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Toxicity Study of Red Ginseng Acidic Polysaccharide (RGAP) : Single and 2-week Repeated Oral Dose Toxicity Study in Rats

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Abstract. The present study was conducted to investigate the single and 2-week repeated dose toxicity of red ginseng acidic polysaccharide (RGAP) in Sprague-Dawley rats. The test article was administered orally to rats at dose levels of 0, and 2000 mg/kg/day for single dose toxicity study and at dose levels of 0, 250, 500, and 1000 mg/kg/day for repeated dose toxicity study. In both studies, there were no treatment-related effects on mortality, clinical signs, food and water consumption, ophthalmoscopy, urinalysis, hematology, serum biochemistry, necropsy findings and organ weights of all animals treated RGAP. Based on these results, it was concluded that the 2-week repeated oral dose of RGAP may have no toxic effect in rats at a dose level of 1000 mg/kg/day. In the condition of this study, the no-observed-adverse-effect level (NOAEL) was considered to be 1000 mg/kg/day for both sexes.

Keywords: Red ginseng acidic polysaccharide (RGAP), Single and 2-week repeated dose toxicity study, no-observed-adverse-effect level (NOAEL), Rats.

INTRODUCTION

Ginseng has been reported to strengthen the organisms resistance to physical, chemical and biological harmful stresses. It is believed that immunomodulatory activities of ginseng is related to its regulatory effect on the immune system, which plays an important role in the protective mechanism of the body. Immunomodulatory activity constitutes a major arm of its biological effects. In the context of immunomodulatory activities of ginseng, more attention was paid on polysaccharide. Polysaccharide from the root of *Panax ginseng* has been known to have mitogenic activities (Eun *et al.*, 1989), hypoglycemic activities (Konno *et al.*, 1984) and antitumor activities (Moon *et al.*, 1983). Furthermore, acidic polysaccharides from ginseng root were found to inhibit toxohormone-L-induced lipolysis (Lee and Okuda, 1990) and reduce the incidence rate of benzo[a]pyrene-induced neoplasm (Lee *et al.*, 1997). Accordingly, acidic polysaccharide may be a primary candidate of medicinal applications of ginseng. Red ginseng acidic polysaccharide (RGAP), isolated from Korean red ginseng (*Panax ginseng*), has found to induce the proliferation of spleen cell, decrease antibody forming cell response to sheep red blood cells and stimulate nitric oxide production of murine macrophages (Park *et al.*, 2000, 2001). And also RGAP has recently been found to show immunomodulating anticancer activity in a murine transplanted cells (Kim *et al.*, 2002). However, while extensive studies on the immunomodulating activities of polysaccharide from red ginseng were carried out (Kim *et al.*, 1990; Tomoda *et al.*, 1993), there is still scarce information about toxicity of red ginseng, the steam-processed form of fresh ginseng. This result has led us to investigate the oral dose toxicity in more detail.

In the present study we report the results of the sin-

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List of abbreviations: RGAP, Red Ginseng Acidic Polysaccharide; AAALAC International, Association for Assessment and Accreditation of Laboratory Animal Care International; KFDA, Korea Food and Drug Administration; NOAEL, no-observed-adverse-effect level; NRC, National Research Council; OECD, Organization for Economic Cooperation and Development

gle and 2-week repeated oral dose toxicity study in Sprague-Dawley rats performed as a part of the preclinical safety evaluation program for RGAP.

MATERIALS AND METHODS

Preparation of Red Ginseng Acidic Polysaccharide (RGAP)

