A REPEATED-DOSE DERMAL TOXICITY STUDY OF HYDROPHOBICALLY MODIFIED HYDROXYPROPYL METHYLCELLULOSE IN RATS

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ABSTRACT — A six-month repeated-dose dermal toxicity study followed by a 30-day recovery test of hydrophobically modified hydroxypropyl methylcellulose (HM-HPMC), a new cellulose derivative used as a thickener for topical pharmaceuticals, was conducted using rats. Aqueous paste of HM-HPMC was applied to the skin of rats once daily at dose levels up to 60 mg/kg/day, which was the highest dose that could be administered. Items checked included general signs, urinalysis, hematology, ophthalmology, and histopathology. One rat died during the administration period owing to a malignant tumor in the hemopoietic system, which was not attributed to the test substance. Statistically significant differences were found in some test results, but those were not dose-dependent and were considered to be incidental or spontaneous. It was concluded that the test substance was not toxic upon chronic dermal administration at dose levels up to 60 mg/kg/day.

KEY WORDS : Hydrophobically modified hydroxypropyl methyl cellulose, Dermal, Repeated administration, Rat

INTRODUCTION

Hydrophobically modified hydroxypropyl methylcellulose (HM-HPMC) is a new cellulose derivative prepared by modification of hydroxypropyl methylcellulose (HPMC), which is widely used as a coating agent and binder for oral solid dosage forms. The difference between HPMC and HM-HPMC is that the latter contains a small amount of long-chain alkyl groups (C₁₆, C₁₈). When HM-HPMC is dissolved in water or in a mixture of water and alcohol, the solution exhibits a very high viscosity owing to

hydrophobic interaction among the long-chain alkyl groups. The polymer has also been reported to enhance the solubility of some active ingredients (Saito *et al.*, 1992a, 1992b). These characteristics imply that the polymer would be useful as a new gel base for topical pharmaceutical dosage forms.

Oral and dermal single-dose toxicity studies on HM-HPMC in rats as well as primary dermal and eye irritability tests in rabbits have revealed no toxicity (Obara *et al.*, 1992a, 1992b). Here we present the results of a 6-month repeated-dose dermal toxicity study of HM-HPMC, followed by a 30-day recovery test, conducted to examine the feasibility of its topical application.

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MATERIALS AND METHODS

Test substance

The HM-HPMC was prepared at the Specialty Chemicals Research Center of Shin-Etsu Chemical Co., Ltd. Its chemical structure and characteristics are shown in Fig. 1. This substance is an odorless white powder which dissolves in water or water-alcohol mixtures to form a highly viscous paste. Its solubility in water depends on the level of long-chain alkyl groups; when the level is less than approximately 1 %, it dissolves in water at room temperature. It does not dissolve in warm water at higher than 50 °C. Increasing the long-chain alkyl level decreases its solubility in water, but it is still soluble in water-alcohol mixtures. The long-chain alkyl level of the sample used in this study was 1.1 %. At this level, which is regarded as representative, the substance is partially soluble in water.

Laboratory animals

Male and female SD rats were used. The animals were purchased from Charles River

Japan Inc. (Kanagawa) at 6 weeks of age and acclimated for one week. Healthy animals that had no abnormalities in appearance and showed normal weight gain were selected and used at 7 weeks of age. The animals were housed in an animal room maintained at 24 ± 2 °C and 55 ± 10 % relative humidity. The room air was ventilated 13 times per hr and a 12 hr/12 hr light-dark cycle was imposed. The animals were given a commercially available pellet diet (MF, Oriental Yeast Co.) and UV-sterilized filtered tap water ad libitum.

Preparation of dosage form

A designated amount of HM-HPMC was weighed and dispersed in warm water at about 70 °C using a stirrer for 20 min. Then the beaker was placed in a constant temperature water bath at 30 °C and stirred continuously for about 30 min until the particles were homogeneously hydrated. When the temperature is low, the hydration is excessive and the dispersion becomes too viscous to administer. Therefore, the prepared dispersion was kept at 30 °C before use.

Dose levels, administration, and groups

HM-HPMC partially dissolves in water, forming a high-viscosity paste. Since this test substance shows high viscosity even at low concentrations compared to other cellulosic polymers, the maximum concentration that can be administered is limited. Even though the viscosity enhancement was suppressed by maintaining the temperature at 30 °C, the highest usable concentration was 3.0 w/v %. The appropriate volume of dispersion for dermal administration was 0.2 ml/100 g-body weight. Therefore, the highest dose was set at 60 mg/kg/day. Doses of 40 mg/kg/day and 20 mg/kg/day were also employed to examine the dose-dependency of any effects, i.e., preparations containing 2.0 and 1.0 w/v % HM-HPMC were administered, respectively. Prior to administration, a part of the back area (about 10% of the body surface) of each rat was shaved, then the test preparation (HM-HPMC dispersion) was applied to an area of 3.5×3.5 cm using a fingerstall. Before subsequent applications, the residual HM-HPMC at the site was wiped off with absorbent cotton and lukewarm water. The animals of the control group were

Methoxyl content : 23.6%

Hydroxypropoxyl content : 6.6%

C₁₆, C₁₈-alkoxyhydroxyl content : 1.1%

Loss on drying : 2.7%

Residue on ignition : 0.12%

Fig. 1. Chemical Structure of HM-HPMC and its characteristic values.

treated with the same volume of distilled water in place of the test preparation, and the application site was wiped in the same way as in the test preparation group. The administration was performed once daily for 6 months. The hair on the application site was removed once a week. Each group comprised 25 males and 25 females. When all administration was finished, 5 males and 5 females in every group were kept for an additional 30 days to observe the reversibility of any effects (recovery test).

Observation and examinations

General signs

Throughout the experimental period, external appearance, behavior and excretion were observed daily. Body weight and food consumption were measured weekly for the initial 3 months and bi-weekly for another 3 months. They were again measured weekly during the recovery period.

Urinalysis

The following items were tested for 5 rats of each sex in each group at the beginning of the experiment, 3 months later, at the end of the administration period, and at the end of the recovery period: urine volume in 24 hrs, color

(appearance), specific gravity (refractometry), sediment (microscopy), sodium and potassium content (flame photometry), and chlorine content (coulombmetry). Using test papers (Multistix(r), Myles-Sankyo, Japan), pH, protein, glucose, urobilinogen, occult blood, bilirubin and ketone bodies in urine were also examined.

Hematology

At three months after the start of the experiment, at the end of the administration period, and at the end of the recovery period, blood samples were obtained from the orbital venous plexus under light ether anesthesia for all animals. The following hematological parameters were determined with an automatic cell counter; leucocyte count, erythrocyte count, platelet count, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC). Myelograms were obtained microscopically, based on differential leucocyte count, reticulocyte count, and differential myelocyte count.

Biochemistry

At the end of the administration period and the recovery period, blood samples were collect-

Fig. 1. Mean body weight of rats during 6-month repeated dermal administration of HM-HPMC followed by 30-day recovery period.

ed from an abdominal vein under ether anesthesia for all animals, and biochemical examination was performed with serum obtained by centrifugation. The examination included the following: GOT, GPT, ALP, LDH, Ch-E, total bilirubin, glucose, uric acid, urea nitrogen, creatinine, total cholesterol, phospholipid, triglyceride, sodium, potassium, chlorine, calcium, inorganic phosphate, total protein, albumin, and albumin-globulin ratio.

Ophthalmology

Examination included the anterior portion of the eye (cornea, conjunctiva, iris, etc.) and ocular fundus (course of blood vessels, edema, hemorrhage, etc.).

Necropsy

Any animal that died during the study period was autopsied immediately. At the end of administration and recovery, all surviving animals assigned to the non-recovery and recovery groups, respectively, were killed for necropsy. The dissected animals were examined macroscopically, then the organs cited below were removed and weighed: brain, pituitary, thyroids, submandibular glands, thymus, heart, lungs, liver, kidneys, adrenals, spleen, prostate, seminal vesicle, testis, epididymis, ovaries, and uterus.

Histopathology

In addition to the above-mentioned organs, the following were examined: skin at the application site, locus of lesions, skin at a non-application site, parathyroid, mammary gland, sternum, femur (with bone marrow), femoral muscle (with sciatic nerve), trachea, tongue, esophagus, stomach, duodenum, small intestine, large intestine, mesenteric lymph node, pancreas, urinary bladder, seminal vesicle, spinal cord and eyeball (right). The samples were fixed with buffered 10% formalin solution, embedded in paraffin, thin-sectioned, stained with hematoxylin-eosin and subjected to histopathological examination. The histopathological examination was conducted only on the animals of the highest dose group and of the control group.

Statistical analysis

The significance of differences in test data between groups was examined by means of the F-test, followed by Student's *t* test or Welch's test, depending on the variance. The data obtained in urinalysis were analyzed by means of the χ^2 test.

RESULTS AND DISCUSSION

General signs observed in animals during

Fig. 3. Mean food consumption of rats during 6-month repeated dermal administration of HM-HPMC followed by 30-day recovery period.

Table 1. Findings in general observation in 6-month repeated dermal toxicity study of HM-HPMC followed by 30-day recovery test.

Dose	Findings	Number of cases	Days from the start of administration
Control	Male Hematuria	3	108-182
	Female Depilation of foreleg	1	26-182
20mg/kg/day	Male Depilation due to injury of the left side of nose Injury and depilation around the right eye	1 1	123-144 132-144
	Female Depilation of foreleg Hypodermal node in the left side of the abdomen →Necrosis at the center of the node →Hemorrhage from the necrotic site →Anemia →Granulation	1 1	124-185 125-169
40 mg/kg/day	Male Small injury on the back Hematuria Hemorrhage from the right knee and anemic state A 3 mm hypodermal node at the base of the right foreleg	1 1 1 1	14-22 116-120 159-184 182
	Female Depilation of the foreleg	1	102-128
60 mg/kg/day	Male Injury around the application site, depilation of foreleg Hematuria Slight erythema at the application site Death	1 1 1 1	7-185 86-180 129-133 144
	Female (No abnormalities)		

After recovery period, hematuria was observed in 3 males in control group.

Other abnormalities were not seen. In addition to the above, a few cases of injury to the eye at blood collection were found.

Table 2. Body weight changes of rats during 6-month repeated dermal administration of HM-HPMC followed by 30-day recovery period.

