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A 13-week subchronic toxicity study of dietary administered morin in F344 rats

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Abstract

A subchronic toxicity study of a flavonoid morin was performed in both sexes of F344 rats with dietary administration at concentrations of 0%, 0.625%, 1.25%, 2.5% and 5% (w/w) for 13 weeks. No mortality or abnormal clinical signs were observed throughout the experimental period in any group. Although a slight tendency for increase in food intake was noted in both sexes of the 2.5% and 5.0% groups, slight non-significant body weight decrease was observed in 5.0% males. Significant increases in alanine transaminase (ALT; over 2.5%), alkali phosphatase (ALP; 1.25% and 5.0%) and relative liver weights (1.25% and 2.5%) in males and in γ-glutamyl transpeptidase (γ-GT), aspartate transaminase (AST), ALT, relative liver weights in the 2.5% and 5.0% females and ALP in 5.0% females were noted. Increased urea nitrogen and relative kidney weights at dose of 1.25% and above and creatinine at 5.0% were observed also in females. On histopathological observation, hepatocyte hypertrophy was detected in 3 of 10 5.0% females. Based on the above findings, the no-observed-adverse-effect level (NOAEL) for both sexes was estimated to be 0.625% (299 and 356 mg/kg b.w./day for males and females, respectively).

Keywords: Morin; F344 rats; Subchronic toxicity

1. Introduction

Morin (2′,3,4′,5,7-pentahydroxyflavone), a constituent of many fruits and herbs, e.g. mulberries, figs and other Moraceae, is a flavonoid which is used as a food additive because of its antioxidant activity (Hanasaki et al., 1994; Ramanathan et al., 1994). In addition, it possesses anti-inflammatory potential through modulatory effects on lipoxygenase and cycloxygenase activities in the arachidonic cascade (Baumann et al., 1980; Galvez et al., 2001; Nakadate et al., 1984). Morin is also reported to be active against phenobarbital promotion of rat liver tumor development after initiation with *N*-nitrosodiethylamine (DEN) (Denda et al., 1989) and to inhibit 4-nitroquinoline 1-oxide (4-NQO)-induced rat tongue carcinogenesis either in the initiation or post-initiation stage, and azoxymethane (AOM)-induced rat colorectal carcinogenesis in the post-initiation stage. These effects are considered to be due to induction of phase II enzymes and/or suppression of cell proliferation activity by morin (Kawabata et al., 1999; Tanaka et al., 1999).

However, quercetin, a positional isomer of morin and a naturally occurring flavonol in plants, induces gene mutations in *Salmonella* with and without exogenous metabolic activation, mutations in *Drosophila*, and chromosomal aberrations and sister chromatid exchange in mammalian cell cultures (Brown, 1980; Carver et al., 1983; IARC, 1983; Meltz and MacGregor, 1981; Nagao et al., 1981; National Toxicology Program, 1992). Renal toxic lesions, with increased severity of chronic nephropathy and tumors of the tubular epithelium, were also found in an oral carcinogenicity study in male rats (Dunnick and Hailey, 1992), although other long-term rat studies indicated no carcinogenic effects (Hirono et al., 1981; Ito et al., 1989).

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Toxicological effects of morin are therefore conceivable, but no data in vivo are available except for the documented pro-oxidant activity in isolated rat liver nuclei (Sahu and Gray, 1997) and oxidative effects on hemoglobin in vitro (Kitagawa et al., 2004). While morin induces gene mutations in *Salmonella*, the activity is weak (Nagao et al., 1981). The present study was therefore conducted to determine toxic effects and to establish a no-observed-adverse-effect level (NOAEL) with oral administration to F344 rats.

2. Material and methods

2.1. Test chemical

The morin sample (CAS no. 480-16-0, Fig. 1) obtained from San-Ei Gen F.F.I. (Osaka, Japan) was a yellowish brown powder with a purity of >95% (w/w), which was extracted from branch, trunk and root parts of *Broussonetia xanthoxylum* MARTIUS using ethanol extraction and purification. The compound is insoluble in water or oil but soluble in ethanol or alkali solutions, and stable at room temperature in a dark place (internal data of San-Ei Gen F.F.I.). For the present study the compound was mixed into powdered basal diet (CRF-1, Oriental Yeast, Tokyo, Japan).

