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Original Article

Four-week repeated dose oral toxicity study of gum ghatti in rats

Ryo Kawahara[1], Kenichiro Watanabe[1], Rina Yamane[1], Hideki Yasui[1], Nao Kikugawa[1], Naoko Mori[1], Ryosuke Akiyama[2], Takeshi Matsubara[2], Miwa Harada[1] and Shinya Kaneda[1]

[1]Naruto Research Institute, Otsuka Pharmaceutical Factory, Inc., 115 Kuguhara, Tateiwa, Muya-cho, Naruto Tokushima 772-8601, Japan
[2]Product Design Laboratory Medical Foods Research Institute OS-1 Division, Otsuka Pharmaceutical Factory, Inc. 115 Kuguhara, Tateiwa, Muya-cho, Naruto Tokushima 772-8601, Japan

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ABSTRACT — We investigated the potential toxicity of gum ghatti, which is added to food for emulsifying, thickening, and stabilizing, after 4 weeks of repeated oral administration at a dose of 8000 mg/kg/day to male and female SD rats. Although food consumption was significantly reduced in males in the gum ghatti group compared with those in the distilled water group from Day 18 onwards, the change was minor, there was no pathological evidence of digestive tract abnormalities, and there were no significant changes in body weight; therefore, the change in food consumption was judged to be of no toxicological significance. Hematology and blood biochemistry revealed statistically significant differences in some parameters between the gum ghatti group and the distilled water group. These changes were all within the normal range of physiological variation and therefore were not considered to represent the effects of gum ghatti. In addition, general signs, body weight, and pathology showed no changes in either sex attributable to gum ghatti. Thus, all changes observed were of no toxicological significance and within the normal range of physiological variation, suggesting gum ghatti has no toxic effects in rats.

Key words: Gum ghatti, Food additive, Toxicity, Rat

INTRODUCTION

Gum ghatti is resin from *Anogeissus latifolia* Wallich, a plant widely distributed throughout India and Sri Lanka. It is a water-soluble polysaccharide consisting of D-glucuronic acid, L-arabinose, D-galactose, D-mannose, and D-xylose, with the main chain composed of D-galactose residues. It also contains 2% to 4% protein.

The status of the country usage of gum ghatti is shown in Table 1. The 84th Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2017 concluded that gum ghatti is unlikely to be a health concern and established an "acceptable daily intake (ADI) not specified" for gum ghatti that complies with the JECFA specifications. In response, the 2019 General Standard for Food Additives (GSFA) included gum ghatti in the "Table 1 Food category: Dietetic foods intended for special medical purpose: Permitted for use in general foods including products without restriction as necessary" for wide-ranging use as an emulsifier, thickener, stabilizer, and carrier.

Gum ghatti is a Generally Recognized As Safe (GRAS) substance approved for use as an emulsifier in the United States, approved as a flavoring in China, and is listed in Japan's specifications and standards for food additives as an existing additive with approval as a thickening stabilizer.

Correspondence: Ryo Kawahara (E-mail: Kawahara.Ryo@otsuka.jp)

Table 1. Status of country usage of gum ghatti.

Country, international organization (References)	Scope and level of use
CAC (Codex Alimentarius Commission, 2019a)	Emulsifier, stabilizer, thickener, carrier
JECFA (World Health Organization & Joint FAO/WHO Expert Committee on Food Additives, 2017)	Thickener, stabilizer ADI (not specified) Concluded that gum ghatti is unlikely to be a health concern and established an "ADI (acceptable daily intake) not specified" that complies with the specifications.Estimated dietary exposure = 12 mg/kg/day
GFSA (Codex Alimentarius Commission, 2019b)	Emulsifier, thickener, stabilizer, carrier
US (FDA) (Electronic Code of Federal Regulations, 2018)	Emulsifier (GRAS) 0.2% in beverages and 0.1% in other foods
China (National Health and Family Planning Committee of the People's Republic of China, 2014)	Flavor Appropriate use according to production demand
Japan (The Japan Food Chemical Research Foundation, 2014)	Thickening stabilizer No restriction on the amount used

Thickening stabilizers are food additives that increase the viscosity and adhesiveness of foods and beverages. Specifically, gum ghatti is classified as a "thickener," which renders food sticky or thick, a "stabilizer (binder)," which adheres food ingredients to prevent food from losing shape, and a "gelling agent," which gelatinizes food. In Japan, gum ghatti is used without restriction for many purposes, including improving texture and the feel of food passing down the throat, as well as providing flavor with emulsion stability.