Red ginseng made by steaming and drying fresh root of Panax ginseng C. A. Meyer was cut to mill. Powdered red ginseng was perchlorinated with 5 volumes of 85% ethanol to extract off ethanol-soluble materials. Remaining residues were repercolated with 5 volumes of distilled water and the water-soluble extracts were concentrated with a vacuum evaporator. The concentrate was dialyzed against running tap water for 7 days to completely cut off small molecules less than 15 KDa. Four volumes of absolute ethanol were added to precipitate the polysacchride in the inner dialysate. The precipitate was dried in a vacuum drying oven and finally used as a red ginseng acidic polysaccharide (RGAP). Chemical composition of RGAP was 56.9% of acidic sugars and 28.3% of neutral sugars as determined by carbazole assay (Chaplin and Kennedy, 1994) and phenol-sulfuric assay, respectively. Protein content of RGAP was below 0.1% as determined by the Lowry method. Less than 0.006 EU (endotoxin unit) of endotoxin was present in 1 mg of RGAP as tested by Limulus amebocyte lysate assay (Sigma, USA). This level of endotoxin did not affect the experimental result obtained by RGAP. The molar composition was identified as 51.8% glucuronic acid, 26.1% glucose, 5.1% galacturonic acid and 1.6% arabinose by GLC analysis. The molecular weight is estimated to be a mixture of 12 KDa and 450 KDa by Sephacryl S-300 gel filtration chromatography (Park *et al.*, 2001).

Animal Husbandry and Maintenance

Twenty-four Sprague-Dawley rats of each sex were obtained from the Japan SLC, Inc. (3371-8 Kotoh-cho, Hamamatsu, Shizuoka, Japan) at 4 weeks of age and used after one week of quarantine and acclimatization. The animals were housed in a room maintained at a temperature of 23±3°C and a relative humidity of 50± 10% with artificial lighting from 08 : 00 to 20 : 00 and with 13~18 air changes per hour. Only healthy animals were assigned to the study. The animals were kept in stainless wire cages and were allowed sterilized tap water and commercial rodent chow (PMI Nutritional International, 505 North 4th Street Richmond, IN, USA) *ad libitum*. This experiment was conducted in facilities approved by AAALAC International, and animals were maintained in accordance with the *Guide for the Care and Use of Laboratory Animals* (NRC, 1996).

Experimental Groups and Test Article Treatment

Single dose toxicity study: RGAP (purity ≥92%) suspended in the distilled water was daily prepared immediately before the treatment. The oral administration was selected in the present study, because the oral route is a clinically intended route. RGAP was administered orally by gavage to rats at dose levels of 0 and 2000 mg/10 ml/kg. The vehicle control rats received distilled water alone. Each group consisted of 5 rats of each sex.

2-week repeated dose toxicity study: RGAP suspended in the distilled water was administered once daily by gavage to rats for 2 weeks at dose levels of 0, 250, 500 and 1000 mg/10 ml/kg. The daily application volume was calculated according to the most recent body weight. Each group consisted of 5 rats of each sex.

Selection of Doses

Single dose toxicity study: A preliminary study with 3 male and 3 female rats was conducted with the dose levels of 1000 and 2000 mg/kg. No toxic effects were observed at these doses. Therefore, a dose of 2000 mg/kg was selected as a limit test dose and vehicle control group received distilled water as suspending agent.

2-week repeated dose toxicity study: In the single dose toxicity study, treatment-related toxicological effect was not observed. Therefore, a dose of 1000 mg/kg was selected for the highest dose in this study. Doses of 500 and 250 mg/kg were selected as middle and low doses, respectively, using a common ratio of 2.

Experimentals

Single dose toxicity study:

(1) Clinical observation and mortality: Through the study, all animals were daily observed for clinical signs of toxicity, moribundity, and mortality.

(2) Body weights: Body weight of each rat was measured shortly before administration and on days 1, 3, 7 and 14 after administration.

(3) Gross findings: On day 14 after the treatment, all surviving animals were euthanized by carbon dioxide overdose and the necropsy was performed with special attention to all vital organs and tissues.

2-week repeated oral dose toxicity study:

(1) Clinical observation and mortality: Through the study, all animals were daily observed for clinical signs of toxicity, moribundity, and mortality. Detailed clinical

observations were recorded and printed by Labcat Computer System (Innovative Programming Associates Inc., NJ, USA), respectively.

(2) Body weights: Body weight of each rat was measured at the initiation of treatment, once a week thereafter, and on the day of scheduled autopsy.

(3) Food consumption: Food consumption were measured per cage at the start of treatment and at weekly intervals thereafter. The amounts of food were calculated before they were supplied to each cage and their remnants were measured next day to calculate the difference which was regarded as a daily food consumption (g/rat/day).