2.2. Experimental animals

A total of 50 male and 50 female 5-week-old F344/DuCrj rats were purchased from Charles River Japan (Kanagawa, Japan) and used after a 1-week acclimatization. The animals were housed five to a polycarbonate cage with soft chip bedding (Sankyo Laboratory Service, Tokyo, Japan) in a room with a barrier system controlled for the light-dark cycle (12–12 h, lights on 7:00–19:00), ventilation (air-exchange rate of 18 times per hour), temperature (24 ± 1 °C) and relative humidity (55 ± 5%) during the study. The cages and the chip bedding were exchanged twice a week. Each animal had free access to powdered diet with or without the test chemical and tap water. At the initiation the animals were randomly allocated to five groups of 10 male and 10 female rats each based on their body weights.

2.3. Study design

In a preliminary 2-week palatability study of morin with dietary administration at doses of 0 (control), 2.5% and 5% (w/w), no test substance-related changes in body weights and food consumption were observed (data not shown). From these results, doses of morin were determined to be 0%, 0.625%, 1.25%, 2.5% or 5.0% (w/w) for both males and females for this 13-week toxicity study.

General conditions and mortality were examined daily and body weights were measured once a week during the study period. The amounts of supplied and residual diet were weighed weekly in order to calculate the average daily food intake for each week, and then the overall means through the entire treatment period were calculated from the determined weekly intake. All rats were fasted overnight at the completion of the treatment period, and then blood samples were collected from the abdominal aorta under ether anesthesia for hematology and serum biochemistry. The following hematological parameters were analyzed using an automatic hematology analyzer M-2000 (Toa Medical Electronics, Hyogo, Japan): red blood cell count (RBC), hemoglobin concentration (Hb), hematocrit (Ht), mean corpuscular volume (MC), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), white blood cell count (WBC), and platelet count (PLT). Blood smears were processed for Giemsa staining and counting of reticulocytes (Ebl) and differential leukocytes using a Microx HEG-120A (Omron Tateishi Electronics, Tokyo, Japan). Serum biochemistry measurements of the following parameters were performed by SRL (Tokyo, Japan): total protein (TP), albumin (Alb), albumin/globulin ratio (A/G), total cholesterol (T-Cho), triglyceride (TG), total bilirubin (T-Bil), γ-glutamyl transpeptidase (γ-GT), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea nitrogen (BUN), creatinine (Cre), calcium (Ca), inorganic phosphorus (P), sodium (Na), potassium (K), and chloride (Cl). Complete necropsy was performed for all animals, and then the brain, heart, lungs, liver, spleen, adrenal glands, kidneys and testes were weighed. These organs and the following organs and tissues were fixed in 10% neutral buffered formalin, and paraffin-embedded sections were routinely prepared and stained with hematoxylin and eosin (H.E.) for histopathological examination: skin, mammary gland, sternum with marrow, femur with marrow, thymus, mandibular and mesenteric lymph nodes, submandibular glands, sublingual glands, aorta, trachea, tongue, esophagus, stomach, small and large intestines, pancreas, urinary bladder, epididymides, seminal vesicles, prostate gland, bulbourethral gland, ovaries, uterus, vagina, pituitary gland, thyroid glands, parathyroid glands, spinal cord, trigeminal nerve, sciatic nerve, eyes, Harderian glands, femoral skeletal muscle and nasal cavity. One of the testes of five animals in each group was fixed in Bouin's fixative of quantitative analysis of seminiferous tubules.

The present study design was basically in accordance with Guidelines for Designation for Food Additives and for Revision of Standards for Use of Food Additives of Japan (1996).

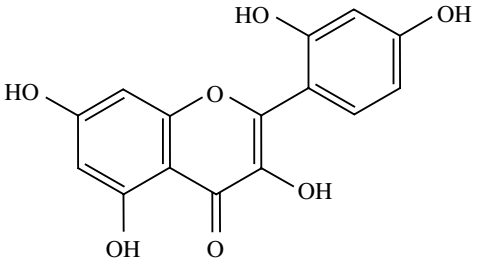

Fig. 1. Chemical structure of morin.

2.4. Statistical analysis

Variance in data for body and organ weights as well as the results of hematology, and serum biochemistry was checked for homogeneity by Bartlett's procedure. When the data were homogeneous, one-way analysis of variance for homogeneity (ANOVA) was used. In the heterogeneous cases, the Kruskal–Wallis test was applied. When statistically significant differences were indicated, the Dunnett's multiple test was employed for comparisons between control and treated groups.