Week	Male				Female			
	Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
Initial	210.4±9.0	213.7±6.9	212.2±8.4	212.8±8.1	172.6±7.8	175.6±7.9	176.0±7.3	173.2±6.8
1	278.2±9.4	279.1±11.3	278.9±10.4	282.2±11.0	200.8±8.5	204.9±10.2	203.9±7.2	201.1±10.7
2	317.4±11.4	316.2±17.7	319.5±14.3	322.0±15.3	223.3±11.0	230.6±11.9*	225.4±8.5	223.8±13.6
3	351.3±16.4	348.0±23.2	352.3±18.2	354.8±16.9	242.0±13.8	248.1±14.2	241.6±10.9	239.4±14.9
4	381.4±19.1	376.8±27.1	382.6±19.8	383.2±20.4	255.5±14.2	263.4±15.5	254.5±13.8	255.9±16.4
5	408.9±21.5	402.4±29.1	409.7±23.6	408.4±25.4	265.9±15.5	277.0±17.8*	267.2±15.1	269.4±17.5
6	430.9±24.3	424.0±32.5	434.8±25.4	434.9±30.2	275.4±16.0	288.8±19.0**	277.4±17.1	277.4±20.0
7	453.0±27.0	446.7±32.0	457.3±26.8	455.1±32.4	283.2±16.3	298.3±21.6**	286.0±17.7	284.8±20.6
8	475.4±28.9	468.2±35.9	480.4±28.2	470.1±37.1	289.9±17.3	305.7±21.2**	293.4±18.4	293.0±22.4
9	492.3±31.4	482.6±38.0	496.6±30.3	491.2±39.5	298.5±18.6	315.3±25.5**	301.6±18.6	299.8±24.1
10	507.4±34.2	497.6±38.9	512.1±33.3	506.6±40.4	302.5±18.8	319.8±25.7**	307.3±22.2	304.6±25.0
11	518.1±37.7	507.5±38.5	521.8±34.0	519.8±43.3	306.0±20.4	326.8±26.9**	309.9±17.5	309.0±25.6
12	531.9±39.2	519.0±39.8	535.0±38.0	531.6±44.7	314.6±19.4	334.4±27.6**	319.8±24.9	316.6±28.3
13	544.0±38.0	531.3±40.2	549.0±38.9	546.4±46.7	320.8±21.4	339.2±29.0*	323.3±24.3	319.8±28.9
15	553.6±40.0	541.4±40.6	558.0±40.7	554.0±45.6	319.8±21.4	340.2±30.2**	326.8±24.9	321.1±29.8
17	565.0±38.9	551.8±42.8	571.3±41.0	568.8±47.6	329.1±23.8	347.3±31.5**	336.7±28.3	327.7±30.4
19	586.1±42.9	572.4±43.7	590.8±45.0	587.8±50.4	341.7±27.4	361.9±32.9*	347.1±29.1	340.7±31.8
21	608.0±47.0	590.1±45.7	605.5±47.7	602.6±57.3	349.2±29.2	374.2±39.2*	355.2±26.4	347.8±36.6
23	623.1±48.7	603.4±49.3	621.4±51.0	622.6±53.1	350.4±27.0	374.8±40.7*	358.8±32.2	352.0±39.5
25	633.0±51.0	615.3±53.4	631.4±53.4	631.5±54.9	354.4±28.9	378.1±38.8*	360.3±32.5	354.2±40.6
26	630.4±49.9	608.8±51.7	628.1±53.5	630.5±56.2	354.1±29.5	379.0±40.8*	363.2±36.4	355.3±42.5
(Recovery) 27	658.8±36.1	620.0±51.8	634.4±49.9	658.0±113.5	349.0±26.5	365.6±24.0	351.6±20.4	339.4±41.9
28	668.0±40.0	628.4±50.2	639.2±50.0	670.3±118.5	353.4±28.9	373.6±24.5	357.6±21.4	348.4±41.1
29	674.0±42.7	632.2±53.5	647.2±51.7	667.5±122.8	357.8±29.4	382.8±26.1	361.6±27.1	354.0±44.5
30	671.2±41.0	627.8±55.7	645.6±51.0	669.3±116.1	354.0±30.8	378.8±27.6	362.0±26.0	352.6±43.5

Unit: g/rat. Data represents mean±S.D. (n=24-25 for administration period, n=4-5 for recovery period). *P<0.05 vs control. **P<0.01 vs control.

Table 3. Food consumption of rats during 6-month repeated dermal administration of HM-HPMC followed by 30-day recovery period.

Week	Male				Female			
	Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
1	25.98±1.16	25.90±1.73	25.76±2.28	26.13±1.79	18.85±0.83	19.12±1.18	19.88±3.17	18.86±1.53
2	25.73±1.52	25.24±2.38	25.51±1.92	26.20±2.02	20.23±1.34	21.41±1.61**	20.99±1.39	20.88±1.94
3	28.07±2.02	27.08±2.45	27.52±1.69	27.57±1.92	22.84±1.81	23.54±1.74	23.24±1.30	23.06±2.06
4	28.63±1.90	27.63±2.42	27.96±1.68	28.24±1.93	23.05±1.51	23.82±1.62	23.40±1.48	23.63±1.89
5	29.44±1.76	28.60±2.36	29.00±1.65	29.19±2.39	21.29±1.38	22.18±1.61*	21.67±1.20	21.73±1.80
6	28.65±2.09	27.66±2.15	28.32±1.53	28.49±2.79	20.78±1.52	22.10±1.64**	21.58±1.40	21.37±2.13
7	28.10±1.80	27.34±1.93	28.33±1.62	28.33±2.39	20.29±1.88	21.61±1.58**	20.90±1.39	20.66±2.11
8	27.73±2.06	27.44±2.29	28.30±1.72	28.34±2.58	20.03±1.96	21.17±1.51*	21.03±1.32*	20.74±2.02
9	27.10±2.00	26.65±2.06	27.41±1.79	27.84±2.35	20.71±1.66	21.90±2.13*	21.58±1.72	20.99±2.14
10	27.32±2.08	26.86±1.93	27.95±2.13	28.05±2.54	20.13±1.53	21.44±1.62**	21.18±1.72*	20.64±1.98
11	26.34±3.93	26.42±2.00	27.46±2.10	27.95±2.66	19.47±2.13	20.86±1.58*	20.67±2.26	20.12±2.21
12	26.72±2.03	25.97±2.05	27.19±2.27	27.82±2.39	20.20±2.27	21.52±2.02*	21.05±2.12	21.00±2.20
13	27.23±2.20	26.31±1.97	27.62±2.26	28.27±2.64	19.91±2.52	20.82±2.20	20.16±1.69	19.61±2.29
15	26.56±1.58	25.95±1.77	26.67±1.96	27.09±2.00	19.40±1.93	20.70±1.72*	20.79±1.57**	20.38±2.24
17	27.88±2.00	27.35±2.09	28.06±2.16	28.63±2.27	20.70±2.25	21.88±1.75*	22.22±1.73**	20.96±1.93
19	27.10±2.09	26.79±1.84	27.56±2.09	27.92±2.23	20.92±2.52	21.94±1.54	21.65±1.95	21.24±2.11
21	27.39±2.19	26.52±1.87	26.77±2.34	27.57±2.76	19.06±2.31	21.17±2.36**	20.36±1.42*	19.80±2.28
23	26.36±2.13	25.86±1.91	26.95±2.40	27.10±2.18	18.30±1.73	19.71±2.40*	19.96±1.98**	19.60±2.42*
25	25.64±1.69	25.66±2.04	26.10±2.27	26.24±2.30	18.20±2.27	19.25±1.78	19.37±1.78*	18.65±1.94
26	24.18±2.13	23.52±2.24	24.60±2.55	24.80±1.97	16.67±1.93	17.85±2.02*	17.99±2.40*	17.84±2.42
(Recovery) 27	26.54±1.87	26.42±2.53	26.62±1.99	27.63±3.18	17.10±2.07	18.52±1.34	18.54±0.86	17.42±1.82
28	25.56±2.11	24.88±2.37	24.84±2.64	27.35±4.62	19.18±1.89	20.56±1.40	20.76±1.22	19.68±1.79
29	25.36±6.17	24.32±2.05	24.46±2.60	27.20±4.88	18.86±2.52	20.52±2.41	18.74±1.40	18.90±2.31
30	23.60±1.33	22.56±1.97	22.60±2.21	24.58±2.87	16.80±1.84	17.46±1.74	16.82±0.57	16.78±2.20

Unit: g/rat/day. Data represents mean ± S.D. (n=24-25 for administration period, n=4-5 for recovery period). *P<0.05 vs control. **P<0.01 vs control.

Table 4. – Urinalysis in rats during and after 6-month repeated dermal administration of HM-HPMC followed by 30-day recovery period (1).

	Dose	Male				Female			
		Initial	3 months	6 months	Recovery	Initial	3 months	6 months	Recovery
Urine volume (ml)	Control	11.32±6.87	8.04±5.41	7.00±4.28	11.50±3.30	16.82±7.12	8.90±4.38	5.76±3.65	7.16±2.08
	20 mg/kg/day	16.36±3.14	4.80±1.46	6.70±3.74	8.52±2.89	23.66±11.24	15.66±5.79	12.10±13.58	6.34±1.34
	40 mg/kg/day	22.96±11.54	7.04±4.08	7.66±2.26	7.06±2.24*	14.88±2.67	8.16±3.44	4.16±2.56	6.26±2.92
	60 mg/kg/day	23.78±14.86	12.40±5.91	11.00±2.69	9.88±3.52	11.60±5.06	9.36±11.81	3.78±2.52	6.68±3.53
Specific gravity	Control	1.052±0.023	1.048±0.013	1.049±0.024	1.044±0.017	1.042±0.029	1.053±0.021	1.078±0.022	1.042±0.014
	20 mg/kg/day	1.052±0.023	1.061±0.012	1.053±0.013	1.057±0.023	1.025±0.006	1.034±0.021	1.056±0.019	1.050±0.025
	40 mg/kg/day	1.044±0.011	1.051±0.032	1.048±0.021	1.064±0.023	1.034±0.018	1.037±0.016	1.071±0.015	1.049±0.022
	60 mg/kg/day	1.049±0.014	1.044±0.013	1.040±0.008	1.052±0.008	1.040±0.019	1.050±0.017	1.067±0.012	1.046±0.017
Sodium (mEq/day)	Control	0.331±0.213	0.228±0.033	0.160±0.103	0.274±0.243	0.273±0.103	0.379±0.143	0.167±0.061	0.206±0.123
	20 mg/kg/day	0.428±0.202	0.226±0.126	0.165±0.070	0.132±0.080	0.327±0.105	0.355±0.187	0.143±0.040	0.219±0.058
	40 mg/kg/day	0.472±0.253	0.160±0.105	0.168±0.096	0.108±0.105	0.359±0.083	0.293±0.116	0.161±0.068	0.164±0.049
	60 mg/kg/day	0.292±0.111	0.230±0.108	0.133±0.052	0.080±0.073	0.306±0.086	0.283±0.045	0.135±0.022	0.259±0.127
Potassium (mEq/day)	Control	1.823±0.466	1.365±0.267	1.560±0.390	1.758±0.259	1.261±0.185	1.024±0.205	0.930±0.238	1.245±0.289
	20 mg/kg/day	1.633±0.472	1.288±0.112	1.537±0.197	1.629±0.155	1.392±0.162	0.944±0.074	0.959±0.193	1.310±0.213
	40 mg/kg/day	1.734±0.171	1.177±0.202	1.799±0.397	1.651±0.381	1.187±0.132	0.894±0.166	0.879±0.161	1.088±0.282
	60 mg/kg/day	1.670±0.272	1.293±0.246	1.589±0.283	1.701±0.467	1.287±0.336	0.792±0.282	0.901±0.321	1.171±0.281
Chlorine (mEq/day)	Control	0.587±0.121	0.347±0.040	0.482±0.328	0.38±0.185	0.359±0.106	0.381±0.141	0.139±0.046	0.193±0.134
	20 mg/kg/day	0.584±0.217	0.251±0.093	0.235±0.144	0.335±0.126	0.485±0.097	0.342±0.125	0.101±0.041	0.243±0.114
	40 mg/kg/day	0.701±0.187	0.212±0.107*	0.321±0.095	0.171±0.083*	0.414±0.093	0.309±1.000	0.131±0.048	0.184±0.102
	60 mg/kg/day	0.555±0.128	0.294±0.107	0.218±0.068	0.213±0.043	0.391±0.088	0.294±0.101	0.150±0.058	0.279±0.093

Data represents mean ± S.D. (n=4-5). *P<0.05 vs control.

