128 ± 12.5	122 ± 20.6	132 ± 22.4	119 ± 19.1	132 ± 17.6	128 ± 37.1
ALT (IU/L)	38 ± 6.8	41 ± 6.1	37 ± 3.6	44 ± 10.5	34 ± 7.4	32 ± 5.5	36 ± 13.6	31 ± 3.4
ALP (IU/L)	200 ± 41.5	228 ± 42.6	213 ± 31.8	195 ± 39.5	130 ± 29.5	125 ± 23.2	134 ± 24.3	128 ± 23.4
GGT (IU/L)	0 ± 0.5	1 ± 0.5	0 ± 0.5	0 ± 0.5	1 ± 0.3	1 ± 0.3	1 ± 0.9	1 ± 0.4
Ca (mg/dL)	10.0 ± 0.32	10.0 ± 0.47	9.6 ± 0.48	9.9 ± 0.53	10.1 ± 0.24	10.1 ± 0.22	9.9 ± 0.27	9.9 ± 0.24
IP (mg/dL)	9.1 ± 0.63	9.5 ± 0.51	8.6 ± 0.44	8.8 ± 0.47	7.8 ± 1.15	8.1 ± 0.79	7.6 ± 0.43	8.5 ± 0.79
Na (mmol/L)	145 ± 1.3	147 ± 1.5	146 ± 1.1	146 ± 2.2	145 ± 1.7	145 ± 0.9	144 ± 1.3	145 ± 1.3
K (mmol/L)	5.7 ± 0.22	5.7 ± 0.44	5.5 ± 0.21	5.8 ± 0.50	5.4 ± 0.23	5.7 ± 0.24	5.6 ± 0.54	5.6 ± 0.24
CI (mmol/L)	101 ± 1.1	102 ± 1.5	102 ± 0.8	101 ± 1.7	101 ± 1.4	102 ± 1.2	101 ± 1.3	102 ± 1.4

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$.

GLU, glucose; UN, urea nitrogen; CRET, creatinine; T PRO, total protein; ALB, albumin; GLOB, globulin; A/G, albumin/globulin ratio; T BILI, total bilirubin; AST, aspartate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase; GGT, gamma glutamyltransferase; Ca, calcium; IP, inorganic phosphorus; Na, sodium ion; K, potassium ion; CI, chlorite ion.

Table 6-2. Summary of clinical chemistry data in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
E ALB (g/dL)	4.5 ± 0.20	4.5 ± 0.28	4.6 ± 0.21	4.8 ± 0.27*	4.9 ± 0.22	4.9 ± 0.23	5.0 ± 0.26	4.8 ± 0.21
E A-1 (g/dL)	0.1 ± 0.00	0.1 ± 0.03	0.1 ± 0.00	0.1 ± 0.06	0.1 ± 0.03	0.1 ± 0.05	0.1 ± 0.05	0.1 ± 0.03
E A-2 (g/dL)	0.5 ± 0.07	0.6 ± 0.11*	0.5 ± 0.06	0.5 ± 0.07	0.5 ± 0.08	0.5 ± 0.09	0.5 ± 0.09	0.5 ± 0.13
E BETA (g/dL)	1.1 ± 0.16	1.0 ± 0.12	1.0 ± 0.09*	1.1 ± 0.07	1.2 ± 0.13	1.0 ± 0.11	1.0 ± 0.11*	1.0 ± 0.13*
E GAMMA (g/dL)	0.2 ± 0.16	0.2 ± 0.07	0.2 ± 0.10	0.2 ± 0.07	0.2 ± 0.08	0.2 ± 0.14	0.2 ± 0.07	0.2 ± 0.09

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$.

E ALB, albumin; E A-1, α1 globulin; E A-2, α2 globulin; E BETA, beta globulin; E GAMMA, gamma globulin.

Table 7. Summary of clinical urinalysis data in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10
U VOL (mL)	25.7 ± 14.62	13.2 ± 8.64	20.3 ± 15.92	15.8 ± 14.35
SP GR	1.012 ± 0.006	1.020 ± 0.007	1.014 ± 0.009	1.018 ± 0.010
U PH	6.9 ± 0.21	7.0 ± 0.28	7.1 ± 0.32	7.0 ± 0.28

Sex	Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10
U VOL (mL)	21.6 ± 16.11	24.3 ± 16.93	21.1 ± 16.91	16.2 ± 13.08
SP GR	1.012 ± 0.011	1.008 ± 0.005	1.011 ± 0.007	1.014 ± 0.009
U PH	7.0 ± 0.37	6.8 ± 0.26	6.6 ± 0.28*	7.3 ± 0.42

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$.