(4) Ophthalmoscopy: External eye examination of all males and females was carried out shortly before the start of treatment and the termination of treatment. The ocular fundus was examined shortly before the termination of treatment using a indirect binocular ophthalmoscope (IO-H, Neitz Instruments Co., Japan) in the vehicle control and highest dose groups. Conjunctiva, sclera, cornea, lens and iris of each eye were also examined.

(5) Urinalysis: During the last week of treatment, urinalysis of all animals was conducted with fresh urine to determine specific gravity, pH, protein, glucose, ketone body, occult blood, bilirubin, urobilinogen, and nitrite by using a CliniTek-100 urine chemistry analyzer (Ames Division, Miles Laboratory, USA). The urine was collected for 17 hours and during the collection, the rats were housed in metabolism cages which allowed for separate collection of urine and feces.

(6) Hematology: Blood samples were drawn from the posterior vena cava by using a syringe with a 24 gauge needle under ether anesthesia. The animals were being fasted overnight prior to necropsy and blood sampling. The blood samples were collected into CBC bottles containing EDTA-2K (Green Cross Medical Industry, Korea), and were analysed within 20 minutes in our laboratory. Red blood cell count (RBC), hemoglobin concentration, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, and white blood cell count (WBC) were determined using a Coulter counter T-540 (Coulter Counter Electronics, USA).

(7) Serum biochemistry: To get the sera for serum biochemistry, blood samples were centrifuged at 3,000 rpm for 10 minutes within 1 hour after collection. The sera were stored in the -80°C freezer before they were analyzed. Serum biochemistry parameters including aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine phosphokinase (CPK), glucose, total protein (TP), albumin, albumin/globulin ratio (A/G ratio), blood urea nitrogen (BUN), creatinine, triglyceride, phospholipid, total cholesterol, total bilirubin, calcium, and inorganic phosphorus were evaluated by an autoanalyzer (Shimadzu CL-7200, Shimadazu Co., Japan). Serum electrolytes such as chloride, sodium, and potassium were measured by an ion autoanalyzer (644 Na/K/Cl Analyzer, Ciba-Corning Co., USA).

(8) Gross findings: At scheduled termination, all surviving animals were anesthetized by ether inhalation for blood sample collection, taken blood samples, and then sacrificed by exsanguination from the aorta. Complete gross postmortem examinations were performed on all terminated animals.

(9) Organ weights: The absolute and relative (organ-to-body weight ratios) weights of following organs were measured in all survivors when they were sacrificed: brain, pituitary gland, adrenal glands, liver, spleen, kidneys, heart, thymus, lung, salivary glands, thyroid glands, testes, epdidymis, seminal vesicles, prostates, ovaries, and uterus.

Statistical Analysis

Statistical analysis was performed by comparing the treatment groups with the vehicle control group using either Labcat Computer System or Statistical Analysis Systems (SAS/STAT User's Guide Version 6.12, NC, USA). Whenever the data were presented as mean±SD, variance of numerical data was checked by Bartlett's test. If the variance was homogeneous, the data was subjected to one-way analysis of variance (ANOVA) and, if not, they were analyzed by the Kruskal-Wallis nonparametric ANOVA. If either of these tests showed a difference between the groups, the data were analyzed by the multiple comparison procedure of the Dunnett's or Scheffe's post-hoc test. Results of urinalysis obtained with reagent strips were analyzed by the Kruskal-Wallis test followed by multiple comparisons using the Scheffe's test. Clinical observations, necropsy findings, and histopathological findings were represented in frequency and were subjected to the Fisher's exact probability test. The level of significance was taken as $p < 0.05$ or $p < 0.01$.

RESULTS

Single Dose Toxicity Study

(1) Mortality and LD_{50} values: No treatment-related death occurred in both sexes during the testing period (Table 1). Consequently, the LD_{50} value of the test item was estimated to be higher than 2000 mg/kg for both sexes.

Table 1. Mortality and clinical signs of rats after single oral administration of RGAP

Dose (mg/kg/day)	Mortality		Clinical signs	
	Male	Female	Male	Female
0	0/5[a]	0/5	-[b]	-
2000	0/5	0/5	-	-

[a]Values are expressed as number of dead animals/total number of animals.
[b]No clinical signs were observed.