Table 5. Urinalysis in rats during and after 6-month repeated dermal administration of HM-HPMC followed by 30-day recovery period (2).

Dose	Urobilinogen	Occult blood	Bilirubin	Ketone body	Glucose	NH ₄ ·Mg·Protein	pH	Phosphate	Epitheliomuscular cell	Leucocytes	Erythrocytes	Sperma
	0.1	1	—	— ± ± ± ±	—	— ± ± ± ±	6 65 7 75 8	— + + + +	— + +	— + + + +	— + + + +	— + + + +
(Male)												
Initial												
Control	5	0	5	0 5 0 0	5	0 0 4 1	0 0 2 3 0	4 1 0 0	0 5 0	4 1 0 0	5 0 0 0	3 1 1 0
20mg/kg/day	5	0	5	2 3 0 0	5	0 1 4 0	0 1 1 3 0	2 2 1 0	0 5 0	5 0 0 0	5 0 0 0	2 3 0 0
40mg/kg/day	5	0	5	0 5 0 0	5	0 0 2 3	0 0 2 3 0	1 1 3 0**	0 5 0	3 2 0 0	4 1 0 0	4 1 0 0
60mg/kg/day	5	0	5	0 5 0 0	5	0 0 3 2	0 0 1 3 1	4 1 0 0	1 4 0	5 0 0 0	5 0 0 0	4 1 0 0
3months												
Control	3	2	3	1 2 1 1	5	0 0 4 1	0 0 2 3 0	1 1 2 1	1 4 0	5 0 0 0	5 0 0 0	1 1 2 1
20mg/kg/day	4	1	5	0 5 0 0	5	0 0 2 3	0 1 0 3 1	1 2 2 0	0 5 0	5 0 0 0	5 0 0 0	1 2 2 0
40mg/kg/day	5	0	4	1 0 0 0*	5	0 0 1 4	0 1 3 0 1	1 2 2 0	0 3 2	4 1 0 0	5 0 0 0	0 3 1 1
60mg/kg/day	3	2	3	2 0 0 0	5	0 0 0 5	0 0 2 2 1	2 1 2 0	0 4 1	3 2 0 0	4 1 0 0	0 2 0 3
6months												
Control	5	0	4	0 0 0 1	5	0 0 3 2	0 1 0 1 3	2 3 0 0	1 4 0	3 1 1 0	4 0 1 0	2 2 0 1
20mg/kg/day	5	0	5	0 0 0 0*	5	0 0 1 4	0 1 0 2 2	0 3 1 1	1 3 1	3 2 0 0	5 0 0 0	1 3 1 0
40mg/kg/day	5	0	3	2 0 0 0	5	0 1 2 2	0 1 0 2 2	2 3 0 0	0 4 1	4 1 0 0	4 1 0 0	2 1 2 0
60mg/kg/day	4	0	4	0 0 0 0	4	0 0 2 2	0 0 0 3 1	1 2 1 0	0 3 1	3 1 0 0	4 0 0 0	0 1 1 2
Recovery												
Control	5	0	3	1 0 0 0	5	0 0 1 4	0 1 1 2 1	2 2 0 0	1 4 0	3 1 0 1	3 1 0 1	0 5 0 0
20mg/kg/day	5	0	4	1 0 0 0	5	0 1 4 0	0 1 0 3 1	3 1 0 1	1 4 0	3 1 1 0	3 2 0 0	0 3 1 1
40mg/kg/day	5	0	4	1 0 0 0	5	0 0 2 3	0 0 0 3 2	2 3 0 0	0 4 1	3 1 1 0	3 2 0 0	1 3 0 1
60mg/kg/day	4	0	2	2 0 0 0	4	0 0 2 2	0 0 0 2 2	2 1 1 0	1 3 0	2 2 0 0	2 2 0 0	2 1 1 0
(Female)												
Initial												
Control	5	0	4	1 0 0 0	5	0 2 3 0	0 0 0 2 3	1 1 3 0	1 4 0	5 0 0 0	5 0 0 0	
20mg/kg/day	5	0	5	0 0 0 0	5	0 3 2 0	0 0 0 0 5	1 4 0 0	0 4 1	5 0 0 0	5 0 0 0	
40mg/kg/day	5	0	5	0 0 0 0	5	0 2 2 1	0 1 0 2 2	1 3 1 0	0 4 1	5 0 0 0	5 0 0 0	
60mg/kg/day	5	0	5	0 0 0 0	5	0 0 5 0	0 0 0 1 4	2 1 2 0	1 4 0	5 0 0 0	5 0 0 0	
3 months												
Control	5	0	4	0 1 0 0	5	0 0 4 1	0 0 1 1 3	1 4 0 0	1 4 0	5 0 0 0	5 0 0 0	
20mg/kg/day	5	0	5	0 0 0 0	5	0 0 4 1	0 0 1 3 1	3 2 0 0	2 3 0	5 0 0 0	5 0 0 0	
40mg/kg/day	4	1	4	1 0 0 0	5	0 0 3 2	0 1 1 3 0	5 0 0 0**	3 2 0	5 0 0 0	4 1 0 0	
60mg/kg/day	4	1	5	0 0 0 0	5	0 2 3 0	0 0 1 2 2	5 0 0 0	3 2 0	5 0 0 0	5 0 0 0	
6 months												
Control	5	0	5	0 0 0 0	5	0 3 2 0	0 2 1 0 2	1 4 0 0	0 5 0	4 1 0 0	5 0 0 0	
20mg/kg/day	5	0	5	0 0 0 0	5	0 2 3 0	0 0 2 1 2	2 3 0 0	0 3 2	5 0 0 0	5 0 0 0	
40mg/kg/day	5	0	4	1 0 0 0	5	0 0 5 0	2 1 0 1 1	1 4 0 0	0 3 2	4 1 0 0	5 0 0 0	
60mg/kg/day	5	0	5	0 0 0 0	5	0 0 4 1	0 0 0 3 2*	1 3 1 0	0 5 0	5 0 0 0	5 0 0 0	
Recovery												
Control	5	0	5	0 0 0 0	5	0 2 2 1	0 1 2 2 0	3 1 0 1	1 4 0	4 1 0 0	5 0 0 0	
20mg/kg/day	5	0	5	0 0 0 0	5	0 2 2 1	0 0 2 2 1	2 2 0 1	2 3 0	5 0 0 0	5 0 0 0	
40mg/kg/day	5	0	5	0 0 0 0	5	0 2 3 0	0 0 1 3 1	3 1 0 1	3 1 1	3 1 1 0	4 1 0 0	
60mg/kg/day	5	0	4	1 0 0 0	5	0 3 1 1	0 0 0 4 1	2 0 1 2	3 2 0	3 2 0 0	4 1 0 0	

Data represents number of rats (4-5 rats per group). *P<0.05 vs control.

**P<0.01 vs control.

Table 6. Hematological findings in rats during and after 6-month repeated dermal administration of HM-HPMC (1).

	Dose	Male			Female		
		3 months	6 months	Recovery	3 months	6 months	Recovery
Erythrocyte ($\times 10^9/\text{mm}^3$)	control	870.0 $\pm$ 60.9	917.6 $\pm$ 48.5	885.2 $\pm$ 67.0	733.3 $\pm$ 79.2	764.0 $\pm$ 61.5	749.6 $\pm$ 30.3
	20 mg/kg/day	895.7 $\pm$ 60.1	860.2 $\pm$ 47.6**	915.4 $\pm$ 36.7	761.6 $\pm$ 72.6	763.0 $\pm$ 47.3	749.4 $\pm$ 44.5
	40 mg/kg/day	886.8 $\pm$ 74.4	886.5 $\pm$ 96.6	898.8 $\pm$ 52.5	752.9 $\pm$ 78.2	739.2 $\pm$ 51.6	769.4 $\pm$ 56.6
Hemoglobin (g/dl)	60 mg/kg/day	856.6 $\pm$ 93.1	891.7 $\pm$ 51.1	898.3 $\pm$ 30.0	745.6 $\pm$ 88.8	759.2 $\pm$ 49.3	775.4 $\pm$ 56.7
	control	14.52 $\pm$ 0.72	14.47 $\pm$ 0.71	14.66 $\pm$ 0.77	13.84 $\pm$ 0.52	13.31 $\pm$ 0.51	13.86 $\pm$ 0.30
	20 mg/kg/day	14.47 $\pm$ 0.51	14.59 $\pm$ 0.57	14.66 $\pm$ 0.46	13.91 $\pm$ 0.48	13.22 $\pm$ 0.60	13.78 $\pm$ 0.55
Hematocrit (%)	40 mg/kg/day	14.60 $\pm$ 0.65	14.16 $\pm$ 1.79	14.74 $\pm$ 0.26	14.07 $\pm$ 0.63	13.37 $\pm$ 0.67	14.36 $\pm$ 0.62
	60 mg/kg/day	14.61 $\pm$ 0.58	14.80 $\pm$ 0.50	14.90 $\pm$ 0.72	13.97 $\pm$ 0.54	13.44 $\pm$ 0.67	14.12 $\pm$ 0.62
	control	42.95 $\pm$ 6.16	45.13 $\pm$ 3.00	45.06 $\pm$ 3.91	43.83 $\pm$ 5.10	42.10 $\pm$ 3.65	43.86 $\pm$ 0.65
Mean corpuscular volume (μm^3)	20 mg/kg/day	43.97 $\pm$ 5.70	44.21 $\pm$ 3.34	47.66 $\pm$ 1.91	46.16 $\pm$ 5.04	42.02 $\pm$ 2.85	45.28 $\pm$ 2.40
	40 mg/kg/day	40.36 $\pm$ 3.73	45.06 $\pm$ 5.36	48.54 $\pm$ 2.31	47.28 $\pm$ 4.55*	42.62 $\pm$ 2.67	49.28 $\pm$ 3.17*
	60 mg/kg/day	38.58 $\pm$ 4.44**	45.40 $\pm$ 3.60	47.12 $\pm$ 2.66	45.74 $\pm$ 5.19	43.85 $\pm$ 3.24	49.00 $\pm$ 2.68*
Mean corpuscular hemoglobin amount (pg)	control	49.2 $\pm$ 5.9	49.3 $\pm$ 2.6	51.0 $\pm$ 1.6	59.7 $\pm$ 1.9	55.2 $\pm$ 2.2	58.6 $\pm$ 2.3
	20 mg/kg/day	49.0 $\pm$ 6.0	51.5 $\pm$ 3.7*	52.2 $\pm$ 2.8	60.6 $\pm$ 2.1	55.1 $\pm$ 2.4	60.6 $\pm$ 2.5
	40 mg/kg/day	45.5 $\pm$ 1.5**	50.8 $\pm$ 2.8	54.2 $\pm$ 1.3**	62.9 $\pm$ 2.6**	57.8 $\pm$ 2.5**	64.0 $\pm$ 3.0*
Mean corpuscular hemoglobin concentration (%)	60 mg/kg/day	45.0 $\pm$ 1.7**	51.0 $\pm$ 2.8*	52.5 $\pm$ 1.7	61.4 $\pm$ 2.2**	57.8 $\pm$ 2.8**	63.2 $\pm$ 3.1*
	control	16.75 $\pm$ 1.11	15.80 $\pm$ 0.96	16.62 $\pm$ 0.89	19.04 $\pm$ 1.58	17.51 $\pm$ 1.12	18.52 $\pm$ 0.79
	20 mg/kg/day	16.22 $\pm$ 1.17	17.00 $\pm$ 0.86**	16.04 $\pm$ 0.86	18.39 $\pm$ 1.61	17.36 $\pm$ 0.90	18.44 $\pm$ 0.99
Platelets ($\times 10^9/\text{mm}^3$)	40 mg/kg/day	16.56 $\pm$ 1.43	15.96 $\pm$ 1.48	16.42 $\pm$ 0.80	18.86 $\pm$ 1.92	18.15 $\pm$ 1.29	18.70 $\pm$ 1.03
	60 mg/kg/day	17.20 $\pm$ 1.44	16.64 $\pm$ 0.89**	16.60 $\pm$ 0.67	18.95 $\pm$ 1.94	17.74 $\pm$ 1.04	18.24 $\pm$ 0.93
	control	34.42 $\pm$ 4.70	32.16 $\pm$ 2.28	32.64 $\pm$ 1.92	31.89 $\pm$ 2.89	31.79 $\pm$ 2.32	31.62 $\pm$ 0.99
Reticulocytes (%)	20 mg/kg/day	33.44 $\pm$ 4.46	33.15 $\pm$ 2.28	30.82 $\pm$ 1.98	30.43 $\pm$ 3.00	31.54 $\pm$ 1.63	30.46 $\pm$ 1.00
	40 mg/kg/day	36.43 $\pm$ 3.55	31.46 $\pm$ 3.03	30.42 $\pm$ 1.28	30.00 $\pm$ 2.87*	31.43 $\pm$ 1.69	29.18 $\pm$ 1.09**
	60 mg/kg/day	38.26 $\pm$ 3.74**	32.75 $\pm$ 2.22	31.65 $\pm$ 1.05	30.86 $\pm$ 3.13	30.75 $\pm$ 1.88	28.88 $\pm$ 1.77*
Mean corpuscular hemoglobin concentration (%)	control	93.26 $\pm$ 16.04	100.59 $\pm$ 11.80	87.38 $\pm$ 7.38	93.58 $\pm$ 10.32	90.94 $\pm$ 14.79	93.78 $\pm$ 5.32
	20 mg/kg/day	100.34 $\pm$ 15.47	92.45 $\pm$ 13.97*	99.50 $\pm$ 11.44	96.66 $\pm$ 12.89	89.36 $\pm$ 17.03	88.04 $\pm$ 3.60
	40 mg/kg/day	98.30 $\pm$ 18.05	101.15 $\pm$ 18.06	99.14 $\pm$ 22.08	95.87 $\pm$ 11.75	89.54 $\pm$ 11.94	84.52 $\pm$ 1.25*
Reticulocytes (%)	60 mg/kg/day	97.20 $\pm$ 17.93	102.52 $\pm$ 19.17	102.03 $\pm$ 15.46	97.20 $\pm$ 17.34	95.16 $\pm$ 13.38	95.58 $\pm$ 6.34
	control	20.7 $\pm$ 3.0	20.5 $\pm$ 2.6	19.0 $\pm$ 2.9	16.9 $\pm$ 4.3	16.5 $\pm$ 4.3	16.4 $\pm$ 3.6
	20 mg/kg/day	21.6 $\pm$ 2.9	18.5 $\pm$ 3.2*	18.6 $\pm$ 3.0	18.1 $\pm$ 3.3	17.8 $\pm$ 4.1	18.0 $\pm$ 1.2
Mean corpuscular hemoglobin concentration (%)	40 mg/kg/day	21.6 $\pm$ 2.2	25.2 $\pm$ 2.2	18.8 $\pm$ 1.3	15.2 $\pm$ 3.1	18.9 $\pm$ 3.3*	21.0 $\pm$ 1.9*
	60 mg/kg/day	21.0 $\pm$ 2.6	19.7 $\pm$ 2.8	18.3 $\pm$ 2.2	16.8 $\pm$ 3.0	18.0 $\pm$ 2.8	18.6 $\pm$ 3.4