Previous studies have performed the following safety tests on gum ghatti: bacterial reverse mutation test, chromosome aberration test, and *in vivo* combined micronucleus/comet assays, which all revealed no genotoxicity (Hobbs *et al*., 2012). Furthermore, there were no toxicological findings in male and female rats after the 90-day administration of gum ghatti mixed in with feed at a maximum of 5% gum ghatti, resulting in an estimated no observed adverse effect level (NOAEL) of 3000-4000 mg/kg/day (Maronpot *et al*., 2013). The NOAEL for gum arabic, which is also derived from resin and similar to gum ghatti in properties and structure, is approximately 3000 mg/kg/day based on the results of a 90-day study of dietary administration (Doi *et al*., 2006).

The present study evaluated the potential toxicity of high exposure to gum ghatti by administering 20% gum ghatti solution at 8000 mg/kg/day by oral gavage for 4 weeks, which corresponds to 2-3 times the NOAEL.

MATERIALS AND METHODS

Animal study and study design

This study was performed using 5-week-old Crl:CD (SD) rats (Charles River Laboratories Japan, Inc., Kanagawa, Japan) of both sexes, in compliance with the "Law for the Humane Treatment and Management of Animals" (Law No. 105, October 1, 1973, partially revised on June 2, 2017), "Standards Relating to the Care and Management of Laboratory Animals and Relief of Pain" (Japanese Ministry of the Environment, Notification No. 88, April 28, 2006, partially revised on August 30, 2013) and "Guideline for Animal Experiment in Facilities under the Jurisdiction of Japanese Ministry of Health, Labour and Welfare" (Notification Kahatsu 0220 No. 1, February 20, 2015), and after receiving approval from the Animal Experiment Committee of Otsuka Pharmaceutical Factory, Inc. Animals were individually housed in polycarbonate cages (206 × 365 × 197 mm; CLEA Japan, Inc., Tokyo, Japan) containing ALPHA-dri bedding (Shepherd Specialty Papers, Inc., Watertown, TN, USA) under controlled environmental conditions: temperature, 23 ± 3°C; humidity, 55 ± 15%; and 12-hr artificial lighting (7:00 to 19:00). Cages were changed once a week. Animals were supplied *ad libitum* with CRF-1 (Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water that had passed through a sterilization filter from a water supply bottle. Water was changed three times weekly. Animals of each sex were divided into two groups of six animals: the distilled water group and gum ghatti 8000 mg/kg/day group. The dosing volume was set at

20 mL/kg/dose after determining the maximum volume for oral administration in rats (Diehl *et al*., 2001) and the physical properties of the dosing solution. The dosing solution was administered orally twice daily (once in the morning and once in the afternoon) for 4 weeks using a stomach tube (Fuchigami Kikai Co. Ltd., Kyoto, Japan) and a Luer lock syringe (Terumo Corporation, Tokyo, Japan). During the treatment period, animals were observed for general signs before and after each dosing, four times daily. Animals were weighed once weekly, and food consumption was measured twice weekly, before the morning dosing. On the day of necropsy, blood for hematology and blood biochemistry was collected via the caudal vena cava from animals fasted for at least 16 hr, while under isoflurane anesthesia. After blood sampling, animals were euthanized by exsanguination and necropsied to macroscopically examine their external appearance, internal organs and tissues, and to sample the following organs: mesenteric lymph nodes, stomach, duodenum, jejunum, ileum, cecum, colon, and rectum. The sampled organs were fixed with 10% neutral buffer formalin solution. Thin paraffin sections were prepared, stained with hematoxylin and eosin, and subjected to histopathology using general methods.