3. Results

Neither mortality nor deterioration in general conditions were observed in any of the groups. A slight reduction of body weight gain was noted in 5.0% males without statistical significance (Table 1). As shown in Table 1, data for food consumption and total chemical intake showed a tendency for increase in both sexes of the 2.5% and 5% groups, resulting in greater than expected doses of the test substance. Hematological data are shown in Tables 2 and 3. WBCs for females of the 2.5% and 5.0% groups were significantly increased. A significant decrease in the proportion of segmented neutrophils was also noted for the 5.0% females. Serum biochemistry data are presented in Tables 4 and 5. In males, significant increase of Alb and significant decrease of TG were observed at 5.0%, A/G was significantly increased in 2.5% and above, and significant

Table 1
Body weight and food intake data for F344 rats fed diet containing morin for 13 weeks

Group (%)	Body weight (g)[a]		Food intake		Total chemical intake (mg/kg/day)
	Initial	Final	(g/rat/total)	(g/rat/day)	
Male					
0	132 ± 8	281 ± 20	173	13.3	0
0.625	133 ± 6	300 ± 11	186	14.4	299
1.25	132 ± 8	291 ± 8	185	14.3	613
2.5	133 ± 8	299 ± 16	209	16.1	1350
5.0	133 ± 7	268 ± 9	240	18.5	3458
Female					
0	100 ± 4	170 ± 9	127	9.8	0
0.625	101 ± 3	167 ± 6	123	9.5	356
1.25	100 ± 3	168 ± 4	124	9.6	712
2.5	101 ± 3	167 ± 7	147	11.4	1701
5.0	100 ± 4	167 ± 8	165	12.7	3802

[a] Mean ± SD.

Table 2
Hematological data for male F344 rats fed diet containing morin for 13 weeks

	Dose level (%)				
	0	0.625	1.25	2.5	5.0
No. of samples	10	10	10	10	10
RBC (10^{10}/l)	922 ± 32	891 ± 27	896 ± 36	894 ± 23	897 ± 47
Hb (g/dl)	15.0 ± 1.0	14.2 ± 0.9	14.5 ± 1.8	14.5 ± 0.7	14.1 ± 0.9
Ht (%)	49.0 ± 2.0	47.3 ± 1.3	48.0 ± 1.8	47.3 ± 1.2	47.3 ± 2.4
MCV (fl)	53.1 ± 0.7	53.1 ± 0.4	53.5 ± 0.4	52.9 ± 0.5	52.7 ± 0.5
MCH (pg)	16.2 ± 0.8	15.9 ± 0.7	16.1 ± 1.6	16.2 ± 0.6	15.7 ± 0.6
MCHC (g/dl)	30.5 ± 1.5	30.0 ± 1.4	30.1 ± 3.1	30.7 ± 1.2	29.8 ± 1.1
Ebl (Count/200WBC)	0.51 ± 0.87	0.80 ± 1.12[a]	0.46 ± 0.70	0.62 ± 1.02	0.20 ± 0.65
WBC (10^8/l)	42.0 ± 6.2	47.4 ± 12.9	38.1 ± 7.9	42.7 ± 6.9	45.2 ± 7.9
PLT (10^{10}/l)	59.4 ± 4.7	62.3 ± 2.9	56.8 ± 4.2	61.1 ± 3.7	63.1 ± 5.5
Differential cell count (%)					
Seg.	16.9 ± 5.0	18.3 ± 5.6[a]	15.1 ± 5.1	18.5 ± 6.0	24.4 ± 7.5
Eosin.	0.60 ± 0.84	1.12 ± 1.28[a]	1.01 ± 1.06	0.90 ± 0.88	0.40 ± 0.70
Lymph	81.7 ± 5.3	79.7 ± 5.6[a]	83.1 ± 5.7	79.9 ± 6.7	74.7 ± 7.1
Mono	0.80 ± 1.14	0.78 ± 1.09[a]	0.86 ± 0.89	0.70 ± 0.67	0.50 ± 0.71

Values are mean ± SD.
[a] Data from nine animals.