Data represents mean $\pm$ S.D. (n=24-25 for administration period, n=4-5 for recovery period). *P<0.05 vs control. **P<0.01 vs control.

Table 7. Hematological findings in rats during and after 6-month repeated dermal administration of HM-HPMC (2).

	Dose	Male			Female		
		3 months	6 months	Recovery	3 months	6 months	Recovery
Prothrombin time (sec)	control	-	14.49 ± 1.09	14.54 ± 0.54	-	13.91 ± 0.52	13.68 ± 0.50
	20 mg/kg/day	-	14.92 ± 1.20	15.96 ± 1.62	-	13.58 ± 0.38*	13.84 ± 0.48
	40 mg/kg/day	-	14.30 ± 1.25	14.42 ± 0.28	-	13.64 ± 0.41	13.42 ± 0.35
	60 mg/kg/day	-	14.51 ± 1.10	15.75 ± 1.06	-	13.68 ± 0.39	13.56 ± 0.36
Activated partial thromboplastin time (sec)	control	-	16.46 ± 1.98	17.18 ± 1.31	-	15.05 ± 1.27	14.68 ± 1.03
	20 mg/kg/day	-	16.68 ± 2.77	18.30 ± 2.12	-	14.73 ± 1.44	14.34 ± 1.84
	40 mg/kg/day	-	16.92 ± 2.13	17.46 ± 1.41	-	14.08 ± 1.35*	14.94 ± 1.62
	60 mg/kg/day	-	16.66 ± 2.04	18.10 ± 1.78	-	13.77 ± 1.74*	14.70 ± 1.61
Leucocytes (× 100/mm ³)	control	108.8 ± 20.3	90.4 ± 15.0	77.0 ± 14.7	76.0 ± 17.0	65.3 ± 14.5	51.8 ± 11.9
	20 mg/kg/day	108.2 ± 24.9	84.8 ± 23.7	90.6 ± 13.9	79.4 ± 25.2	71.4 ± 20.3	46.0 ± 8.1
	40 mg/kg/day	108.7 ± 19.8	92.5 ± 27.7	98.8 ± 28.9	76.9 ± 20.2	70.4 ± 17.3	58.4 ± 15.5
	60 mg/kg/day	102.9 ± 18.5	85.6 ± 15.4	99.3 ± 38.9	81.4 ± 18.4	67.0 ± 11.3	91.2 ± 59.6
Basocytes (%)	control	0.0 ± 0.0	0.3 ± 0.5	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.2	0.0 ± 0.0
	20 mg/kg/day	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
	40 mg/kg/day	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
	60 mg/kg/day	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.3	0.0 ± 0.0
Eosinocytes (%)	control	1.0 ± 0.9	1.0 ± 1.4	1.4 ± 0.5	1.2 ± 1.1	1.2 ± 1.1	1.0 ± 1.0
	20 mg/kg/day	0.8 ± 1.0	0.8 ± 1.0	1.4 ± 0.9	0.9 ± 0.9	1.2 ± 1.3	1.4 ± 1.3
	40 mg/kg/day	0.8 ± 0.9	0.9 ± 1.0	0.4 ± 0.9	1.0 ± 1.2	1.4 ± 1.3	0.6 ± 0.5
	60 mg/kg/day	1.1 ± 0.9	0.4 ± 0.5*	1.5 ± 1.7	1.7 ± 2.3	1.3 ± 1.3	1.2 ± 0.8
Stab form leucocytes (%)	control	0.8 ± 0.9	1.0 ± 1.0	0.6 ± 0.9	0.2 ± 0.6	0.6 ± 0.9	0.6 ± 0.9
	20 mg/kg/day	0.9 ± 0.9	0.8 ± 0.9	1.0 ± 0.7	0.2 ± 0.4	0.7 ± 0.8	0.0 ± 0.0
	40 mg/kg/day	0.9 ± 1.1	0.8 ± 1.3	0.6 ± 0.9	0.0 ± 0.0	0.5 ± 0.7	0.2 ± 0.4
	60 mg/kg/day	0.8 ± 0.9	1.0 ± 0.9	0.5 ± 0.6	0.2 ± 0.5	0.6 ± 0.9	0.6 ± 0.9
Segmented leucocytes (%)	control	8.2 ± 3.8	11.6 ± 4.9	11.8 ± 3.1	10.1 ± 3.3	11.4 ± 6.3	16.6 ± 7.3
	20 mg/kg/day	8.8 ± 3.5	11.0 ± 4.4	10.0 ± 4.1	8.0 ± 2.8*	9.9 ± 5.2	12.6 ± 3.4
	40 mg/kg/day	7.3 ± 3.5	11.4 ± 4.6	10.6 ± 5.0	9.2 ± 4.2	10.4 ± 4.2	16.4 ± 5.8
	60 mg/kg/day	9.2 ± 4.1	10.3 ± 4.7	15.5 ± 5.4	8.3 ± 3.4	10.0 ± 5.5	12.0 ± 7.0
Lymphocytes (%)	control	84.4 ± 5.5	81.3 ± 8.2	76.2 ± 6.0	80.4 ± 5.3	83.2 ± 6.4	78.6 ± 8.1
	20 mg/kg/day	85.8 ± 4.2	80.4 ± 5.5	79.0 ± 5.0	82.3 ± 4.9	83.4 ± 6.8	80.0 ± 3.9
	40 mg/kg/day	86.9 ± 4.6	81.6 ± 6.4	80.6 ± 3.8	79.9 ± 5.9	83.4 ± 6.0	77.8 ± 7.8
	60 mg/kg/day	85.4 ± 4.8	81.7 ± 5.9	71.0 ± 5.0	80.7 ± 5.1	83.7 ± 6.5	81.2 ± 6.8
Monocytes (%)	control	5.7 ± 2.7	4.8 ± 3.8	10.0 ± 6.9	8.2 ± 3.8	3.5 ± 2.2	3.2 ± 3.3
	20 mg/kg/day	3.7 ± 1.9**	7.0 ± 3.1*	8.6 ± 2.7	8.6 ± 3.3	4.7 ± 2.4	6.0 ± 2.0
	40 mg/kg/day	4.1 ± 2.8*	5.2 ± 3.2	7.6 ± 1.3	9.9 ± 4.1	4.2 ± 3.0	5.0 ± 2.7
	60 mg/kg/day	3.5 ± 1.8**	6.5 ± 2.7	11.5 ± 4.7	9.1 ± 3.1	4.3 ± 2.1	5.0 ± 3.4

Data represents mean ± S.D. (n=24-25 for administration period, n=4-5 for recovery period).

*P<0.05 vs control. **P<0.01 vs control.

Table 8. Blood chemical findings of rats after 6-month repeated dermal administration of HM-HPMC.