Table 2. Changes of body weights in rats after single oral administration of RGAP

Sex	Days after treatment	Dose (mg/kg/day)	
		0	2000
Male	0	103.0 ± 4.97[a]	104.2 ± 6.49
	1	125.3 ± 6.17	126.3 ± 4.95
	3	145.6 ± 6.93	147.7 ± 5.89
	7	182.7 ± 8.93	185.6 ± 6.16
	14	245.5 ± 11.9	257.6 ± 17.6
	Gain	142.5 ± 10.3	153.3 ± 16.6
Female	0	96.7 ± 3.98	96.7 ± 2.48
	1	117.1 ± 5.30	118.0 ± 4.49
	3	133.9 ± 9.28	133.6 ± 6.48
	7	154.2 ± 14.1	152.5 ± 10.1
	14	187.9 ± 19.8	188.8 ± 14.3
	Gain	91.2 ± 16.4	92.1 ± 14.6

[a]Values are presented as mean ± S.D.

Table 3. Gross findings of necropsy in rats after single oral administration of RGAP

Dose (mg/kg/day)	Male	Female
0	0/5[a]	0/5
2000	0/5	0/5

[a]Values are expressed as number of abnormal animals/total number of animals.

Table 4. Mean body weight gains of male and female rats after 2-week repeated oral adminstion of RGAP

	Dose (mg/kg/day)			
	0	250	500	1000
Male				
Day 2	10.7 ± 0.67[a]	11.5 ± 0.99	8.20 ± 2.03	5.80 ± 2.98**
Day 9	57.6 ± 4.21	54.2 ± 6.34	51.4 ± 5.64	53.3 ± 5.51
Total gain	68.4 ± 4.69	65.7 ± 5.77	59.6 ± 5.55	59.0 ± 6.07*
Female				
Day 2	5.00 ± 1.79	7.70 ± 4.87	8.10 ± 2.69	4.90 ± 2.73
Day 9	30.4 ± 3.11	34.7 ± 3.77	28.3 ± 3.08	30.4 ± 5.17
Total gain	35.4 ± 3.60	42.5 ± 4.66	36.4 ± 4.97	35.3 ± 7.00

[a]Values are presented as means ± SD (g).
* and ** indicate significant difference at $p < 0.05$ and $p < 0.01$ levels, respectively, when compared with the control group.

Table 5. Urinalysis findings in male and female rats after 2-week repeated oral administration of RGAP

		Male (mg/kg/day)				Female (mg/kg/day)			
		0	250	500	1000	0	250	500	1000
Glucose	-	5	5	5	5	5	5	4	5
	+/-	0	0	0	0	0	0	1	0
Bilirubin	-	5	5	5	5	4	3	4	5
	1+	0	0	0	0	1	0	1	0
Ketone	-	5	5	4	5	4	4	4	5
	+/-	0	0	1	5	1	1	1	0
Specific gravity	≤1.005	0	0	0	0	0	1	0	1
	1.010	2	1	0	1	0	2	3	2
	1.015	0	1	2	2	3	1	0	1
	1.020	3	3	3	2	2	0	1	1
	1.025	0	0	0	0	0	1	0	0
	≤1.030	0	0	0	0	0	0	1	0
pH	6.5	0	0	0	0	0	0	1	0
	7.0	0	0	0	0	0	0	1	0
	7.5	0	0	0	0	0	0	1	0
	8.0	0	0	1	0	1	1	0	1
	8.5	5	5	4	5	3	3	1	3
	≤9.0	0	0	0	0	1	1	1	1
Protein	-	4	5	1	4	5	1	3	5
	+/-	1	0	2	1	0	4	1	0
	1+	0	0	2	0	0	0	1	0
Urobilinogen	0.1	5	5	5	5	5	5	5	5
Nitrite	-	5	5	5	4	5	3	5	5
	+	0	0	0	1	0	2	0	0
Occult blood	-	5	5	5	5	4	5	5	5
	+	0	0	0	0	1	0	0	0
Color	Yellow	5	5	5	5	5	5	5	5

(2) Clinical signs: No treatment-related clinical signs were observed in all dose groups throughout the study period (Table 1).

(3) Body weight changes: Normal body weight gains were observed in all dose groups during the study period (Table 2).