U VOL, urinary volume; SP GR, specific gravity; U PH, urinary pH.

Table 8-1. Summary of organ weight (absolute organ weight) data in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
Body weight[a] (g)	321.1 ± 22.3	311.6 ± 21.6	309.6 ± 17.7	294.0 ± 25.6	211.1 ± 13.8	195.8 ± 16.3	202.4 ± 11.6	178.3 ± 15.6*
Adrenals (mg)	59.8 ± 9.1	57.4 ± 5.1	61.3 ± 5.1	53.5 ± 5.6	69.7 ± 7.3	68.7 ± 3.3	67.2 ± 9.4	56.8 ± 6.3*
Brain[b] (g)	1.99 ± 0.06	1.97 ± 0.04	1.97 ± 0.11	1.91 ± 0.08	1.85 ± 0.05	1.89 ± 0.06	1.86 ± 0.07	1.79 ± 0.05
Heart (g)	1.26 ± 0.13	1.20 ± 0.10	1.26 ± 0.12	1.16 ± 0.11	0.895 ± 0.102	0.851 ± 0.122	0.862 ± 0.104	0.748 ± 0.070*
Kidneys (g)	2.64 ± 0.22	2.67 ± 0.22	2.67 ± 0.18	2.72 ± 0.33	1.82 ± 0.17	1.74 ± 0.14	1.74 ± 0.06	1.62 ± 0.21
Liver (g)	10.11 ± 0.74	9.76 ± 0.90	9.79 ± 0.73	10.04 ± 1.16	7.28 ± 0.69	6.63 ± 0.68	6.76 ± 0.48	6.16 ± 0.57*
Ovaries[c] (mg)	NA	NA	NA	NA	133 ± 25	127 ± 13	137 ± 24	118 ± 13
Pituitary (mg)	11.9 ± 1.3	11.4 ± 1.9	11.9 ± 1.4	11.5 ± 1.5	13.8 ± 3.3	13.8 ± 1.6	14.0 ± 1.5	12.4 ± 2.0
Salivary glands[d] (mg)	718 ± 74	708 ± 82	730 ± 66	668 ± 44	483 ± 64	466 ± 41	471 ± 51	454 ± 55
Spleen (mg)	708 ± 59	733 ± 98	667 ± 85	667 ± 107	487 ± 47	483 ± 106	484 ± 63	487 ± 115
Testes (g)	2.96 ± 0.14	2.99 ± 0.26	2.93 ± 0.23	2.94 ± 0.21	NA	NA	NA	NA
Thyroids[e] (mg)	20.2 ± 5.8	20.0 ± 3.5	19.3 ± 1.9	20.4 ± 3.7	17.3 ± 2.5	18.1 ± 1.9	16.4 ± 2.3	16.9 ± 4.6
Thymus (mg)	619 ± 90	672 ± 118	626 ± 120	576 ± 128	565 ± 125	552 ± 110	566 ± 98	503 ± 118

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$. NA, Not applicable.

[a] Terminal body weight. [b] brain with brainstem. [c] ovary and fallopian tubes. [d] mandibular gland. [e] thyroid with parathyroid.