Test	unit	Male				Female			
		control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
GOT	IU/l	96.8±24.1	84.0±14.1*	88.8±22.5	86.5±12.5	102.4±42.5	87.2±21.6	141.5±114.1	130.3±94.2
GPT	IU/l	29.0±14.9	22.4±3.8	23.8±5.4	24.8±5.4	32.6±22.2	26.6±13.4	55.9±58.0	43.1±34.1
ALP	IU/l	144.6±29.6	136.8±18.0	136.0±22.7	135.6±22.3	50.9±11.6	48.7±13.6	47.1±10.2	49.1±9.7
LDH	IU/l	1083.2±491.4	864.3±420.3	1042.0±656.0	968.6±325.6	722.0±242.5	647.6±291.8	810.1±343.7	737.0±252.8
Ch-E	×10 ² mg/dl	8.81±3.14	8.13±2.55	8.02±2.52	8.70±2.72	84.02±21.08	75.57±24.17	82.44±21.46	76.61±16.60
T-Bill	×10 ² mg/dl	6.1±2.4	5.8±2.0	6.6±1.8	4.9±1.5	8.7±2.3	8.0±2.6	9.0±2.4	8.8±2.8
GLU	mg/dl	122.7±12.6	112.8±8.9**	119.0±11.4	118.6±11.4	107.8±11.5	107.7±6.5	106.7±8.8	112.0±8.2
UA	mg/dl	1.081±0.265	0.989±0.211	1.138±0.277	1.074±0.194	1.159±0.173	1.068±0.141	1.074±0.248	1.128±0.172
BUN	mg/dl	14.85±1.91	15.53±2.27	14.23±1.34	14.11±1.68	16.87±2.67	16.33±2.38	16.79±3.42	16.55±2.90
CREA	mg/dl	0.52±0.05	0.51±0.04	0.49±0.03*	0.49±0.06	0.58±0.06	0.55±0.06	0.53±0.07*	0.53±0.06*
T-cho	mg/dl	60.8±15.9	47.3±12.5**	54.8±9.3	52.1±9.3*	59.5±10.4	53.6±10.9	55.4±11.9	62.5±14.6
P-lip	mg/dl	88.4±16.6	72.7±16.1**	81.1±10.2	77.6±11.1*	129.1±18.5	120.5±19.5	120.1±22.9	128.0±26.1
TG	mg/dl	53.9±14.2	52.9±21.0	56.7±18.8	53.2±18.7	38.4±12.3	39.7±10.6	35.0±9.2	40.0±16.2
Na	mEq/l	146.3±1.5	146.0±1.6	145.5±2.0	145.0±2.1*	144.6±2.4	143.5±1.7	143.5±2.6	145.4±1.3
K	mEq/l	4.08±0.21	3.98±0.17	4.17±0.25	4.09±0.24	3.83±0.74	3.57±0.21	3.80±0.59	3.71±0.21
Cl	mEq/l	104.6±1.6	104.2±1.5	104.4±1.3	104.1±1.9	105.0±1.4	105.0±1.2	105.0±1.8	106.0±1.6*
Ca	mg/dl	9.15±0.13	9.06±0.15*	9.06±0.12*	8.89±0.11**	9.38±0.17	9.26±0.13*	9.27±0.13*	9.29±0.13
IP	mg/dl	5.54±0.47	5.54±0.45	5.73±0.66	5.61±0.40	5.11±1.09	5.30±0.78	5.03±0.59	5.24±1.08
TP	mg/dl	6.61±0.25	6.50±0.16	6.58±0.35	6.60±0.22	7.20±0.43	7.06±0.48	7.02±0.34	7.36±0.43
ALB	g/dl	3.76±0.22	3.76±0.16	3.83±0.33	3.73±0.27	4.72±0.54	4.54±0.50	4.62±0.37	4.84±0.40
A/G		1.331±0.184	1.376±0.142	1.407±0.187	1.314±0.188	1.932±0.372	1.859±0.431	1.960±0.321	1.943±0.305
Protein fraction									
A/G		0.955±0.172	0.985±0.067	1.012±0.133	1.048±0.146	1.600±0.189	1.659±0.168	1.534±0.208	1.528±0.252
ALB	%	48.46±4.45	49.56±1.74	50.07±3.43	50.94±3.82	61.33±3.09	62.25±2.43	60.29±3.16	60.08±3.88
α	%	22.71±2.77	21.57±2.90	20.98±2.67	20.84±1.52*	13.94±1.95	12.74±1.96	13.59±1.01	14.11±1.55
β	%	5.07±0.91	5.23±1.06	5.00±1.28	4.92±0.72	4.22±1.11	3.95±0.67	4.05±0.81	3.86±0.87
γ	%	23.77±3.30	23.64±2.00	23.96±2.47	23.31±2.77	20.02±2.82	21.06±2.58	22.07±2.83*	21.94±3.33

Data represents mean ± S.D. (n=20). *P<0.05 vs control.

GOT: glutamate oxaloacetate transaminase, GPT: glutamate pyruvate transaminase,

ALP: alkaline phosphatase, LDH: lactate dehydrogenase, Ch-E: choline esterase, T-Bill: total bilirubin,

GLU: glucose, UA: uric acid, BUN: blood urea-nitrogen, CREA: creatinine, T-cho: total cholesterol,

P-lip: phospholipid, TG: triglyceride, Na: sodium, K: potassium, Cl: chlorine, Ca: calcium,

IP: inorganic phosphorus, TP: total protein, ALB: albumin, A/G: albumin-globulin ratio.

Table 9. Blood chemical findings of rats after recovery period.

Test	unit	Male				Female			
		control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
GOT	IU/l	82.2±7.6	82.4±15.5	74.2±5.3	86.0±6.2	111.0±26.8	81.6±10.2	99.6±35.5	116.4±57.0
GPT	IU/l	22.0±2.5	26.0±9.1	21.8±1.3	23.8±2.2	39.4±16.6	26.0±6.3	32.8±17.2	33.6±22.2
ALP	IU/l	113.6±11.3	107.0±21.2	114.8±23.5	129.0±21.3	42.2±9.8	42.8±5.7	48.0±8.2	48.4±10.1
LDH	IU/l	694.2±271.5	557.2±183.1	646.6±252.6	741.5±393.3	801.8±204.1	581.4±296.7	486.6±120.8*	764.4±218.1
Ch-E	×10 ⁻² mg/dl	9.18±2.31	7.68±3.29	5.92±0.93*	7.30±0.93	85.76±28.47	93.68±20.96	108.08±28.93	75.92±8.12
T-Bill	×10 ⁻² mg/dl	6.0±0.7	4.6±0.9*	5.6±1.1	6.0±1.2	8.4±0.9	7.6±1.5	8.0±0.7	9.6±2.2
GLU	mg/dl	134.4±5.7	124.4±11.4	124.2±8.6	119.0±11.0*	108.6±7.6	113.0±8.3	111.4±11.1	112.4±6.4
UA	mg/dl	0.910±0.157	0.966±0.124	0.970±0.154	0.863±0.142	1.100±0.244	1.066±0.121	0.932±0.160	1.108±0.266
BUN	mg/dl	11.16±1.97	11.72±1.13	11.62±1.33	11.73±0.76	14.90±4.13	14.04±0.92	12.58±1.57	13.34±2.28
CREA	mg/dl	0.48±0.04	0.50±0.00	0.48±0.04	0.43±0.05	0.52±0.04	0.50±0.07	0.52±0.04	0.48±0.04
T-cho	mg/dl	70.8±19.9	61.0±8.7	60.0±12.8	60.5±2.9	58.4±10.1	68.8±18.1	57.2±10.7	76.6±20.5
P-lip	mg/dl	99.0±25.8	86.0±9.0	88.4±16.5	85.3±6.0	120.8±13.1	135.8±21.0	118.2±18.7	136.4±26.1
TG	mg/dl	53.4±19.0	47.8±11.9	54.6±7.4	41.0±6.5	33.4±5.8	34.0±6.6	30.2±6.3	32.6±9.2
Na	mEq/l	146.2±0.8	146.6±1.1	145.2±1.5	145.5±0.6	144.4±1.3	143.6±1.5	145.2±1.6	145.4±0.5
K	mEq/l	4.14±0.17	4.00±0.24	4.00±0.10	4.00±0.08	3.62±0.19	3.36±0.30	3.34±0.19	3.62±0.11
Cl	mEq/l	104.6±1.1	104.6±1.1	104.2±0.4	104.0±0.8	104.8±0.4	105.0±2.0	105.8±1.8	106.6±1.7
Ca	mg/dl	9.26±0.09	9.10±0.12*	9.06±0.15*	9.03±0.15*	9.42±0.08	9.40±0.10	9.40±0.07	9.28±0.08*
IP	mg/dl	5.58±0.36	5.72±0.26	5.36±0.19	5.95±0.26	4.76±0.89	4.90±0.54	5.20±0.61	5.16±0.63
TP	mg/dl	6.78±0.27	6.60±0.23	6.66±0.15	6.58±0.15	7.18±0.34	7.38±0.37	7.34±0.22	7.26±0.50
ALB	g/dl	3.82±0.15	3.56±0.15*	3.78±0.23	3.68±0.05	4.82±0.33	4.96±0.35	4.88±0.29	4.82±0.38
A/G		1.302±0.138	1.170±0.046	1.328±0.194	1.270±0.093	2.058±0.269	2.068±0.280	1.998±0.270	1.976±0.110
Protein fraction									
A/G		0.974±0.061	1.066±0.094	1.032±0.082	0.990±0.166	1.618±0.122	1.818±0.192	1.580±0.226	1.564±0.173
ALB	%	49.34±1.58	51.54±2.28	50.74±2.01	49.48±4.16	61.74±1.84	64.38±2.33	60.98±3.38	60.84±2.85
α	%	22.18±1.26	20.94±2.86	21.36±2.33	20.28±2.14	12.42±1.49	12.20±1.00	12.16±0.92	12.20±0.37
β	%	4.94±0.55	5.18±0.69	5.10±0.32	5.12±0.64	3.72±0.78	3.76±0.39	4.40±0.50	3.92±0.47
γ	%	23.54±1.94	22.34±2.79	22.80±1.33	25.12±2.92	22.12±3.44	19.66±2.59	22.46±2.65	23.04±3.03

Data represents mean ± S.D. (n=4-5). *P<0.05 vs control.

Abbreviations: See Table 8.

Table 10. Myelogram of rats after 6-month repeated dermal administration of HM-HPMC.