(4) Gross findings: No treatment-related effects were found in all dose groups when the animals were macroscopically examined in the necropsy (Table 3).

2-Week Repeated Dose Toxicity Study

(1) Clinical signs and mortality: There were no

Table 6. Hematological findings in male and female rats after 2-week repeated oral administration of RGAP

	Dose (mg/kg/day)			
	0	250	500	1000
Male				
Leukocytes ($\times10^9$/l)	7.21 ± 1.43[a]	8.62 ± 1.57	7.68 ± 1.81	7.79 ± 1.09
Erythrocytes ($\times10^{12}$/l)	6.69 ± 0.22	6.78 ± 0.23	6.91 ± 0.43	6.64 ± 0.26
Hemoglobin (g/dl)	14.5 ± 0.18	14.8 ± 0.40	14.8 ± 0.57	14.6 ± 0.39
Hematocrit (%)	41.7 ± 0.81	42.0 ± 1.05	42.5 ± 2.02	41.5 ± 1.33
MCV (fl)	62.3 ± 1.07	62.1 ± 1.21	61.6 ± 1.37	62.5 ± 0.60
MCH (pg)	21.7 ± 0.46	21.8 ± 0.54	21.4 ± 0.55	22.0 ± 0.39
MCHC (g/dl)	34.8 ± 0.26	35.2 ± 0.28	34.8 ± 0.43	35.3 ± 0.36
Platelets ($\times10^9$/l)	1216 ± 100	1178 ± 83.5	1174 ± 114	1111 ± 84.5
Female				
Leukocytes ($\times10^9$/l)	6.42 ± 1.58	6.92 ± 1.71	5.93 ± 0.78	5.70 ± 1.03
Erythrocytes ($\times10^{12}$/l)	6.99 ± 0.34	6.95 ± 0.28	7.15 ± 0.29	7.22 ± 0.34
Hemoglobin (g/dl)	15.4 ± 0.50	15.1 ± 0.43	15.4 ± 0.34	15.2 ± 0.55
Hematocrit (%)	43.5 ± 1.22	42.8 ± 1.63	43.6 ± 1.00	43.8 ± 1.65
MCV (fl)	62.2 ± 1.72	61.6 ± 1.74	61.1 ± 1.72	60.7 ± 1.52
MCH (pg)	22.1 ± 0.58	21.8 ± 0.53	21.5 ± 0.87	21.0 ± 0.72
MCHC (g/dl)	35.4 ± 0.31	35.4 ± 0.46	35.2 ± 0.59	34.7 ± 0.36
Platelets ($\times10^9$/l)	1180 ± 132	1267 ± 121	1272 ± 108	1168 ± 151

[a]Values are presented as means ± SD. MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

Table 7. Serum biochemical findings in female rats after 2-week repeated oral administration of RGAP