increases of ALT and P and decrease of Na were observed at 2.5%. The ALP value was significant increased in the 1.25% and 5.0% groups along with T-Bil at 1.25%. In females, significant increases of T-Bil and ALP, and significant decreases of T-Cho and Cr were observed at 5.0%. A significant decrease of TP and significant increases of γ-GT, AST, ALT were also observed at 2.5% and 5.0%. Significant increase of BUN was evident at 1.25% and above. Cl showed significant changes at 1.25% and higher, but without dose dependence. Organ weight data are shown in Tables 6 and 7. In males, sporadic changes in absolute and relative weights were found for the heart, kidney and spleen, but no dose-dependence was apparent. The liver absolute and relative weights were increased in the 1.25% and 2.5%, and the 1.25% and 5.0% groups, respectively. In females, statistically significant decreases in the absolute weights of the thymus, heart, kidneys and adrenal glands, and significant increase liver values were observed at 5%. The relative organ weights for liver and kidneys were significantly increased in 2.5% and 1.25%, respectively, and over, but decreased for the thymus and heart. On the macroscopic examination, there were no obvious findings noted in either sex of any group. Histopathological examination demonstrated focal accumulation of mononuclear cells in the myocardium, and proximal tubular eosinophilic bodies and basophilic tubules in the kidneys, as spontaneously occurring lesions in many of the males. In females, focal hepatocyte hypertrophy was detected in 3 of 10 rats receiving the 5.0% dose. However, the incidence did not differ from the control value. Other lesions were also sporadically detected, but no significant alteration in incidence was apparent in the 5.0% group (data not shown). In addition, there were no lesions in ovaries, uterus, adrenal glands and bone marrow attributable to the morin treatment.

Table 3
Hematological data for female F344 rats fed diet containing morin for 13 weeks

	Dose level (%)				
	0	0.625	1.25	2.5	5.0
No. of samples	10	10	10	10	10
RBC (10^{10}/l)	823 ± 45	849 ± 59	813 ± 75	794 ± 26[a]	822 ± 32
Hb (g/dl)	13.4 ± 1.6	13.8 ± 1.4	13.7 ± 1.5	13.2 ± 1.2[a]	13.4 ± 1.0
Ht (%)	45.9 ± 2.5	47.4 ± 3.3	45.3 ± 4.3	44.4 ± 1.6[a]	46.0 ± 1.9
MCV (fl)	55.8 ± 0.4	55.8 ± 0.4	55.7 ± 0.4	55.9 ± 0.4	55.9 ± 0.3
MCH (pg)	16.2 ± 1.6	16.3 ± 1.3	16.8 ± 0.7	16.6 ± 1.2	16.3 ± 1.3
MCHC (g/dl)	29.1 ± 2.8	29.2 ± 2.3	30.1 ± 1.2	29.7 ± 2.0	29.1 ± 2.3
Ebl (Count/200WBC)	1.85 ± 1.63	1.12 ± 0.89	2.01 ± 1.96	1.87 ± 1.80	1.14 ± 1.60
WBC (10^8/l)	34.3 ± 4.7	41.0 ± 7.6	38.5 ± 6.2	42.7 ± 5.2[a,*]	44.2 ± 7.2[**]
PLT (10^{10}/l)	66.6 ± 4.6	68.1 ± 2.9	68.6 ± 7.8	66.8 ± 6.7[a]	65.7 ± 4.4
Differential cell count (%)					
Seg.	19.9 ± 9.0	17.6 ± 2.4	16.2 ± 4.8	15.7 ± 5.8	12.8 ± 3.4[#]
Eosin.	0.81 ± 0.93	1.20 ± 0.92	1.02 ± 0.99	1.41 ± 1.19	1.22 ± 0.80
Lymph	77.8 ± 8.8	80.1 ± 2.1	81.2 ± 4.9	81.3 ± 6.2	85.0 ± 3.1
Mono	1.42 ± 0.88	1.10 ± 0.88	1.57 ± 1.02	1.64 ± 1.47	1.01 ± 0.83

[*] Significantly different from the controls (0%) at $p < 0.05$ (parametric method).
[**] Significantly different from the controls (0%) at $p < 0.01$ (parametric method).
[#] Significantly different from the controls (0%) at $p < 0.05$ (non-parametric method).
[a] Data from nine animals.