	Male			Female					
unit	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	
MYE-BLA	%	0.10±0.31	0.20±0.41	0.20±0.41	0.25±0.44	0.50±0.61	0.45±0.69	0.30±0.47	0.65±0.81
PROMYEL	%	2.35±1.60	2.50±1.85	2.15±1.31	2.25±1.29	1.55±1.43	1.35±1.23	1.25±0.97	0.80±0.89
NEU-MYEL	%	4.85±2.74	5.25±2.29	5.45±2.82	4.90±2.79	14.10±4.90	11.85±4.34	11.24±2.34*	8.50±2.78**
NEU-META	%	8.78±4.09	11.58±3.18*	15.10±4.55**	17.50±6.47**	5.50±2.04	4.90±2.31	4.95±1.90	4.95±1.32
NEU-STAB	%	2.05±1.54	1.65±1.39	1.35±1.46	1.00±1.12*	1.25±1.07	1.20±1.15	0.20±0.41**	0.75±0.85
NEU-SEG	%	18.77±3.55	19.43±4.01	17.90±4.12	15.90±4.44*	14.15±4.30	14.00±3.51	14.09±5.04	15.75±4.01
EOS-MYEL	%	2.00±1.65	1.80±1.36	2.05±1.19	1.75±1.25	0.30±0.66	0.00±0.00	0.20±0.52	0.85±0.93
EOS-META	%	0.45±0.60	0.30±0.47	0.60±0.88	0.50±0.61	0.55±0.76	0.40±0.60	0.50±0.83	0.95±1.00
EOSINO	%	0.60±0.82	0.45±0.60	0.55±0.69	1.05±0.69	2.40±1.35	2.05±1.32	1.75±1.55	1.00±0.97*
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	0.0±0.0	0.0±0.0
MYE-MITO	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PROEb	%	0.70±0.98	0.15±0.37*	0.00±0.00	0.30±0.47	1.80±1.11	1.95±1.57	1.00±1.08*	1.00±0.79*
BASO-Eb	%	1.30±1.26	1.05±1.10	1.15±1.04	1.15±1.31	11.90±4.34	8.60±3.15**	12.33±3.62	9.50±3.00*
POLY-Eb	%	11.83±4.70	18.82±7.89**	24.30±5.81**	30.05±8.31**	17.80±6.09	17.25±4.76	19.67±4.22	16.55±5.72
NORM-Eb	%	23.95±5.70	18.68±7.03*	19.00±5.93*	12.60±8.22**	9.70±5.91	9.60±5.43	9.53±3.91	14.55±5.87*
Eb-MITO	%	0.15±0.37	0.25±0.55	0.25±0.55	0.35±0.59	0.40±1.79	0.05±0.22	0.10±0.31	0.00±0.00
PROLYM	%	1.00±0.86	0.80±0.70	0.50±0.89	0.15±0.49**	0.05±0.22	0.15±0.37	0.30±0.57	0.50±0.61**
LYM	%	18.22±6.24	14.08±5.66*	6.50±3.24**	6.90±6.14**	16.10±4.58	24.75±6.77**	21.37±5.15**	22.25±5.42**
PRO-MONO	%	0.05±0.22	0.00±0.00	0.10±0.31	0.05±0.22	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
MONO	%	0.65±0.75	0.45±0.83	0.25±0.64	0.55±0.83	0.15±0.37	0.00±0.00	0.05±0.22	0.00±0.00
PLA-C	%	0.45±0.6	0.75±1.02	0.6±0.88	0.55±0.89	0.35±0.67	0.45±0.83	0.25±0.44	0.50±0.69
MACROPH	%	1.50±1.00	1.30±1.22	1.30±1.30	1.80±0.52	0.65±0.67	0.45±0.51	0.35±0.49	0.20±0.41*
MAST-C	%	0.20±0.41	0.25±0.44	0.35±0.59	0.30±0.47	0.45±0.69	0.20±0.52	0.30±0.57	0.25±0.44
PRO-MEGA	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
MEGAK	%	0.10±0.31	0.20±0.41	0.25±0.44	0.15±0.37	0.35±0.49	0.25±0.55	0.30±0.66	0.50±0.69
MITO-C	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Data represents mean ± S.D. (n=20). *P<0.05 vs control. **P<0.01 vs control.

Data represents mean ± S.D. (n=20). *P<0.05 vs control. **P<0.01 vs control.

MYE-BLA: myeloblasts, PROMYEL: promyelocytes, NEU-MYEL: neutrophilic myelocytes, EOS-MYEL: eosinophilic myelocytes, EOS-META: eosinophilic metamyelocytes, NEU-META: neutrophilic metamyelocytes, NEU-STAB: neutrophilic stab form leucocytes, NEU-SEG: neutrophilic segmented leucocytes, EOS-MYEL: eosinophilic myelocytes, EOS-META: eosinophilic metamyelocytes, EOSINO: eosinophils, BASO: basophils, MYE-MITO: mitotic myelocytes, PROEb: proerythroblasts, BASO-Eb: basophilic erythroblasts, POLY-Eb: polychromatophilic erythroblasts, NORM-Eb: normochromic erythroblasts, PROLYM: prolymphocytes, LYM: lymphocytes, PRO-MONO: promonocytes, MONO: monocytes, PLA-C: platelets, MACROPH: macrophages, MAST-C: mast cells, PRO-MEGA: promegakaryocytes, MEGAK: megakaryocytes, MITO-C: mitochondria.

Table 11. Myelogram of rats after recovery period.

		Male				Female			
	unit	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
MYE-BLA	%	0.40±0.55	0.20±0.45	0.60±0.89	0.00±0.00	0.40±0.55	0.40±0.55	0.00±0.00	0.40±0.89
PROMYEL	%	4.40±1.95	3.00±1.87	3.20±2.28	3.25±0.96	1.00±0.71	0.20±0.45	0.00±0.00	0.20±0.45
NEU-MYEL	%	9.00±1.22	4.40±0.89**	5.40±1.14**	7.00±2.83	14.00±9.22	11.78±4.19	10.60±2.07	11.80±5.81
NEU-META	%	18.00±2.83	16.60±4.28	18.20±5.45	15.50±6.35	7.60±1.67	8.56±1.98	9.40±4.93	6.60±2.07
NEU-STAB	%	1.60±1.14	0.60±0.55	0.80±0.84	0.75±0.5	1.20±0.84	0.80±0.84	0.60±0.89	0.60±0.89
NEU-SEG	%	13.00±1.87	16.40±8.17	15.60±4.67	16.25±4.65	14.20±2.77	15.58±3.32	14.40±4.34	10.40±5.68
EOS-MYEL	%	1.80±2.05	1.00±1.41	1.60±1.52	2.00±0.82	0.40±0.89	0.00±0.00	0.00±0.00	1.80±1.30
EOS-META	%	1.20±1.10	0.80±0.45	1.20±1.64	1.00±0.82	0.40±0.55	0.40±0.55	0.60±1.34	1.60±1.14
EOSINO	%	1.40±0.89	1.00±1.22	1.00±1.73	0.50±0.58	2.20±1.79	1.60±1.52	2.00±1.22	0.80±0.84
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	0.0±0.0	0.0±0.0
MYE-MITO	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PROEb	%	0.20±0.45	0.0±0.0	0.0±0.0	0.50±0.58	1.20±0.84	0.60±0.55	0.60±0.89	0.89±1.10
BASO-Eb	%	0.60±0.89	0.80±1.30	1.40±1.14	0.00±0.00	6.60±1.82	7.98±2.03	9.00±3.00	7.80±4.38
POLY-Eb	%	36.6±1.14	41.4±3.51*	36.8±6.30	38.0±6.00	14.40±2.30	16.56±3.52	15.00±5.24	12.80±4.44
NORM-Eb	%	4.60±1.14	5.40±2.41	5.60±2.7	7.00±4.08	13.40±2.30	9.58±2.29*	12.00±4.00	18.20±3.90*
Eb-MITO	%	0.20±0.45	0.60±0.55	0.00±0.00	0.25±0.50	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PROLYM	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.20±0.45	0.00±0.00	0.20±0.45	0.20±0.45
LYM	%	4.00±1.41	4.40±1.67	4.20±2.95	3.00±2.45	21.20±7.92	25.14±6.63	23.80±3.27	22.80±9.01
PRO-MONO	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
MONO	%	0.80±1.10	0.60±0.89	1.20±0.84	1.00±0.82	0.20±0.45	0.00±0.00	0.00±0.00	0.00±0.00
PLA-C	%	0.60±1.34	0.80±0.84	1.20±1.64	1.25±0.96	0.20±0.45	0.00±0.00	0.40±0.55	1.20±1.64
MACROPH	%	1.00±1.00	1.40±0.55	1.00±0.71	1.25±1.89	0.60±0.89	0.40±0.89	0.20±0.45	0.20±0.45
MAST-C	%	0.20±0.45	0.20±0.45	0.60±0.55	0.50±1.00	0.00±0.00	0.20±0.45	0.20±0.45	1.20±1.10
PRO-MEGA	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
MEGAK	%	0.40±0.55	0.20±0.45	0.40±0.55	0.75±0.50	0.60±0.89	0.20±0.45	0.60±0.55	0.60±0.89
MITO-C	%	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Data represents mean ± S.D. (n=4-5). *P<0.05 vs control. **P<0.01 vs control.

Abbreviations: See Table 10.

Table 12. Organ weights (absolute) in rats after 6-month repeated dermal administration of HM-HPMC.

	unit	Male				Female			
		control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
Brain	g	2.183±0.072	2.233±0.107	2.206±0.090	2.210±0.097	1.981±0.091	2.014±0.074	1.982±0.113	1.977±0.069
Pituitary	mg	11.65±1.79	12.24±1.67	13.00±1.83*	12.99±1.90*	22.44±6.53	22.18±5.72	22.12±5.23	23.17±5.82
Thyroid(R)	mg	13.66±3.14	12.75±2.58	13.17±3.13	14.37±3.01	10.06±2.19	10.38±3.63	9.17±2.49	9.27±1.80
Thyroid(L)	mg	12.04±2.28	11.67±1.70	11.75±2.95	13.01±2.96	9.59±1.76	9.37±2.58	8.92±1.37	8.84±2.74
Thymus	g	0.159±0.045	0.157±0.051	0.178±0.056	0.161±0.031	0.136±0.036	0.160±0.043	0.136±0.033	0.139±0.030
Submandibular (R)g	g	0.372±0.048	0.371±0.039	0.389±0.035	0.375±0.059	0.223±0.020	0.228±0.022	0.229±0.022	0.234±0.023
Submandibular (L)g	g	0.367±0.048	0.371±0.042	0.382±0.037	0.376±0.058	0.220±0.019	0.226±0.021	0.229±0.024	0.230±0.025
Heart	g	1.686±0.158	1.626±0.120	1.714±0.166	1.709±0.159	1.016±0.014	1.072±0.106	1.071±0.092	1.022±0.087
Lungs	g	1.712±0.165	1.679±0.138	1.750±0.167	1.749±0.173	1.217±0.116	1.265±0.101	1.282±0.110	1.253±0.114
Liver	g	13.151±1.816	12.965±2.136	13.536±1.649	13.609±1.274	7.516±0.826	7.827±0.908	7.822±0.822	7.587±0.889
Kidney(R)	g	1.632±0.169	1.565±0.174	1.637±0.144	1.660±0.137	0.937±0.078	0.954±0.064	0.964±0.096	0.905±0.077
Kidney(L)	g	1.620±0.157	1.546±0.139	1.603±0.128	1.632±0.144	0.914±0.076	0.917±0.072	0.931±0.090	0.883±0.076
Adrenal (R)	mg	28.75±4.83	29.19±4.86	29.48±4.34	31.47±4.71	30.73±5.16	30.31±5.46	32.48±5.52	30.04±5.68
Adrenal(L)	mg	30.87±5.23	30.61±4.86	30.70±3.98	32.62±4.45	31.83±4.97	32.06±6.11	32.44±5.03	31.49±6.54
Spleen	g	0.748±0.120	0.757±0.108	0.771±0.106	0.762±0.102	0.481±0.078	0.551±0.084**	0.532±0.074*	0.472±0.063
Epididymis (R)	g	0.712±0.066	0.717±0.060	0.725±0.077	0.716±0.062				
Epididymis (L)	g	0.699±0.072	0.698±0.053	0.711±0.068	0.704±0.064				
Prostate gland	g	0.476±0.130	0.440±0.108	0.503±0.108	0.507±0.113				
Seminal vesicle	g	1.518±0.245	1.611±0.233	1.580±0.232	1.661±0.246				
Testis (R)	g	1.713±0.116	1.686±0.100	1.698±0.150	1.699±0.109				
Testis (L)	g	1.709±0.116	1.675±0.092	1.695±0.151	1.691±0.110				
Ovary (R)	g					32.62±11.24	39.76±12.18	32.67±10.57	32.52±9.42
Ovary (L)	g					31.72±10.42	38.75±13.40	34.94±12.76	31.52±10.55
Uterus	g					0.648±0.168	0.695±0.216	0.698±0.184	0.648±0.159

Data represents mean±S.D.(n=20). *P<0.05 vs control. **P<0.01 vs control.