Table 8-2. Summary of organ weight (organ-to-body weight percentage) data in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
Body weight[a] (g)	321.1 ± 22.3	311.6 ± 21.6	309.6 ± 17.7	294.0 ± 25.6	211.1 ± 13.8	195.8 ± 16.3	202.4 ± 11.6	178.3 ± 15.6*
Adrenals (mg%)	18.7 ± 2.9	18.5 ± 1.9	19.8 ± 1.8	18.2 ± 1.8	33.1 ± 3.7	35.2 ± 2.6	33.2 ± 4.2	32.1 ± 4.7
Brain[b] (mg%)	623 ± 40	634 ± 40	638 ± 48	653 ± 50	878 ± 53	972 ± 80*	921 ± 70	1012 ± 73
Heart (mg%)	393 ± 39	385 ± 30	407 ± 43	395 ± 34	424 ± 38	434 ± 38	425 ± 40	420 ± 28
Kidneys (mg%)	821 ± 43	858 ± 56	863 ± 49	924 ± 61*	864 ± 72	891 ± 56	861 ± 55	913 ± 103
Liver (g%)	3.15 ± 0.15	3.13 ± 0.18	3.16 ± 0.20	3.41 ± 0.13*	3.45 ± 0.18	3.39 ± 0.18	3.34 ± 0.11	3.46 ± 0.27
Ovaries[c] (mg%)	NA	NA	NA	NA	62.9 ± 11.2	65.3 ± 7.2	67.7 ± 11.8	66.6 ± 6.8
Pituitary (mg%)	3.7 ± 0.3	3.6 ± 0.5	3.9 ± 0.4	3.9 ± 0.6	6.6 ± 1.6	7.1 ± 0.7	6.9 ± 0.8	6.9 ± 0.9
Salivary glands[d] (mg%)	224 ± 16	227 ± 18	236 ± 18	228 ± 14	229 ± 29	238 ± 18	233 ± 29	255 ± 25
Spleen (mg%)	221 ± 15	235 ± 26	215 ± 20	226 ± 25	232 ± 27	245 ± 39	239 ± 28	272 ± 54
Testes (mg%)	926 ± 63	964 ± 114	946 ± 64	1007 ± 111	NA	NA	NA	NA
Thyroids[e] (mg%)	6.3 ± 1.6	6.4 ± 1.1	6.3 ± 0.8	7.0 ± 1.4	8.2 ± 1.0	9.3 ± 1.1	8.1 ± 1.1	9.5 ± 2.5
Thymus (mg%)	193 ± 22	216 ± 38	203 ± 46	197 ± 47	267 ± 51	281 ± 48	279 ± 37	282 ± 64

Values are means ± S.D. Significantly different from the control: * $p \leq 0.05$. NA, Not applicable.

[a] Terminal body weight. [b] brain with brainstem. [c] ovary and fallopian tubes. [d] mandibular gland. [e] thyroid with parathyroid.

Statistically significant differences for serum protein fractions, as measured by serum protein electrophoresis, were considered incidental because the differences were very small and inconsistent. In addition, there were no correlative clinical or anatomic pathology findings.

Anatomic pathology

Terminal body weights were lower for males and females fed the 5.0% (w/w) diet. The mean absolute organ weight of adrenals, heart, and liver of females fed the 5.0% (w/w) L-phenylalanine diet were significantly lower than animals fed the basal diet (Table 8). In addition, the mean organ-to-body weight percentage of kidneys and liver of males fed the 5.0% (w/w) L-phenylalanine diet were significantly higher relative than animals fed the basal diet. There were no L-phenylalanine-related changes for macroscopic or microscopic findings (Tables 9 and 10). Changes in organ weights are not considered adverse, because there were no correlative clinical, or macroscopic or microscopic pathology findings.

DISCUSSION

Based on the results of this study, when fed to male

Table 9. Incidence of Macroscopic Observations in 28-day dietary toxicity study with L-phenylalanine in rats

Sex	Male				Female			
Dose (% w/w)	0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats	10	10	10	10	10	10	10	10
Kidney								
Mottled	0	0	0	0	0	0	0	1
Irregular shape	0	0	0	0	0	0	0	1
Large pelvis	0	0	1	0	0	0	0	1
Thymus								
Red focus/area	0	0	0	0	0	1	0	0
Stomach, glandular								
Red focus/area	0	0	0	0	0	0	0	1
Lymph node, mandibular								
Large	2	2	1	0	2	2	0	0
Mottled	0	0	2	0	0	1	0	0
Diffusely red	0	1	0	1	0	0	0	0
Skin								
Crusted area	0	0	0	1	0	0	0	0
Uterus								
Lumen filled with fluid	NA	NA	NA	NA	1	0	0	0
Ureter								
Large	0	0	0	0	0	0	0	1
Lymph node, renal								
Large	0	0	0	0	0	0	0	1

NA, Not applicable.

and female Crl:CD®(SD)IGS BR rats in the diet at levels of 0.5, 1.5, or 5.0% (w/w) for at least 28 days, L-phenylalanine did not result in toxic effects on clinical or ophthalmic observations, on organ weights, or on macroscopic or microscopic findings.

L-phenylalanine-related effects were noted in mean body weights and mean body weight gains for females fed 0.5% and for males and females fed 5.0% (w/w) L-phenylalanine diets, which were consistent with lower food consumption for animals in these groups. L-phenylalanine-related effects were also noted in mean food consumption for males and females fed the 5% (w/w) L-phenylalanine diet. Marked suppression of body weight gains and decreased food consumption are known to occur in weanling rats fed diets containing excessive levels (5.0%) of L-phenylalanine (Muramatsu *et al*.,1971) or in rats averaging 170 g at study start and fed 5% L-phenylalanine diets (Peng *et al*., 1973). The similar, but less profound, effects of L-phenylalanine on a different strain of rats approximately 6-weeks old observed in the current study are consistent with these earlier investigations. Similarly, intragastric preloads of 0.50 and 1.00 g/kg L-phenylalanine in monkeys produced marked suppressions of food consumption (Gibbs *et al*., 1960) (body weight is not reported in the study).