Table 4
Serum chemistry data for male F344 rats fed diet containing morin for 13 weeks

	Dose level (%)				
	0	0.625	1.25	2.5	5.0
No. of samples	10	10	10	10	10
TP (g/dl)	6.28 ± 0.17	6.24 ± 0.20	6.33 ± 0.16	6.33 ± 0.23	6.38 ± 0.33
Alb (g/dl)	4.32 ± 0.14	4.31 ± 0.13	4.40 ± 0.11	4.45 ± 0.15	4.57 ± 0.23[**]
A/G	2.22 ± 0.12	2.25 ± 0.12	2.30 ± 0.12	2.38 ± 0.08[**]	2.53 ± 0.09[**]
T-Cho (mg/dl)	61.2 ± 4.4	62.0 ± 5.1	62.0 ± 4.2	65.1 ± 5.8	68.4 ± 18.4
TG (mg/dl)	109 ± 38	85.3 ± 20.6	96.8 ± 29.1	98.5 ± 17.1	68.8 ± 30.2[**]
T-Bil (mg/dl)	0.11 ± 0.03	0.10 ± 0.00	0.16 ± 0.05[#]	0.10 ± 0.00	0.12 ± 0.04
γ-GT (IU/l)	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0
AST (IU/l)	77.9 ± 12.0	81.2 ± 10.0	80.8 ± 13.0	78.7 ± 13.7	91.4 ± 30.6
ALT (IU/l)	56.0 ± 4.6	58.3 ± 7.5	59.1 ± 4.4	73.0 ± 20.5[#]	84.3 ± 35.5
ALP (IU/l)	430 ± 59	364 ± 32	707 ± 74[##]	556 ± 83	808 ± 319[#]
BUN (mg/dl)	19.0 ± 2.5	21.2 ± 1.6	19.8 ± 1.7	19.6 ± 2.3	19.8 ± 2.6
Cre (mg/dl)	0.31 ± 0.03	0.31 ± 0.03	0.31 ± 0.03	0.30 ± 0.02	0.32 ± 0.03
Ca (mg/dl)	10.1 ± 0.2	10.3 ± 0.4	10.3 ± 0.2	10.1 ± 0.2	10.2 ± 0.2
P (mg/dl)	5.50 ± 0.38	6.18 ± 0.98	5.92 ± 0.42	6.15 ± 0.42[#]	5.62 ± 0.47
Na (mEQ/l)	148 ± 1	148 ± 1.6	148 ± 1	146 ± 1[##]	147 ± 2
K (mEQ/l)	4.71 ± 0.36	4.99 ± 0.50	4.87 ± 0.33	4.55 ± 0.28	4.79 ± 0.32
Cl (mU/dl)	110 ± 2	110 ± 3	109 ± 1	109 ± 1	111 ± 2

Values are mean ± SD.
[**] Significantly different from the controls (0%) at $p < 0.01$ (parametric method).
[#] Significantly different from the controls (0%) at $p < 0.05$ (non-parametric method).
[##] Significantly different from the controls (0%) at $p < 0.01$ (non-parametric method).

4. Discussion

In the present study of morin administration in the diet at concentrations of 0%, 0.625%, 1.25%, 2.5% or 5.0% to male and female F344 rats for 13 weeks, a number of test substance-related changes were observed in organ weights and parameters in hematology, serum biochemistry and histopathology. For example, the 2.5 and 5.0% females exhibited significantly increased WBCs and the 5.0% females significantly decreased segmented neutrophils. Although adverse nutritional effects should be avoided according to the guideline that the concentration of the test substance in the diet does not usually exceed 5% (w/w), nutritional imbalance, if any, might contribute to these subtle effects. These were not considered to be toxicologically significant, however, because histopathological

Table 5
Serum chemistry data for female F344 rats fed diet containing morin for 13 weeks