Table 13. Organ weights (absolute) in rats after recovery period.

	unit	Male				Female			
		control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
Brain	g	2.159±0.129	2.195±0.080	2.201±0.084	2.171±0.057	1.972±0.075	2.016±0.078	2.092±0.124	1.981±0.047
Pituitary	mg	13.28±2.18	13.44±0.84	13.92±1.14	14.03±0.93	25.82±6.34	27.54±6.62	23.82±2.94	25.34±6.30
Thyroid(R)	mg	10.96±2.42	11.64±2.00	12.42±2.55	12.37±1.54	10.30±1.13	9.92±2.06	9.66±1.25	11.26±2.38
Thyroid(L)	mg	10.84±2.31	11.76±2.14	11.92±1.59	12.12±2.28	10.40±2.07	9.26±1.37	9.20±1.86	10.04±0.85
Thymus	g	0.128±0.032	0.112±0.028	0.146±0.045	0.127±0.035	0.097±0.013	0.120±0.028	0.110±0.016	0.115±0.034
Submandibular (R)g		0.388±0.033	0.389±0.036	0.387±0.036	0.412±0.100	0.244±0.030	0.233±0.013	0.238±0.028	0.227±0.027
Submandibular (L)g		0.398±0.031	0.384±0.040	0.388±0.039	0.408±0.084	0.245±0.033	0.231±0.014	0.230±0.016	0.231±0.023
Heart	g	1.649±0.071	1.626±0.105	1.643±0.096	1.671±0.188	1.014±0.071	1.061±0.094	1.082±0.053	1.078±0.107
Lungs	g	1.648±0.067	1.679±0.124	1.775±0.076*	1.737±0.168	1.268±0.053	1.372±0.118	1.335±0.048	1.428±0.353
Liver	g	14.516±1.403	13.534±2.666	14.820±1.445	14.747±2.744	7.767±1.174	8.164±1.020	7.873±0.743	7.788±1.167
Kidney(R)	g	1.747±0.104	1.637±0.135	1.807±0.158	1.853±0.281	0.951±0.076	0.985±0.125	0.956±0.046	1.011±0.094
Kidney(L)	g	1.684±0.154	1.672±0.145	1.719±0.193	1.860±0.345	0.925±0.067	0.941±0.105	0.927±0.064	0.980±0.107
Adrenal (R)	mg	30.24±2.30	27.88±1.94	30.06±3.14	30.73±7.37	37.06±8.19	35.64±4.16	36.82±6.05	34.80±5.04
Adrenal(L)	mg	32.42±2.07	29.80±3.57	31.82±3.79	32.12±6.03	40.46±6.08	39.26±5.48	37.40±5.18	36.60±5.62
Spleen	g	0.797±0.156	0.734±0.129	0.772±0.094	0.749±0.167	0.438±0.062	0.462±0.085	0.487±0.066	0.518±0.098
Epididymis (R)	g	0.682±0.069	0.679±0.042	0.731±0.064	0.726±0.066				
Epididymis (L)	g	0.661±0.063	0.692±0.038	0.701±0.051	0.690±0.061				
Prostate gland	g	0.604±0.173	0.528±0.092	0.490±0.090	0.540±0.105				
Seminal vesicle	g	1.534±0.222	1.654±0.234	1.662±0.209	1.712±0.122				
Testis (R)	g	1.718±0.070	1.749±0.173	1.754±0.202	1.756±0.057				
Testis (L)	g	1.694±0.088	1.759±0.199	1.734±0.191	1.725±0.047				
Ovary (R)	g					28.74±5.04	28.42±10.57	35.66±7.74	35.64±13.46
Ovary (L)	g					31.38±10.00	29.90±8.70	35.06±10.30	35.64±14.20
Uterus	g					0.718±0.270	0.969±0.149	0.964±0.581	0.692±0.110

Data represents mean ± S.D. (n=5). *P<0.05 vs control. **P<0.01 vs control.

Table 14. Organ weights (relative) in rats after 6-month repeated dermal administration of HM-HPMC.

	unit	Male				Female			
		control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
Body weight	g	597.6±49.7	583.1±55.9	605.8±57.4	603.0±42.1	337.8±30.2	363.0±42.5*	346.6±37.4	337.8±41.9
Brain	g/100g-BW	0.37±0.03	0.38±0.04	0.37±0.03	0.37±0.03	0.59±0.05	0.56±0.06	0.58±0.07	0.59±0.07
Pituitary	mg/100g-BW	1.96±0.29	2.10±0.26	2.15±0.28*	2.16±0.35*	6.67±1.95	6.17±1.65	6.42±1.53	6.90±1.70
Thyroid(R)	mg/100g-BW	2.29±0.48	2.18±0.42	2.17±0.46	2.39±0.52	3.01±0.75	2.86±0.93	2.65±0.73	2.76±0.52
Thyroid(L)	mg/100g-BW	2.03±0.43	2.01±0.35	1.93±0.40	2.17±0.54	2.86±0.58	2.58±0.61	2.59±0.45	2.62±0.76
Thymus	g/100g-BW	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.04±0.01	0.04±0.01	0.04±0.01	0.04±0.01
Submandibular (R)	g/100g-BW	0.06±0.01	0.06±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.07±0.01
Submandibular (L)	g/100g-BW	0.06±0.01	0.06±0.01	0.06±0.01	0.06±0.01	0.07±0.01	0.06±0.01	0.07±0.01	0.07±0.01
Heart	g/100g-BW	0.28±0.02	0.28±0.02	0.28±0.03	0.28±0.02	0.30±0.02	0.30±0.03	0.31±0.03	0.30±0.03
Lungs	g/100g-BW	0.29±0.03	0.29±0.03	0.29±0.02	0.29±0.02	0.36±0.03	0.35±0.03	0.37±0.04	0.38±0.04
Liver	g/100g-BW	2.19±0.18	2.21±0.23	2.23±0.12	2.26±0.15	2.23±0.18	2.16±0.17	2.27±0.21	2.25±0.15
Kidney(R)	g/100g-BW	0.27±0.02	0.27±0.03	0.27±0.02	0.28±0.02	0.28±0.02	0.27±0.03	0.28±0.03	0.27±0.02
Kidney(L)	g/100g-BW	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02	0.27±0.02	0.25±0.02*	0.27±0.03	0.26±0.02
Adrenal (R)	mg/100g-BW	4.82±0.73	5.01±0.82	4.88±0.66	5.22±0.67	9.16±1.68	8.47±1.88	9.42±1.54	8.96±1.57
Adrenal(L)	mg/100g-BW	5.16±0.71	5.26±0.81	5.09±0.66	5.41±0.63	9.50±1.71	8.91±1.87	9.43±1.55	9.41±2.00
Spleen	g/100g-BW	0.12±0.02	0.13±0.02	0.13±0.01	0.13±0.02	0.14±0.02	0.15±0.03	0.15±0.02*	0.14±0.02
Epididymis (R)	g/100g-BW	0.12±0.01	0.12±0.02	0.12±0.01	0.12±0.01				
Epididymis (L)	g/100g-BW	0.12±0.01	0.12±0.02	0.12±0.01	0.12±0.01				
Prostate gland	g/100g-BW	0.08±0.02	0.08±0.02	0.08±0.01	0.08±0.02				
Seminal vesicle	g/100g-BW	0.25±0.04	0.28±0.05	0.26±0.04	0.26±0.08				
Testis (R)	g/100g-BW	0.29±0.02	0.29±0.03	0.28±0.03	0.28±0.02				
Testis (L)	g/100g-BW	0.29±0.02	0.29±0.03	0.28±0.03	0.28±0.02				
Ovary (R)	mg/100g-BW					9.62±3.11	10.99±3.06	9.48±3.17	9.64±2.59
Ovary (L)	mg/100g-BW					9.39±2.88	10.77±3.60	10.11±3.74	9.44±3.23
Uterus	g/100g-BW					0.19±0.05	0.19±0.06	0.21±0.07	0.19±0.04

Data represents mean ± S.D.(n=20). *P<0.05 vs control. **P<0.01 vs control.

Table 15. Organ weights (relative) in rats after recovery period.

		Male				Female			
	unit	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
Body weight	g	645.2±38.5	603.8±55.4	618.0±49.6	643.5±118.5	331.2±29.0	356.4±26.5	337.8±26.6	330.0±42.6
Brain	g/100g-BW	0.33±0.02	0.37±0.04	0.36±0.03	0.35±0.06	0.60±0.06	0.57±0.03	0.62±0.06	0.61±0.07
Pituitary	mg/100g-BW	2.06±0.33	2.24±0.18	2.26±0.17	2.22±0.34	7.80±1.91	7.71±1.65	7.05±0.68	7.70±1.69
Thyroid(R)	mg/100g-BW	1.70±0.37	1.93±0.32	2.01±0.33	1.98±0.42	3.15±0.60	2.79±0.55	2.86±0.32	3.42±0.61
Thyroid(L)	mg/100g-BW	1.67±0.31	1.96±0.42	1.93±0.19	1.94±0.51	3.19±0.78	2.60±0.38	2.75±0.66	3.08±0.44
Thymus	g/100g-BW	0.02±0.00	0.02±0.00	0.02±0.01	0.02±0.01	0.03±0.01	0.03±0.01	0.03±0.01	0.04±0.01
Submandibular (R)	g/100g-BW	0.06±0.00	0.07±0.01	0.06±0.00	0.06±0.01	0.07±0.01	0.07±0.01	0.07±0.00	0.07±0.01
Submandibular (L)	g/100g-BW	0.06±0.01	0.06±0.01	0.06±0.00	0.07±0.01	0.07±0.01	0.07±0.01	0.07±0.00	0.07±0.01
Heart	g/100g-BW	0.26±0.01	0.27±0.02	0.27±0.03	0.26±0.03	0.31±0.03	0.30±0.01	0.32±0.01	0.33±0.04
Lungs	g/100g-BW	0.26±0.01	0.28±0.03	0.29±0.02**	0.28±0.03	0.39±0.03	0.38±0.01	0.40±0.04	0.44±0.12
Liver	g/100g-BW	2.25±0.25	2.23±0.30	2.39±0.11	2.30±0.16	2.33±0.17	2.29±0.16	2.33±0.06	2.36±0.25
Kidney(R)	g/100g-BW	0.27±0.02	0.27±0.02	0.29±0.01	0.29±0.02	0.29±0.02	0.28±0.02	0.28±0.02	0.31±0.03
Kidney(L)	g/100g-BW	0.26±0.03	0.28±0.02	0.28±0.01	0.29±0.01	0.28±0.01	0.27±0.02	0.27±0.03	0.30±0.03
Adrenal (R)	mg/100g-BW	4.69±0.36	4.66±0.62	4.87±0.39	4.75±0.27	11.23±2.38	9.99±0.81	10.93±1.81	10.65±1.86
Adrenal(L)	mg/100g-BW	5.04±0.44	4.97±0.72	5.15±0.50	4.99±0.06	12.29±2.05	11.00±1.22	11.12±1.72	11.19±1.97
Spleen	g/100g-BW	0.13±0.03	0.12±0.01	0.13±0.02	0.12±0.02	0.13±0.01	0.13±0.02	0.14±0.01	0.16±0.01*
Epididymis (R)	g/100g-BW	0.11±0.01	0.11±0.02	0.12±0.01	0.12±0.02				
Epididymis (L)	g/100g-BW	0.10±0.00	0.12±0.01	0.11±0.01*	0.11±0.02				
Prostate gland	g/100g-BW	0.09±0.02	0.09±0.02	0.08±0.01	0.09±0.02				
Seminal vesicle	g/100g-BW	0.24±0.04	0.28±0.04	0.27±0.05	0.27±0.04				
Testis (R)	g/100g-BW	0.27±0.01	0.29±0.02	0.29±0.04	0.28±0.04				
Testis (L)	g/100g-BW	0.26±0.01	0.29±0.03	0.28±0.04	0.27±0.05				
Ovary (R)	mg/100g-BW					8.68±1.32	7.92±2.83	10.60±2.48	10.33±3.05
Ovary (L)	mg/100g-BW					9.50±2.83	8.38±2.37	10.58±3.53	10.61±3.36
Uterus	g/100g-BW					0.22±0.08	0.27±0.06	0.29±0.19	0.21±0.05

Data represents mean ± S.D. (n=5). *P<0.05 vs control.