	Dose (mg/kg/day)				normal range[b]
	0	250	500	1000	
Aspartate aminotransferase (IU/l)	81.1 ± 4.83[a]	87.6 ± 7.19	94.9 ± 15.4	92.9 ± 12.7	-
Alanine aminotransferase (IU/l)	36.5 ± 3.50	36.9 ± 3.33	38.1 ± 3.67	42.3 ± 1.68*	44 ± 23.9
Alkaline phosphatase (IU/l)	845 ± 107	868 ± 97.7	924 ± 81.1	794 ± 74.6	-
Blood urea nitrogen (mg/dl)	18.4 ± 1.41	15.5 ± 1.40	16.9 ± 1.49	16.2 ± 3.21	-
Creatinine (mg/dl)	0.35 ± 0.05	0.34 ± 0.05	0.42 ± 0.02	0.38 ± 0.04	-
Glucose (mg/dl)	135 ± 11.2	132 ± 13.7	128 ± 7.67	133 ± 13.4	-
Total cholesterol (mg/dl)	106 ± 14.1	91.9 ± 21.2	88.4 ± 10.4	89.4 ± 9.95	-
Total bilirubin (mg/dl)	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	-
Total protein (g/dl)	5.91 ± 0.09	5.66 ± 0.22	5.84 ± 0.10	5.91 ± 0.23	-
Albumin (g/dl)	4.13 ± 0.04	4.04 ± 0.15	4.15 ± 0.12	4.15 ± 0.15	-
Creatine phosphokinase (IU/l)	372 ± 77.8	445 ± 145	650 ± 355	456 ± 160	-
Triglyceride (mg/dl)	31.6 ± 16.3	25.0 ± 6.44	32.5 ± 14.1	26.2 ± 11.4	-
Calcium (mg/dl)	9.68 ± 0.44	9.25 ± 0.22	9.67 ± 0.67	9.31 ± 0.54	-
Inorganic phosphate (mg/dl)	8.50 ± 0.44	9.01 ± 0.38	9.00 ± 0.58	8.75 ± 0.69	-
Phospholipid (mg/l)	150 ± 27.1	131 ± 24.1	121 ± 11.2	123 ± 11.6	-
Albumin/Globulin (ratio)	2.32 ± 0.06	2.50 ± 0.16	2.46 ± 0.18	2.36 ± 0.08	-
Sodium (nmol/l)	141 ± 0.80	140 ± 0.90	141 ± 1.10	141 ± 0.50	-
Potassium (nmol/l)	4.40 ± 0.41	4.43 ± 0.30	4.64 ± 0.26	4.46 ± 0.22	-
Chloride (nmol/l)	104 ± 0.80	103 ± 1.50	104 ± 1.10	104 ± 0.40	-

[a]Values are presented as means ± SD.
*Indicates significant difference at $p < 0.05$ level when compared with the control group.
[b]Worford *et al.* (1986).

treatment-related clinical signs and mortality in animals treated with RGAP at 250, 500 and 1000 mg/kg for 2-weeks (data not shown).

(2) Body weight changes: Body weight gains of male rats were significantly decreased in 1000 mg/kg groups (Table 4). Body weight gains of female rats were not affected by the test article.

(3) Food consumption: Food consumption of both sexes did not differ between the groups during the course of the study (data not shown).

(4) Ophthalmoscopy: Ophthalmologic examinations did not reveal ocular lesions in any of the animals (data not shown).

(5) Urinalysis: No significant difference between treatment groups and vehicle control group was seen for any urinary parameters (Table 5).

(6) Hematology: No significant difference between treatment groups and vehicle control group was seen for hematology parameters (Table 6).

(7) Serum biochemistry: The alanine aminotransferase (ALT) level of females at 1000 mg/kg group was significantly increased when compared with the vehicle control group (Table 7). In males, significant differences were not observed in any of serum biochemical values between the groups (data not shown).

(9) Gross findings: At necropsy, one female rat at 500 mg/kg group showed the hernia of the liver, but there were no treatment-related gross pathological findings in other males and females of treatment groups (data not shown).

(10) Organ weights: The relative and absolute weight of left thyroid in male 500 mg/kg group (Table 8) and the relative and absolute weight of uterus in female 500 mg/kg group were significantly increased compared to the vehicle control group (Table 9).

DISCUSSION

In the present study we reported the results of the single and 2-week repeated oral administration of

Table 8. Organ weights in male rats after 2-week repeated oral administration of RGAP