	Dose level (%)				
	0	0.625	1.25	2.5	5.0
No. of samples	10	10	10	10	10
TP (g/dl)	6.37 ± 0.17	6.25 ± 0.14	6.30 ± 0.29	6.06 ± 0.07##	6.07 ± 0.17##
Alb (g/dl)	4.65 ± 0.15	4.54 ± 0.08	4.63 ± 0.24	4.52 ± 0.08	4.55 ± 0.13
A/G	2.72 ± 0.18	2.68 ± 0.21	2.77 ± 0.16	2.96 ± 0.13*	3.00 ± 0.21**
T-Cho (mg/dl)	77.8 ± 10.1	77.5 ± 6.9	77.6 ± 6.7	76.9 ± 3.9	67.7 ± 4.8**
TG (mg/dl)	33.8 ± 12.6	24.5 ± 3.3	26.1 ± 7.2	24.1 ± 4.3	52.1 ± 32.0
T-Bil (mg/dl)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00	0.13 ± 0.05#
γ-GT (IU/I)	2.0 ± 0.0	2.0 ± 0.0	2.0 ± 0.0	2.3 ± 0.7#	5.9 ± 2.0##
AST (IU/I)	75.1 ± 6.2	92.8 ± 18.5	85.1 ± 8.4	143 ± 32##	119 ± 50##
ALT (IU/I)	41.3 ± 4.9	47.2 ± 6.6	53.0 ± 9.4	88.0 ± 26.3##	88.3 ± 38.9##
ALP (IU/I)	271 ± 62	294 ± 44	301 ± 38	314 ± 48	690 ± 242##
BUN (mg/dl)	18.5 ± 2.4	19.8 ± 2.3	22.2 ± 2.9*	22.5 ± 4.2*	25.2 ± 2.3**
Cre (mg/dl)	0.33 ± 0.02	0.33 ± 0.02	0.33 ± 0.01	0.32 ± 0.03	0.29 ± 0.02**
Ca (mg/dl)	10.0 ± 0.3	9.9 ± 0.2	10.1 ± 0.3	10.0 ± 0.2	10.0 ± 0.2
P (mg/dl)	5.23 ± 0.56	5.33 ± 0.44	5.79 ± 0.70	5.59 ± 0.57	5.72 ± 0.50
Na (mEQ/I)	147 ± 2	146 ± 1	146 ± 2	145 ± 1	148 ± 1
K (mEQ/I)	4.46 ± 0.38	4.39 ± 0.27	4.59 ± 0.29	4.61 ± 0.51	4.71 ± 0.23
Cl (mU/dl)	111 ± 1	111 ± 1	109 ± 1**	108 ± 1**	113 ± 1**

Values are mean ± SD.
* Significantly different from the controls (0%) at $p < 0.05$ (parametric method).
** Significantly different from the controls (0%) at $p < 0.01$ (parametric method).
# Significantly different from the controls (0%) at $p < 0.05$ (non-parametric method).
Significantly different from the controls (0%) at $p < 0.01$ (non-parametric method).

Table 6
Organ weights for male F344 rats fed diet containing morin for 13 weeks

	Dose level (%)				
	0	0.625	1.25	2.5	5.0
Body weight (g)	281 ± 20	300 ± 11	291 ± 8	299 ± 16	268 ± 9
Absolute (g)					
Brain	1.88 ± 0.07	1.91 ± 0.03	1.88 ± 0.06	1.88 ± 0.06	1.89 ± 0.04
Thymus	0.156 ± 0.032	0.173 ± 0.026	0.150 ± 0.026	0.160 ± 0.039	0.139 ± 0.015
Heart	0.783 ± 0.057	0.890 ± 0.069**	0.775 ± 0.043	0.840 ± 0.049	0.785 ± 0.043
Lung	0.936 ± 0.082	0.980 ± 0.054	0.900 ± 0.074	0.937 ± 0.055	0.878 ± 0.041
Liver	6.91 ± 0.84	7.12 ± 0.29	8.15 ± 0.38##	7.88 ± 0.53#	7.70 ± 0.73
Kidney	1.69 ± 0.13	1.84 ± 0.08**	1.71 ± 0.12	1.79 ± 0.07	1.70 ± 0.06
Adrenal	0.036 ± 0.006	0.036 ± 0.005	0.033 ± 0.004	0.033 ± 0.004	0.036 ± 0.006
Spleen	0.546 ± 0.032	0.612 ± 0.054**	0.520 ± 0.037	0.563 ± 0.025	0.509 ± 0.027
Testis	2.89 ± 0.29	2.95 ± 0.29	3.01 ± 0.09	3.01 ± 0.16	2.98 ± 0.10
Relative (g/100 gB.W.)					
Brain	0.673 ± 0.037	0.638 ± 0.027	0.647 ± 0.029	0.630 ± 0.043*	0.708 ± 0.029
Thymus	0.050 ± 0.021	0.058 ± 0.009	0.051 ± 0.008	0.053 ± 0.012	0.052 ± 0.005
Heart	0.279 ± 0.012	0.296 ± 0.015	0.266 ± 0.012	0.281 ± 0.020	0.293 ± 0.017
Lung	0.334 ± 0.026	0.327 ± 0.015	0.309 ± 0.023	0.314 ± 0.022	0.328 ± 0.019
Liver	2.46 ± 0.19	2.38 ± 0.11	2.80 ± 0.14##	2.63 ± 0.09	2.87 ± 0.24##
Kidney	0.602 ± 0.039	0.613 ± 0.021	0.586 ± 0.041	0.598 ± 0.038	0.635 ± 0.032
Adrenal	0.013 ± 0.002	0.012 ± 0.002	0.011 ± 0.001	0.011 ± 0.001	0.013 ± 0.002
Spleen	0.195 ± 0.012	0.204 ± 0.014	0.178 ± 0.009**	0.188 ± 0.007	0.190 ± 0.007
Testis	1.04 ± 0.12	0.98 ± 0.10	1.04 ± 0.04	1.01 ± 0.09	1.11 ± 0.06