Test agent

Gum ghatti was purchased from San-Ei Gen F.F.I., Inc. (Osaka, Japan). It was dissolved in pure water to prepare a 20% gum ghatti solution, which was used as the dosing solution. The 20% gum ghatti solution had a viscosity of approximately 300 mPa·s, eliminating concerns about dosing feasibility.

Statistical analysis

Body weight, food consumption, hematology, and blood biochemistry findings were analyzed using Statistical Analysis System versions 9.4 (SAS Institute Inc., Cary, NC, USA). The means and standard deviations were determined for the distilled water group and gum ghatti group, and analyzed for homoscedasticity using the F-test. When homoscedasticity was shown, the Student's *t*-test was used for subsequent analysis, and when heteroscedasticity was shown, the Aspin-Welch *t*-test was used. Differential WBC and reticulocyte ratios were analyzed using the Wilcoxon rank sum test. All analyses were performed with a significance level of 5%.

Table 2. Effect of 4-week oral administration of gum ghatti on body weight in rats.

Sex	Male		Female	
Test agent	Distilled water	Gum ghatti	Distilled water	Gum ghatti
Dose (mg/kg)	0	8000	0	8000
Day				
0	213.9 ± 7.7	214.0 ± 10.1	152.6 ± 6.6	151.2 ± 6.7
7	274.9 ± 15.2	271.6 ± 20.1	178.5 ± 7.0	175.1 ± 7.7
14	340.9 ± 25.6	328.9 ± 26.9	207.8 ± 14.2	203.4 ± 9.4
21	390.3 ± 27.8	375.6 ± 29.4	223.7 ± 16.9	222.9 ± 14.7
27	429.8 ± 31.9	411.2 ± 36.3	238.2 ± 22.1	240.6 ± 12.5

Values are the mean body weights (g) ± SD (n = 6). There was no significant difference in values between groups.

Table 3. Effect of 4-week oral administration of gum ghatti on food consumption in rats.

Sex	Male		Female	
Test agent	Distilled water	Gum ghatti	Distilled water	Gum ghatti
Dose (mg/kg)	0	8000	0	8000
Day				
-1	22.7 ± 1.9	23.1 ± 2.3	14.8 ± 1.9	16.0 ± 1.7
0-4	22.4 ± 2.1	22.2 ± 1.7	15.5 ± 0.8	14.5 ± 0.8
4-7	23.7 ± 1.9	23.0 ± 2.8	15.4 ± 1.3	15.1 ± 1.2
7-11	25.7 ± 2.2	23.7 ± 2.3	16.5 ± 1.9	16.4 ± 1.2
11-14	26.3 ± 2.9	24.2 ± 2.5	16.4 ± 1.5	15.9 ± 1.3
14-18	27.8 ± 3.1	24.8 ± 2.1	16.7 ± 2.0	16.5 ± 1.6
18-21	27.5 ± 2.0	24.7 ± 1.8*	16.6 ± 1.8	16.5 ± 2.2
21-25	28.1 ± 2.4	25.1 ± 2.0*	16.9 ± 2.2	16.8 ± 1.8
25-27	29.3 ± 2.6	25.2 ± 2.2*	17.2 ± 2.8	17.2 ± 1.6

Values are the mean food consumption (g) ± SD (n = 5-6). *$p < 0.05$ analyzed by Student's *t*-test.

Table 4. Effect of 4-week oral administration of gum ghatti on hematology in rats.