The mean food consumption of females fed 1.5 or 0.5% (w/w) L-phenylalanine diets also was, intermittently, significantly lower than of females fed the basal diet. These differences in food consumption and mean body weights for females fed the 0.5 or 1.5% L-phenylalanine diets were not dose-related and were not considered adverse. The lower food consumption and lower body weights of the males and females fed the 5.0% L-phenylalanine diet were considered to be signs of mild toxicity.

In conclusion, the no-observed-effect level (NOEL) of dietary exposure of male rats to L-phenylalanine is considered to be 1.5% (w/w) L-phenylalanine. The NOEL of dietary exposure of female rats to L-phenylalanine is considered to be less than 0.5% (w/w) L-phenylalanine. However, the no-observed-adverse-effect level (NOAEL) for males and females is considered to be 1.5% (w/w) L-phenylalanine.

Conflict of interest---- The authors declare that there is no conflict of interest.

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Table 10. Incidence of microscopic observations in 28-day dietary toxicity study with L-phenylalanine in rats

Sex		Male				Female			
Dose (% w/w)		0 (Control)	0.5	1.5	5.0	0 (Control)	0.5	1.5	5.0
Number of rats		10	10	10	10	10	10	10	10
Brain	Number examined	10	0	0	10	10	0	0	10
Extramedullary hematopoiesis		1	0	0	0	0	0	0	0
Kidney	Number examined	10	0	1	10	10	0	0	10
Cast, proteinaceous		0	0	0	1	0	0	0	0
Dilatation, pelvic		0	0	1	0	0	0	0	1
Infarct		0	0	0	0	0	0	0	1
Mineralization, tubular		0	0	0	0	1	0	0	1
Pyelonephritis		0	0	0	0	0	0	0	1
Liver	Number examined	10	0	0	10	10	0	0	10
Inflammation, chronic		4	0	0	3	3	0	0	1
Hemorrhage		0	0	0	1	0	0	0	0
Necrosis, hepatocellular		0	0	0	1	0	0	0	0
Lung	Number examined	10	0	0	10	10	0	0	10
Mineralization, vascular		0	0	0	1	0	0	0	0
Thrombus		0	0	0	0	1	0	0	0
Thyroid	Number examined	10	0	0	10	10	0	0	10
Cyst, ultimobronchial		1	0	0	0	1	0	0	1
Ectopic thymus		2	0	0	2	0	0	0	2
Thymus	Number examined	10	0	0	10	10	1	0	10
Hemorrhage		1	0	0	3	1	1	0	0
Stomach, gl[a]	Number examined	10	0	0	10	10	0	0	10
Edema		1	0	0	1	2	0	0	2
Stomach, non-gl[a]	Number examined	10	0	0	10	10	0	0	10
Edema		0	0	0	1	0	0	0	0
Rectum	Number examined	10	0	0	10	10	0	0	10
Parasitism		2	0	0	0	0	0	0	0
LN[b], mandibular	Number examined	10	2	3	10	10	3	0	10
Hemorrhage		1	1	1	4	1	0	0	0
Hyperplasia, lymphocytic		1	2	1	0	0	1	0	0
Skin	Number examined	10	0	0	10	10	0	0	10
Edema		0	0	0	1	0	0	0	0
Erosion		0	0	0	1	0	0	0	0
Fibrosis		0	0	0	1	0	0	0	0
Hemorrhage		0	0	0	1	0	0	0	0
Inflammation, acute		0	0	0	1	0	0	0	0
Urinary bladder	Number examined	10	0	0	10	10	0	0	10
Hyperplasia, transitional cell		0	0	0	0	0	0	0	1
Inflammation, chronic-active		0	0	0	0	0	0	0	1
Uterus	Number examined	NA	NA	NA	NA	10	0	0	10
Dilatation						3	0	0	1
Ureter	Number examined	NA	NA	NA	NA	0	0	0	1
Dilatation						0	0	0	1
Hyperplasia						0	0	0	1
Inflammation, chronic-active						0	0	0	1

[a] glandular

[b] Lymph node

NA, Not applicable

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