Values are mean ± SD.
* Significantly different from the controls (0%) at $p < 0.05$ (parametric method).
** Significantly different from the controls (0%) at $p < 0.01$ (parametric method).
# Significantly different from the controls (0%) at $p < 0.05$ (non-parametric method).
Significantly different from the controls (0%) at $p < 0.01$ (non-parametric method).

lesions like inflammatory lesions or increase in bone marrow cellularity, which might provide an explanation, were not found.

Similarly, the significant increases in Alb and A/G rations at high dose and the decrease in TP might not be toxicologically significant again because of the lack of

Table 7
Organ weights for female F344 rats fed diet containing morin for 13 weeks

	Dose level (%)				
	0	0.625	1.25	2.5	5.0
Body weight (g)	170 ± 9	167 ± 6	168 ± 4	167 ± 7	167 ± 8
Absolute (g)					
Brain	1.75 ± 0.04	1.80 ± 0.11	1.79 ± 0.04	1.76 ± 0.05	1.76 ± 0.04
Thymus	0.173 ± 0.022	0.157 ± 0.028	0.153 ± 0.025	0.154 ± 0.016	0.141 ± 0.016**
Heart	0.543 ± 0.034	0.541 ± 0.022	0.545 ± 0.034	0.540 ± 0.020	0.502 ± 0.028**
Lung	0.696 ± 0.042	0.725 ± 0.075	0.721 ± 0.042	0.695 ± 0.059	0.661 ± 0.031
Liver	3.64 ± 0.25	3.59 ± 0.21	3.74 ± 0.14	3.92 ± 0.27	4.84 ± 0.78##
Kidney	1.02 ± 0.06	1.03 ± 0.06	1.09 ± 0.09	1.07 ± 0.05	1.10 ± 0.05*
Adrenal	0.041 ± 0.006	0.042 ± 0.003	0.043 ± 0.005	0.037 ± 0.004	0.033 ± 0.004**
Spleen	0.386 ± 0.041	0.404 ± 0.016	0.409 ± 0.028	0.404 ± 0.029	0.363 ± 0.028
Relative (g/100 gB.W.)					
Brain	1.04 ± 0.05	1.08 ± 0.06	1.07 ± 0.03	1.05 ± 0.05	1.06 ± 0.04
Thymus	0.102 ± 0.011	0.094 ± 0.015	0.092 ± 0.015	0.092 ± 0.010	0.083 ± 0.007**
Heart	0.320 ± 0.017	0.324 ± 0.011	0.325 ± 0.019	0.323 ± 0.016	0.301 ± 0.014*
Lung	0.411 ± 0.019	0.434 ± 0.042	0.430 ± 0.026	0.415 ± 0.029	0.396 ± 0.011
Liver	2.14 ± 0.09	2.15 ± 0.08	2.23 ± 0.08	2.34 ± 0.10#	2.89 ± 0.36##
Kidney	0.603 ± 0.034	0.618 ± 0.023	0.648 ± 0.042*	0.643 ± 0.036*	0.658 ± 0.024**
Adrenal	0.024 ± 0.003	0.025 ± 0.002	0.026 ± 0.003	0.022 ± 0.003	0.020 ± 0.003**
Spleen	0.228 ± 0.025	0.242 ± 0.012	0.244 ± 0.014	0.242 ± 0.014	0.218 ± 0.012