Sex	Male		Female	
Test agent	Distilled water	Gum ghatti	Distilled water	Gum ghatti
Dose (mg/kg)	0	8000	0	8000
WBC, 10^2/μL	135.3 ± 15.8	78.2 ± 17.8**	59.2 ± 19.3	68.0 ± 14.9
RBC, 10^4/μL	747 ± 31	738 ± 14	726 ± 22	721 ± 35
HGB, g/dL	14.9 ± 0.4	14.4 ± 0.2*	14.1 ± 0.4	14.3 ± 0.6
HCT, %	42.2 ± 1.0	40.5 ± 0.7*	39.6 ± 1.2	40.2 ± 1.4
MCV, fL	56.5 ± 1.9	54.9 ± 1.0	54.7 ± 2.2	55.9 ± 2.3
MCH, pg	19.9 ± 0.6	19.5 ± 0.4	19.4 ± 0.5	19.8 ± 0.5
MCHC, g/dL	35.2 ± 0.4	35.5 ± 0.2	35.5 ± 0.7	35.4 ± 0.9
PLT, 10^4/μL	118.9 ± 17.1	121.8 ± 8.9	104.8 ± 11.2	107.0 ± 13.9
NEUT%	12.4 ± 2.7	14.9 ± 2.0	11.8 ± 3.5	16.4 ± 4.9
LYMPH%	83.5 ± 3.6	80.9 ± 2.2	84.2 ± 5.0	79.6 ± 4.8
MONO%	3.1 ± 1.0	3.3 ± 0.5	2.4 ± 0.9	2.8 ± 0.6
EO%	1.1 ± 0.4	0.8 ± 0.1	1.5 ± 0.8	1.2 ± 0.3
BASO%	0.0 ± 0.1	0.0 ± 0.1	0.0 ± 0.0	0.1 ± 0.1
Ret.#, 10^4/μL	35.72 ± 4.40	32.53 ± 5.67	27.34 ± 5.54	29.76 ± 3.60
Ret.%	4.77 ± 0.41	4.40 ± 0.72	3.78 ± 0.82	4.15 ± 0.61
PT, sec	10.9 ± 0.8	12.3 ± 2.6	9.9 ± 0.2	10.1 ± 0.3
APTT, sec	17.5 ± 1.3	18.6 ± 3.4	14.9 ± 1.5	16.5 ± 0.7*

Values are the mean ± SD (n = 5-6). *$p < 0.05$, **$p < 0.01$ analyzed by Student's *t*-test.
WBC: white blood cells. RBC: red blood cells. HGB: hemoglobin. HCT: hematocrit. MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration. PLT: platelet. NEUT: neutrophil. LYMPH: lymphocyte. MONO: monocyte. EO: eosinophil. BASO: basophil. Ret: reticulocyte. PT: prothrombin time. APTT: activated partial thromboplastin time.

RESULTS

No animals had died by the time of necropsy or showed abnormalities at any time point during the treatment period. There was no significant difference in body weight between groups at any time point during the treatment period (Table 2). As shown in Table 3, food consumption was significantly reduced in males in the gum ghatti group compared with those in the distilled water group from Day 18 onwards. For females, there was no difference in food consumption between groups at any time point. As shown in Table 4, hematology revealed significantly lower levels of WBC, Hgb, and Hct in males in the gum ghatti group compared with males in the distilled water group. In addition, APTT was significantly prolonged in females in the gum ghatti group compared with females in the distilled water group. Other hematology parameters showed no group differences for either sex. As shown in Table 5, blood biochemistry showed significantly higher levels of ALT and T-BIL and significantly lower levels of CRE in females in the gum ghatti group compared with females in the distilled water group. Other blood biochemistry parameters showed no group differences for either sex. Necropsy and histopathology demonstrated no abnormalities in either group of either sex.

DISCUSSION

Gum ghatti is used as a food additive for emulsifying, thickening, and stabilizing. The use of gum ghatti is restricted in many countries: in China, only a small amount is permitted to be used as a flavoring, and in the United States it can only be used as an emulsifier in liquids at a concentration of up to 0.2%. Concerning safety, a previous study showed that the NOAEL was 3000-4000 mg/kg/day based on the results of a 90-day administration of gum ghatti mixed in with feed to rats (Maronpot *et al*., 2013). The present study investigated the potential toxicity in rats receiving gum ghatti by oral gavage at 8000 mg/kg/day, which corresponds to 2-3 times the NOAEL, for 28 days.