Organ	Dose (mg/kg/day)			
	0	250	500	1000
Body weight	222 ± 4.79[a]	215 ± 11.7	212 ± 7.06	211 ± 11.8
Brain (g)	1.87 ± 0.036	1.87 ± 0.056	1.87 ± 0.054	1.85 ± 0.071
per body weight (%)	0.840 ± 0.028	0.869 ± 0.050	0.881 ± 0.031	0.881 ± 0.076
Hypophysis (g)	0.007 ± 0.001	0.007 ± 0.001	0.007 ± 0.001	0.006 ± 0.001
per body weight (%)	0.003 ± 0.0005	0.003 ± 0.0004	0.003 ± 0.0005	0.003 ± 0.0004
Adrenal glands (g)	0.047 ± 0.004	0.048 ± 0.006	0.044 ± 0.006	0.040 ± 0.005
per body weight (%)	0.021 ± 0.002	0.022 ± 0.002	0.021 ± 0.003	0.019 ± 0.003
Liver (g)	7.63 ± 0.48	7.27 ± 0.75	6.94 ± 0.41	7.32 ± 0.39
per body weight (%)	3.43 ± 0.219	3.38 ± 0.175	3.27 ± 0.150	3.48 ± 0.103
Spleen (g)	0.56 ± 0.10	0.58 ± 0.10	0.53 ± 0.05	0.48 ± 0.02
per body weight (%)	0.252 ± 0.045	0.268 ± 0.034	0.252 ± 0.021	0.229 ± 0.008
Kidneys (g)	1.79 ± 0.08	1.70 ± 0.19	1.72 ± 0.16	1.66 ± 0.09
per body weight (%)	0.804 ± 0.037	0.788 ± 0.043	0.811 ± 0.079	0.788 ± 0.059
Heart (g)	0.85 ± 0.04	0.86 ± 0.05	0.84 ± 0.06	0.82 ± 0.07
per body weight (%)	0.380 ± 0.017	0.399 ± 0.017	0.393 ± 0.032	0.390 ± 0.019
Testes (g)	2.59 ± 0.18	2.44 ± 0.17	2.50 ± 0.12	2.44 ± 0.14
per body weight (%)	1.16 ± 0.092	1.13 ± 0.042	1.18 ± 0.072	1.16 ± 0.111
Prostates (g)	0.122 ± 0.032	0.098 ± 0.024	0.128 ± 0.037	0.121 ± 0.020
per body weight (%)	0.055 ± 0.014	0.046 ± 0.010	0.060 ± 0.017	0.058 ± 0.012
Lung (g)	1.06 ± 0.053	1.13 ± 0.092	1.13 ± 0.086	1.12 ± 0.103
per body weight (%)	0.522 ± 0.017	0.526 ± 0.016	0.530 ± 0.033	0.533 ± 0.037
Thymus (g)	0.591 ± 0.084	0.613 ± 0.060	0.542 ± 0.079	0.555 ± 0.095
per body weight (%)	0.266 ± 0.038	0.285 ± 0.022	0.256 ± 0.036	0.263 ± 0.036
Thyroid glands-Left (g)	0.005 ± 0.0004	0.005 ± 0.0011	0.007 ± 0.0008*	0.004 ± 0.0011
per body weight (%)	0.002 ± 0.0004	0.002 ± 0.0007	0.003 ± 0.0004*	0.002 ± 0.0004
Thyroid glands-right (g)	0.005 ± 0.0014	0.005 ± 0.0018	0.006 ± 0.0016	0.005 ± 0.0017
per body weight (%)	0.002 ± 0.0009	0.002 ± 0.0009	0.003 ± 0.0008	0.002 ± 0.0005
Epididymides (g)	0.358 ± 0.042	0.297 ± 0.043	0.345 ± 0.026	0.308 ± 0.048
per body weight (%)	0.161 ± 0.018	0.138 ± 0.019	0.163 ± 0.015	0.147 ± 0.026

[a]Values are presented as means ± SD.
*Indicates significant difference at $p < 0.05$ level when compared with the control group.

Table 9. Organ weights in female rats after 2-week repeated oral administration of RGAP