Table 16. Items showing possible dose-dependent significant differences in urinalysis, hematological analysis, myelogram and organ weight in 6-month repeated dermal toxicity test of HM-HPMC.

Time of inspection	Items	Male	Female
3 months	Urinalysis		↓
	Hematology	↓	↑
6 months	Ammonium magnesium phosphate		
	Mean corpuscular volume	↓	↑
	Hematocrit	↓	
	Mean corpuscular hemoglobin concentration		
	Monocytes	↓	
	Hematology		
	Mean corpuscular volume	↑	↑
	Mean corpuscular hemoglobin concentration	↑	
	Activated partially thromboplastin time		↓
	Eosinophils	↓	
	Creatinine		↓
	Calcium	↓	↓
	Myelogram		
	Neutrophilic metamyelocytes	↑	
	Neutrophilic myelocytes		↓
	Neutrophilic stab form leukocytes	↓	
	Neutrophilic segmented leukocytes	↓	
	Eosinophilic myelocytes		↑
	Eosinophils		↓
	Proerythroblasts		↓
Absolute organ weight	Polychromatophilic erythroblasts	↑	
	Normochromic erythroblasts	↓	↑
	Prolymphocytes	↓	↑
	Lymphocytes	↓	↑
Relative organ weight	Pituitary	↑	
	Pituitary	↑	
After recovery period	Hematology		
	Calcium	↓	↓
	Mean corpuscular volume		↑
	Hematocrit		↑
	Mean corpuscular hemoglobin concentration		↓

↑ : increase ↓ : decrease

Table 17. Autopsy findings in rats after 6-month repeated dermal administration of HM-HPMC.

Organ	Finding	Male			Female				
		Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
Pituitary	Enlargement Hematoma				1			1	
Thyroid	Hypertrophy Atrophy	1 1			1				
Lung	Diffuse white patch (black at the center)	1							2
	Diffuse white spots (black at the center)		1						
	Diffuse white spots			1				2	
	Gray spots	1		2		1			
	Light green patches	1							
Kidney	Pyelectasis	2			1				2
	Cyst			1					
	Obscure boundary between the cortex and medulla.						1		
	Gray-white spots								
	Rough surface		1						
	Decoloration				1				
Adrenal	Hematoma								1
Liver	White necrotic change	1							2
	Gray-white node	1	1		2			5	3
	Gray-white patch						1		
	Hypogenesis of papillae								
	Hernia				1				
	Decoloration		1						
	Thickening		1						
Epididymis	Yellow node		1						
	Degeneration of adipose tissue			1					
Ovary	Hematoma							1	
Hypodermis	Node			1			1		

Data represents number of cases.

Table 18. Autopsy findings in rats after recovery period.

Organ	Finding	Male				Female			
		Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day	Control	20 mg/kg/day	40 mg/kg/day	60 mg/kg/day
Pituitary	Slight enlargement						1		
Lung	Adhesion with diaphragm	1							
	Gray-white spots					1		1	1
	Light green spots								
	Dark red change								1
Kidney	Cyst			1					
	Decoloration				1				
	Yellowish white spots		1	1					
Adrenal	Hematoma					1			
Liver	Gray-white spots	1	1			2	2	1	
	Hernia					1		1	
Spleen	Slight enlargement	1							

Data represents number of cases.

Table 19. Histopathological findings in rats after 6-month repeated dermal administration of HM-HPMC.

Organs	Findings	Male		Female	
		Control	60 mg/kg/day	Control	60 mg/kg/day
Pituitary	Cyst			2	
Pineal gland	Cyst		1		
Thyroid	Small cyst surrounded by squamous epithelium Cystic adenoma Cyst	2	1	1	3
Heart	Cell aggregation in the myocardium (small)		2	1	
Lung	Calcification of the pulmonary arterial wall in the lung (single focus) Small granuloma (histocyte origin) Aggregation of small inflammatory cells	2	3 1	6	3
Kidney	Small cell aggregate in the stroma Nidal chronic pyelonephritis Calcium deposition in the renal pelvis A few casts in the renal tubules Cyst	1 1 3	1 5	1 2	2
Adrenal	Hematoma			1	1
Liver	Fatty degeneration Single nodal focus of clear altered cells Focal fibrosis and pigment-ingesting macrophages Hepatocyte-necrotic focus	13	19 2	7 3 1	3 6 1
Prostate gland	Acute prostatitis	2	2		
Urinary bladder	Cystitis Dilatation of the inside	1 1			
Uterus	Dilatation of the inside			1	1

Data represents number of cases.

the administration period are summarized in Table 1. A few signs, such as hematuria, were found from the middle of the administration period; however, they showed no dose-dependency and seemed to occur spontaneously or incidentally. Changes in mean body weight are shown in Fig. 2 and Table 2, and food consumption is presented in Fig. 3 and Table 3. Group mean values of body weight and food consumption in females of the 20 mg/kg/day dosing group were significantly higher than those of the control group from week 5 to 26 (including animals for the recovery test). However, the differences were not significant throughout the recovery period. The same trend was observed in food consumption in females of the 40 mg/kg/day group. However, those changes were neither systematic nor dose-dependent, and therefore they were not considered to be attributable to the test substance.

One male in the 60 mg/kg/day group died on the 144th day of administration. At necropsy, significant enlargement of the liver and spleen, and a dark reddish change in the small intestine (a sign of severe peritoneal hemorrhage) were observed. Histopathological findings were as follows: malignant lymphoma, with tumor cells (including infiltration) in bone marrow, liver, spleen, lymph nodes, and heart. It was considered that a malignant tumor of the hemopoietic system, which is occasionally found in old rats, had developed incidentally at an early age. No other animals developed such tumors, hence it was considered that the tumor was not attributable to the test substance.

The results of urinalysis are shown in Tables 4 and 5, those for hematological analysis in Tables 6-9, those for myelocyte analysis in Tables 10 and 11, and those for organ weight in Tables 12-15. Table 16 summarizes those examination items for which the data in the test preparation groups were significantly different from those of the control group and may have been dose-dependent. This table includes the direction of shift. In some cases, an increase was noted in males but a decrease in females of the same dose group, or vice versa. Since the changes were not systematic or consistent, they seemed to be incidental. Even if the test substance really affected the animals physiological-

ly in some way, many of the changes could not be considered as adverse effects. For example, in the urinalysis, urinary pH in the females of the 60 mg/kg/day group was slightly higher than that in the control group, but was still within the normal range. A difference in ammonium magnesium phosphate was found in the males of the 40 mg/kg/day group at the start of administration and in the females of the 40 and 60 mg/kg/day groups in the interim examination, but these changes seemed to be toxicologically insignificant. In the hematological examination, a decrease in hematocrit was found in the males of the 60 mg/kg/day group in the interim examination, but no change was found at the end of administration and so the interim decrease is considered to be incidental. At the end of the recovery period, increases in hematocrit value were observed in the females of the 40 and 60 mg/kg/day groups, but the values were all within the normal range. Although changes in the mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration were observed, no marked changes in erythrocyte count, hematocrit or hemoglobin concentration were found, and the changes are not considered toxicologically significant. Some significant dose-dependent changes were observed in the myelogram, i.e., neutrophilic metamyelocytes, polychromatophilic erythroblasts, and normochromic erythroblasts in males, and lymphocytes in males and females.

The percentages of these cells also seemed to be different between the administration period and recovery period, but this is not considered to be attributable to HM-HPMC, since the control group also showed such a difference. No changes were observed in leucocytes or in their differential examination, and no abnormalities were observed in histopathological examination of the bone marrow, so the observed differences are not considered to be related to the test substance. Significant differences in the absolute and relative weights of the hypophysis were observed in males of the 40 and 60 mg/kg/day groups. However, these differences were considered to be physiological changes within the range of normal fluctuation. A tendency toward a dose-dependent decrease in the amount of serum cal-

cium was observed; this may suggest some physiological change, but cannot be regarded as a toxic effect.

Macroscopic findings in necropsy are summarized in Tables 17 and 18. No findings suggested an adverse effect of the test substance. Further, no ophthalmological abnormalities were seen throughout the test period, except for a few cases of injury to the eye during blood sample collection.

Findings of histopathological examination are shown in Table 19. Fatty degeneration, necrotic foci, fibrous proliferation and aggregation of pigment-ingesting cells were observed in the liver. Since such changes are spontaneous and were also found in the control group, they seemed to be independent of the test substance. Nodal foci consisting of hepatocytes with translucent changes were found frequently in the females of the test-preparation groups in particular. Although this type of change, called a clear cell-altered focus, does not attract attention in human cases, it is thought to be important in animal studies. In the present histopathological examination, this type of change was also found in the control group. However, it was not tumorous, and did not appear to be attributable to the test substance. Although aggregation of cells in the myocardium seemed to be pathological, this appeared to be unrelated to the administration of the test substance. Granuloma in the lung was reactive and apparently caused by deglutition. Cysts in the thyroid, hypophysis and pineal body were small and occurred singly, and again appeared to be independent of the test substance. The base material of the test substance is HPMC, which has long been used as a coating agent and as an excipient for sustained-release solid dosage forms in the pharmaceutical industry. It is also widely used in cosmetics and foods as a thickening agent. The biological safety of HPMC has been generally recognized for long time, and many toxicological studies on other water-soluble cellulosic polymers, including hydroxyethylcellulose, hydroxypropyl cellulose, methylcellulose, and carboxymethyl cellulose have also been conducted and reviewed (Cosmetic Toiletry and Fragrance Association, 1986). Such cellulosic polymers have been reported to be practically non-toxic when

administered by inhalation or by oral, intraperitoneal, subcutaneous, or dermal routes. Our study shows that HM-HPMC can also be regarded as a safe material, like other cellulose derivatives. Preparation of HM-HPMC involves introducing a small amount of long-chain alkyl groups. A typical long-chain alkyl group level of 1 % by weight is equivalent to approximately 0.006 per glucose unit in molecular ratio. The present results indicate that such a low degree of modification does not influence the biological safety of the polymer, although it greatly affects the solution characteristics.

CONCLUSION

No toxic effects attributable to the test substance were found in a six-month repeated-dose dermal administration study of HM-HPMC in rats at dose levels up to 60 mg/kg/day, which was the highest amount possible to administer. This substance is therefore considered to be a safe material, like other cellulosic polymers, at least in dermal application.

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