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Evaluation of 90-day oral rat toxicity studies on the food additive, gum ghatti

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ABSTRACT

Gum ghatti, a polysaccharide of natural origin, is used in foods as a thickening, gelling, emulsifying and stabilizing agent. In a 90-day toxicity study following Organization for Economic Co-operation and Development (OECD) Guideline #408, male and female Sprague–Dawley rats were exposed to 0 (control), 0.5, 1.5 and 5% gum ghatti in AIN-93M basal diet. Expected changes included increased full and empty cecal weights in 5% groups. Incidentally 2/10 females from the 5% gum ghatti group had a single colon ulcer with associated acute inflammation. In a second 90-day study increased cecal weights were present in Sprague–Dawley females exposed to 5% gum ghatti in AIN-93M and NIH-07 basal diets. A single colon ulcer with associated acute inflammation occurred in 1/20 control females given AIN-93M basal diet. The colon ulcers were considered a sporadic change possibly attributable to AIN-93M basal diet. In the second study a few statistically significant alterations in clinical chemistry were considered sporadic and unrelated to treatment. Feed consumption among treated and control groups was similar for each sex. Gum ghatti intake at the 5% dietary level ranged from 3044 to 3825 mg/kg body weight/day. The 5% dietary administration was a NOAEL in both studies. NOAELs for males and females in the first study were 3044 and 3309 mg/kg/day, respectively. NOAELs for females in the second study were 3670 and 3825 mg/kg/day for AIN-93M and NIH-07 diets, respectively.

1. Introduction

Gum ghatti is one of several gums used commercially in foods as emulsifiers, thickeners, and stabilizers. Polysaccharide gums are a source of soluble fiber and are important additives to replace calorific foods. Gum ghatti is a complex polysaccharide obtained as an exudate of the *Anogeissus latifolia* tree, native to the dry deciduous forests of India (Sakai et al., 2012). It was originally considered as a substitute for gum arabic in the early 1900s but was not commercially developed because of batch-to-batch variability (Kaur et al., 2009). Gum ghatti naturally exists as mixed salts of calcium, magnesium, potassium, and sodium (Kaur et al., 2009). Hydrolysis produces L-arabinose, D-galactose, D-mannose, D-xylose, D-glucuronic acid (48:29:10:5:10 M ratios), and less than 1% L-rhamnose (Aspinall et al., 1965; Kaur et al., 2009; Sakai et al., 2012; Tischer et al., 2002). More recently a new gum ghatti product, Gatifolia, derived by a non-chemical physical procedure of spray-drying the dissolved, filtered, and sterilized starting material has been produced. This process yields a product with consistent batch-to-batch quality, superior rheological properties, acid resistance, and salt tolerance (Al-Assaf et al., 2008, 2009; Ido et al., 2008; Pszczola and Banasiak, 2006). The new gum ghatti product also has greater water solubility compared to many other polysaccharides (Kaur et al., 2009). Consequently, gum ghatti with its low viscosity has excellent emulsification properties at lower concentrations than gum arabic (Al-Assaf et al., 2008, 2009; Sakai et al., 2012) with potentially important applications in the food industry.

As a complex polysaccharide with an approximate molecular weight of 12,000 Da, gum ghatti is expected to escape degradation in the stomach and small intestine of humans and animals and undergo anaerobic microbial fermentation in the cecum and colon. The feeding of similar organic gums, including gum arabic, guar gum, rhamsan gum as well as other oligosaccharides, to laboratory rats results in increased cecal weight and size (Ali et al., 2009; Doi et al., 2006; Hagiwara et al., 2010; Levrat et al., 1991; Phillips, 1998; Tulung et al., 1987), increased blood flow to the cecum (Tulung et al., 1987; Younes et al., 1995), increased cecal bacterial proliferation (May et al., 1994; Walter et al., 1988), and bacterial

Abbreviations: AAALAC, Association for Assessment and Accreditation of Laboratory Animal Care International; ANOVA, Analysis of variance; FEMA, Flavor and Extract Manufacturers Association; GLP, Good Laboratory Practices; ILS, Integrated Laboratory Systems; NOAEL, No observable adverse effect level; OECD, Organization for Economic Co-operation and Development; PWG, Pathology Working Group.

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fermentation to short chain volatile fatty acids with a cecal pH decrease to 6 (Levrat et al., 1991; McLean Ross et al., 1984; Walter et al., 1988). Complete cecal fermentation of gum arabic has been noted in humans (Phillips, 1998). Both gum arabic and gum ghatti are fermented in the human large intestine by *Bacteriodes longum* (Salyers et al., 1977).

Gum ghatti is marketed as a food additive in Japan without limitation (JMHLW, 2009) and was assigned GRAS status in the United States in 1965 by the Flavor and Extract Manufacturers Association (FEMA No. 2519; Hall and Oser, 1965) and in 1977 by the FDA (21CFR184.1333; FDA, 2010). Because of limited toxicity data on gum ghatti, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has not as yet set dietary intake limits. Utilizing an internationally adopted battery of tests to evaluate potential somatic and germ cell genetic risks to humans, gum ghatti was recently reported to have no evidence of genotoxic potential when given at the maximum OECD recommended guidelines (Hobbs et al., 2012).

The purpose of this report is to document the lack of systemic and organ specific toxicity of gum ghatti following administration to rats at up to 5% in the diet.