There were no changes in general signs, body weight, or pathological findings, attributable to gum ghatti in either sex. From Day 18 of treatment onwards, food consumption was significantly reduced in males in the gum ghatti group compared with the distilled water group. However, this change was judged to be of no toxicological significance because changes in food consumption

Table 5. Effect of 4-week oral administration of gum ghatti on blood biochemistry in rats.

Sex	Male		Female	
Test agent	Distilled water	Gum ghatti	Distilled water	Gum ghatti
Dose (mg/kg)	0	8000	0	8000
AST, U/L	88 ± 15	90 ± 8	95 ± 20	85 ± 10
ALT, U/L	35 ± 9	34 ± 6	23 ± 6	33 ± 7*
ALP, U/L	519 ± 123	616 ± 108	343 ± 81	347 ± 22
T-P, g/dL	5.56 ± 0.28	5.53 ± 0.14	5.59 ± 0.26	5.70 ± 0.33
ALB, g/dL	3.02 ± 0.11	3.06 ± 0.12	3.23 ± 0.18	3.39 ± 0.26
A/G	1.20 ± 0.07	1.25 ± 0.12	1.36 ± 0.06	1.47 ± 0.16
GLU, mg/dL	132 ± 17	126 ± 7	126 ± 23	118 ± 15
TG, mg/dL	55 ± 25	44 ± 15	19 ± 7	30 ± 18
T-CHO, mg/dL	68 ± 14	67 ± 17	67 ± 12	68 ± 22
PL, mg/dL	105 ± 19	104 ± 20	115 ± 13	123 ± 39
T-BIL, mg/dL	0.07 ± 0.01	0.06 ± 0.02	0.07 ± 0.01	0.09 ± 0.02*
UN, mg/dL	20.8 ± 4.5	18.8 ± 6.9	23.1 ± 5.0	18.0 ± 4.0
CRE, mg/dL	0.29 ± 0.03	0.32 ± 0.03	0.36 ± 0.06	0.30 ± 0.03*
Ca, mg/dL	10.0 ± 0.4	9.8 ± 0.1	9.8 ± 0.4	10.2 ± 0.5
P, mg/dL	8.6 ± 0.4	8.7 ± 0.9	7.8 ± 1.0	8.0 ± 1.1
Na, mEq/L	141.9 ± 1.0	142.3 ± 0.8	141.3 ± 0.6	141.6 ± 1.8
K, mEq/L	4.75 ± 0.11	4.63 ± 0.21	4.26 ± 0.28	4.21 ± 0.34
Cl, mEq/L	100.7 ± 1.0	100.6 ± 1.6	102.0 ± 0.6	100.3 ± 2.6

Values are the mean ± SD (n = 6). *$p < 0.05$ analyzed by Student's *t*-test.
AST: aspartate aminotransferase. ALT: alanine aminotransferase. ALP: alkaline phosphatase. T-P: total protein. ALB: albumin. A/G: albumin/globulin, GLU: glucose. TG: triglyceride. T-CHO: total cholesterol. PL: phospholipid. T-BIL: total bilirubin. UN: urea nitrogen. CRE: creatinine. Ca: calcium. P: Phosphorus. Na: sodium. K: potassium. Cl: chloride.

were minor, there were no significant changes in body weight, and no pathological evidence for digestive tract abnormalities was observed. Hematology and blood biochemistry revealed statistically significant differences in some parameters between the gum ghatti group and the distilled water group. However, these changes were all within the normal range of physiological variation and were therefore not considered to represent the effects of gum ghatti.

This study reports that the changes observed in male and female rats receiving 4-week repeated oral administration of gum ghatti at a dose of 8000 mg/kg/day were of no toxicological significance and within the normal range of physiological variation, suggesting gum ghatti at this dose is not toxic.

The present study demonstrated that gum ghatti is safe at doses higher than those currently used, providing a rationale for the revision of the current maximum permitted level of use in each country.

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

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