Organ	Dose (mg/kg/day)			
	0	250	500	1000
Body weight	153.8 ± 6.92[a]	161.8 ± 6.25	156.9 ± 9.07	154.6 ± 7.05
Brain (g)	1.735 ± 0.072	1.781 ± 0.054	1.713 ± 0.036	1.713 ± 0.080
per body weight (%)	1.130 ± 0.074	1.103 ± 0.047	1.095 ± 0.075	1.111 ± 0.093
Hypophysis (g)	0.008 ± 0.002	0.008 ± 0.001	0.008 ± 0.002	0.007 ± 0.001
per body weight (%)	0.005 ± 0.001	0.005 ± 0.001	0.006 ± 0.001	0.004 ± 0.001
Adrenal glands (g)	0.051 ± 0.007	0.050 ± 0.004	0.051 ± 0.005	0.048 ± 0.005
per body weight (%)	0.017 ± 0.002	0.015 ± 0.001	0.017 ± 0.001	0.016 ± 0.001
Liver (g)	4.962 ± 0.152	5.342 ± 0.307	5.187 ± 0.215	5.037 ± 0.448
per body weight (%)	3.229 ± 0.084	3.301 ± 0.135	3.309 ± 0.121	3.253 ± 0.146
Spleen (g)	0.366 ± 0.051	0.378 ± 0.043	0.375 ± 0.029	0.320 ± 0.043
per body weight (%)	0.237 ± 0.029	0.233 ± 0.018	0.239 ± 0.010	0.207 ± 0.021
Kidneys (g)	1.267 ± 0.073	1.385 ± 0.078	1.299 ± 0.090	1.287 ± 0.096
per body weight (%)	0.824 ± 0.028	0.856 ± 0.021	0.829 ± 0.053	0.832 ± 0.035
Heart (g)	0.611 ± 0.034	0.658 ± 0.046	0.638 ± 0.041	0.649 ± 0.051
per body weight (%)	0.398 ± 0.020	0.406 ± 0.022	0.407 ± 0.017	0.420 ± 0.014
Ovary (g)	0.059 ± 0.011	0.060 ± 0.008	0.059 ± 0.004	0.062 ± 0.008
per body weight (%)	0.038 ± 0.006	0.037 ± 0.005	0.038 ± 0.003	0.040 ± 0.005
Uterus (g)	0.224 ± 0.058	0.239 ± 0.021	0.390 ± 0.120**	0.248 ± 0.058
per body weight (%)	0.145 ± 0.032	0.148 ± 0.018	0.247 ± 0.069**	0.161 ± 0.038
Lung (g)	0.917 ± 0.070	0.925 ± 0.051	0.910 ± 0.043	0.914 ± 0.053
per body weight (%)	0.597 ± 0.049	0.572 ± 0.030	0.580 ± 0.017	0.591 ± 0.017
Thymus (g)	0.464 ± 0.078	0.554 ± 0.060	0.524 ± 0.057	0.520 ± 0.126
per body weight (%)	0.303 ± 0.055	0.343 ± 0.039	0.334 ± 0.036	0.334 ± 0.069
Thyroid glands (g)	0.008 ± 0.0023	0.009 ± 0.0017	0.010 ± 0.0023	0.010 ± 0.0019
per body weight (%)	0.005 ± 0.002	0.006 ± 0.001	0.006 ± 0.002	0.007 ± 0.001

[a]Values are presented as means ± SD.
**Indicates significant difference at $p < 0.01$ level when compared with the control group.

RGAP to Sprague-Dawley rats performed as a part of the preclinical safety evaluation program for RGAP. For a single dose toxicity study, RGAP was administered by gavage to Sprague-Dawley rats at dose levels of 0 and 2000 mg/kg/day and evaluations during the 14-day observation period including mortality, clinical signs, body weight changes, and gross findings were performed. For 2-week repeated dose toxicity study, RGAP was administered by gavage to rats at dose levels of 0, 250, 500 and 1000 mg/kg/day for 2 weeks.

In the single dose toxicity study, no treatment-related effects on mortality, clinical signs, body weight changes and gross findings were observed. In the 2-week repeated dose toxicity study, the significant reduction of body weight gains of male 1000 mg/kg groups was considered as temporary, because they did not exhibit a dose-response relationship. It occurred in one sex only, and was seen only at the end of the treatment period. A statistically significant increase of ALP in females of 1000 mg/kg group was not dose-related and were within the limits of normal biological variation (Wolford *et al.*, 1986; Kang *et al.*, 1995). The liver hernia of one female rat in 500 mg/kg group did not show a dose-response relationship, therefore it was judged to be an accidental finding. The statistically increased relative and absolute weight of left thyroid in male 500 mg/kg group and the relative and absolute weight of uterus in female 500 mg/kg group were negligible and not associated with the gross findings, so these changes in the above organs are of doubtful toxicological significance.

Based on these results, it was concluded that the single oral dose of RGAP produced no toxic effects in SD rats at the dose level of 2000 mg/kg/day, and that the LD_{50} value was estimated to be over 2000 mg/kg for both sexes. Also, 2-week repeated oral dose of RGAP did not cause any toxic effect to SD rats at the dose level of 1000 mg/kg/day. In the condition of this study, the NOAEL of RGAP is considered to be 1000 mg/kg/day for both sexes.

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