Values are mean ± SD.
* Significantly different from the controls (0%) at $p < 0.05$ (parametric method).
** Significantly different from the controls (0%) at $p < 0.01$ (parametric method).
# Significantly different from the controls (0%) at $p < 0.05$ (non-parametric method).
Significantly different from the controls (0%) at $p < 0.01$ (non-parametric method).

inflammatory change and also because the extent of fluctuation was very small. The significant decrease in TG in the 5.0% males was unlikely to have any toxicological significance because there was an opposite tendency for increase in females. Although T-Cho in the 5.0% females showed significant decrease, the degree of change was slight and hemolytic anemia or hyperthyroidism were not detected. However, the possibility that the decrease was due to hepatic dysfunction cannot be precluded. While males and females showed significant fluctuation in P and Na, and Cl and T-Bil, respectively, the lack of any dose-relation indicated no relation to the test substance. Significant increases in ALT (over 2.5%), ALP (1.25% and 5.0%) and relative liver weight (1.25% and 2.5%) in males were considered to be test substance-related, but hepatotoxicity was clearly weak because no histopathological changes were observed in the livers. The significantly increased γ-GT, AST, ALT in the 2.5% and 5.0% females and ALP in 5.0% females were also considered to be test substance-related, given the associated increases in relative liver weights and hepatocyte hypertrophy detected in some animals. The increased BUN, Cre and relative kidney weights in females can also be considered to be toxicologically significant. However, no histopathological lesions were detected in kidney, suggesting only weak toxic effects.

Although the absolute and relative thymus, heart and adrenal glands weights in the 5.0% females were significantly decreased, no histopathological changes were apparent and they cannot be considered to be adverse effects of the test substance.

Quercetin, a positional isomer of morin, is a major bioflavonoid in the human diet. An increase of renal tubular adenomas was seen in male receiving 4% quercetin in an earlier long term study, but this was not the case in females (National Toxicology Program, 1992). Greater sensitivity of males to quercetin toxicity is apparently due to more extensive spontaneous nephropathy during aging and exacerbation of the disease by chemical administration. The National Toxicology Program trial also found a dose-related decrease in mammary fibroadenomas in treated animals (9/50 in the high-dose group as compared with 29/50 in the controls). Rutin, a glycoside of quercetin, is extracted from buckwheat or red beans, known to have pharmacological activity, decreasing blood pressure and capillary reinforcement (Becker et al., 1985). Potential carcinogenicity has been examined in inbred ACI strain rats and golden hamsters, but no increase in the tumor incidence was evident (Hirono et al., 1981; Morino et al., 1982). Enzymatically decomposed rutin, generated by naringinase/hesperinase/rhamnosidase reactions followed by purification exhibited no toxicological changes on 13-weeks dietary administration to Wistar rats (Hasumura et al., 2004). Enzymatically modified isoquercitrin, another glycoside of quercetin, caused decrease of body weight gain in 2.5% males, and increase of γ-GT in females and of BUN in both sexes of rats treated for 13 weeks, but all these parameters had returned to within the normal ranges after a 4-week recovery period (Tamano et al., 2001). A recent study found that F344 rats consuming diet containing up to 1.5% enzymatically modified isoquercitrin for 104

weeks were not at increased risk of cancer (Salim et al., 2004). Although rutin and enzymatically decomposed rutin possess mutagenic potential (Hayashi et al., 2000), there is thus no unequivocal evidence of carcinogenicity.

In conclusion, the present 13-week dietary toxicity study of morin in F344 rats demonstrated significant increases in ALT (over 2.5%), ALP (1.25% and 5.0%) and relative liver weights (1.25% and 2.5%) in males and increased γ-GT, AST, ALT, and relative liver weights in the 2.5% and 5.0% females and ALP in 5.0% females. Increased BUN and relative kidney weights in females receiving 1.25% or over, and Cre at 5.0% were also observed. Based on these results the no-observed-adverse-effect level (NOAEL) and the no-observed-effect level (NOEL) of morin in F344 rats were both estimated to be 0.625% in both males and females (299 and 356 mg/kg/day, respectively).

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