2. Materials and methods

2.1. Animal study and study design

A standard 90-day Good Laboratory Practices (GLP) toxicity study was conducted in male and female Sprague Dawley rats according to OECD guideline #408 in which gum ghatti was prepared in AIN-93M diet. Based upon incidentally occurring colon ulcers in two females in the highest-dose (5% in diet), a second 90-day Good Laboratory Practices (GLP) toxicity study was conducted in females using the 5% high dose in two different diets, AIN-93M and NIH-07. Both studies were conducted within the same Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC)-accredited specific pathogen free facility. All procedures were in compliance with Animal Welfare Act Regulations (9 CFR 1-4) and the *Guide for the Care and Use of Laboratory Animals* (ILAR, 2011) and with approval by the Integrated Laboratory Systems (ILS), Inc. Institutional Animal Care and Use Committee. In the first study groups of 10 males and 10 females were given gum ghatti at 0, 0.5, 1.5 and 5% in diet for at least 90 days. In the second study groups of 20 females were given gum ghatti in AIN-93M and NIH-07 at 0 and 5% for at least 90 days. Study parameters were essentially identical with minor exceptions (Table 1). Rats ordered for the second study were specifically requested to be non-littermates.

2.2. Test agent

Gum ghatti (Gatti Gum SD, previously called GATIFOLIA SD) was provided by San-Ei Gen, F.F.I., Inc. (Osaka, Japan), as a gray to reddish-gray powder and mixed in diet at Research Diets, Inc. (New Brunswick, NJ). For the first study, two batches of dosed diet were prepared taking into account a 58-day stability in feed. Similarly, two batches of dosed diet were prepared for use in the second 90-day study. The same lot of gum ghatti was used in both studies. Dose formulation analyses were conducted by OpAns, LLC (Durham, NC) and were within acceptable ranges in both studies.

2.3. Statistical analysis

Group mean, and standard deviations were calculated using Microsoft® Excel. All data were analyzed (final body weight, body weight gain, feed consumption [g/kg/day], neurotoxicity endpoints, absolute and relative tissue weights, and hematology, urinalysis and clinical pathology endpoints) using Statistical Analysis System versions 9.1 or 9.2 (SAS Institute, Cary, NC). First, studentized residual plots were used to detect possible outliers in the data; outliers that were the result of collection errors were resolved. On remaining data, statistical analysis was subsequently performed. Homogeneity of variance was then analyzed using Levene's test. In case of heterogeneous data, transformation of the data was performed and re-analyzed for homogeneity of variance. Homogenous data were then analyzed using a one way analysis of variance (ANOVA) with treated groups compared to the appropriate control group using Dunnett's test. Data that could not be transformed to be homogeneous either through log, square root, or multiplicative inverse conversions were analyzed using a Kruskal–Wallis test and treated groups compared to the appropriate control group using Dunn's test. Dunn's test was applied to the analysis of absolute and relative ovary weights. Tests were two-tailed and unpaired with a level of significance of $p < 0.05$. Dose dependent changes in the first study were evaluated using a SAS linear regression model. *T*-tests were used to compare gum ghatti exposed animals to controls in the second study.

3. Results

3.1. First study

All animals survived until scheduled euthanasia with no clinical signs of toxicity in any animal. The majority of measured parameters did not differ significantly between gum ghatti-exposed and control rats. Mean feed consumption (Fig. 1A and Table 2), body weight (Fig. 1B), body weight gains and tissue weights (adrenal, brain, epididymides, heart, kidney, liver, lung, ovaries, pituitary, prostate, salivary glands, seminal vesicles, spleen, testes, thymus, thyroid/parathyroid and uterus/cervix) were not affected by treatment. Mean gum ghatti consumption is presented in Table 2. Mean hematological measurements and clinical chemistry analytes are presented in Tables 3a, 3b, 4a, and 4b. Ophthalmologic and neurological examination, urinalysis, plasma clotting times, histopathology of tissues from 40 sites and clinical chemistry endpoints were unaffected by gum ghatti. A statistically significant ($p < 0.05$) increase in monocytes was flagged in treated females due to a low control value but was within normal range for this measurement. No other hematological effects attributable to gum ghatti exposure were identified.

Exposure to high dose (5%) gum ghatti produced minimal to moderate changes in the cecum of male and colon of female rats. Cecal crypt hyperplasia was observed in 6/10 male rats but evaluation was problematic in that cecal mucosa orientation was not consistently perpendicular. No significant lesions were present in female cecum. The 5% dose of gum ghatti was also associated with significant increases in full and empty cecum weights (Fig. 1C and D). Focal ulceration with acute inflammation of the colon was present in 2/10 female rats exposed to the highest dose (Fig. 2); however no significant lesions were observed in male colon. A thorough re-examination of fixed colon wet tissues from all rats failed to identify any additional lesions. Because of suspicion that the focal colonic ulcerations may have been associated with the AIN-93M diet or the chance that intrinsically susceptible littermates were randomly assigned to the same group, a second study was undertaken to specifically focus on histopathology of female colon.

3.2. Second study

The purpose of this study was to determine if exposure to 5% dietary gum ghatti for at least 90 days was causally associated with colon changes in female rats. Differences in study parameters from the first study included use of only female rats, exposure to only 0 and 5% gum ghatti, and use of two diets, viz., AIN-93M and NIH-07, with 20 rats per group. Neuropathology assessment and urinalysis were not carried out in the second study and histopathology assessment was restricted to cecum and colon. To maximize cecal and colonic mucosal tissue orientation, these tissues were opened and fixed flat on cardstock. Otherwise, study parameters were similar to those in the first study.

All animals survived until scheduled euthanasia with no clinical signs of toxicity in any animal. No gum ghatti-associated changes were observed in feed consumption (Fig. 3A and Table 5), final body weight (Fig. 3B), body weight gain, and ophthalmological parameters. Feed consumption was measured weekly and showed that the amount of test item consumed in the 2nd study (Table 5) was equivalent to the amount consumed in the 5% dose group of the 1st study (See Table 2). Mean gum ghatti consumption is presented in Table 5. Hematological measurements were similar be-