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A subchronic dietary toxicity study of rice hull fiber in rats

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ABSTRACT

We conducted a 90-day feeding study to investigate subchronic toxicity of rice hull fiber. Sprague Dawley rats were randomly divided into four groups; each received a diet containing 0%, 2.5%, 3.75% and 5.0% (w/w) rice hull fiber for 90 days. Clinical observations were carried out daily, with weekly measurements of body weight and food consumption. We performed ophthalmic and histological examinations at termination. Blood and urine samples were collected to measure hematology and clinical chemistry parameters. No mortality, ophthalmic abnormalities, or adverse treatment-related effects were seen during clinical observations, hematological tests, or analyses of urine. Macroscopic or microscopic examinations of organs revealed no treatment related abnormalities. The only treatment related significant changes were reduced concentrations of fasting blood glucose (up to 17.6%) and cholesterol (up to 22.0%), typical benefits of dietary fiber, in males treated with 3.75 and 5% rice hull fiber. The no-observed-adverse-effect-level (NOAEL) for rice hull fiber was 5.0% for both genders (females, 3.80 g/kg body weight/day; males, 4.11 g/kg body weight/day).

1. Introduction

Industrialized nations have high rates of obesity, coronary heart disease, and large bowel disorders (e.g., constipation, irritable bowel syndrome, diverticular disease, colon cancer). Dietary fiber is an effective prophylactic for such disorders and is used with increasing frequency (Gestel et al., 1994). It generally comes from plant foods and consists of the part of the plant that is not digested by humans; for example, cellulose is a dietary fiber and rice hull is a high-fiber food.

Rice hull is agricultural waste that accounts for about one-fifth of annual gross rice output. China produces approximately 40 million tons of rice hull; the worldwide figure is 80 million tons (Lu et al., 2008; Li and Wang, 2008). The product contains abundant floristic fiber, protein, and some functional food components (e.g., carboxyl, hydroxyl, amidogen) (Nakbanpote et al., 2007). Rice fiber is a widely used dietary product in many countries. Prior research shows that it enhances the formation of short chain fatty acids (SCFA) (Fernando et al., 2008, 2010), which are associated with reduced risk of diseases that include irritable bowel syndrome, inflammatory bowel disease, cardiovascular disease, and cancer (Roediger, 1980; Jenkins et al., 1999; Floch and Hong-Curtiss, 2002). Benno et al. have studied probiotics and co-cultures, and have also investigated the role of rice fiber in the growth of human fecal microflora (Benno et al., 1989).

Recent data on the effects of environmental factors on the adhesion of probiotics on rice hull fiber indicate that rice hull fibers are suitable hosts for certain probiotics (Fernando et al., 2012). The product is also widely used as an additive in bread (Hu et al., 2002), biscuits (Cheng et al., 2003; Ge et al., 2003) and other baked items. These findings suggest that rice hull fiber may not only be a good prebiotic for probiotic foods, but also an adaptable additive that can be used in food technology or related fields.

Systematic research about possible toxicity of rice hull fiber has yet to be carried out. To determine the safety of drugs and plant products for human use, toxicological evaluation is carried out in animal models. Findings from these studies are used to predict toxicity and provide guidelines for 'safe' dosing in humans (Rhiouani et al., 2008). This study was performed to determine body weight, biochemical, hematological, and histopathological toxicity of rice hull fiber after subchronic oral administration in Sprague-Dawley rats. Its aim was to determine the safety of rice hull fiber and provide guidance for safe dosing in humans.

2. Materials and methods

2.1. Study design

This 90-day subchronic toxicity study was performed in accordance with FDA Redbook 2000: chapter IV.C.4.a Subchronic Toxicity Studies with Rodents.

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2.2. Chemicals

Rice hull fiber (Rice Fiber 300) was provided by SunOpta Ingredients (Chelmsford, USA), which was extract from normal rice. The composiiton of the rice hull fiber are shown in Table 1 (data from Medallion Labs, Minneapolis, MN, USA).

2.3. Animals

Sprague-Dawley rats (115 ± 15 g) came from the Experimental Animal Center of Shandong Luye Pharmaceutical Co., Ltd. (SPF grade, Certificate No. 2009009). During the entire study period, the animals were housed in cages under hygienic conditions and placed in a controlled environment with a 12-h light/dark cycle at 23 ± 3 °C and 40–70% humidity.

Each animal was examined for clinical signs of ill health on receipt and observed within 7 days of arrival. All procedures were in accordance with the Guidelines of the Animal Care and Use of Laboratory Animals from the Association of Laboratory Animal Science and the Center for Laboratory Animal Science of Yantai University.

2.4. Treatment

Rats were weighed prior to treatment, stratified by weight, and randomized to one of four groups. They were fed diets containing 0% (control), 2.5%, 3.75% and 5.0% rice hull fiber. Group assignments are outlined in Table 2. Beijing Keao Xieli Feed Co., Ltd. (Beijing, China) produced the diet in compliance with FDA Redbook 2000: chapter IV.C.4.a Subchronic Toxicity Studies with Rodents and in accordance with GB 14924.3-2010 (China).

2.5. Parameters investigated

2.5.1. Clinical observations, body weight, and feed consumption

All animals were observed twice daily for clinical signs of toxicity. Body weight was measured pre-test, then weekly and at sacrifice after fasting. We determined food consumption weekly.

We used an indirect ophthalmoscope for pre-test ophthalmic examinations on all animals. At termination, rats in the control (0%) and high-dose groups (5.0%) were examined again. All rats were tested if eye changes possibly associated with the test substance were discovered.

2.5.2. Urinalysis

During the last week of the study, we collected urine from surviving rats in urine collection cages. Parameters analyzed with an autoanalyzer (FA-100, ShanXi Ya-Sen Industrtal Co., LTD., ShanXi, China) included pH, specific gravity, leukocytes, nitrite, protein, glucose, ketones, urobilinogen, and bilirubin. We also examined appearance, color, and volume.

2.5.3. Hematology

The animals were fasted overnight before blood collection. Samples were drawn into separate tubes containing ethylenediamin-etetraacetic acid (EDTA). A HemaVet 950FS Hematology Analyzer (Drew Scientific Inc., Dallas, USA) was used to determine hemoglobin (HGB), hematocrit (HCT), red blood cells (RBC), total leukocyte count (WBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count (PLT), differential leukocytes count (neutrophils, N; lymphocytes,L; monocytes, M), and blood clotting time (CT, performed by the capillary tube method). If effects on the hematopoietic system were noted, reticulocyte counts and terminal bone marrow cytology were examined microscopically.

2.5.4. Chemistry

Blood samples were centrifuged to separate plasma. Clinical chemistry parameters analyzed using a biochemical analyzer (TBA-FR40; TOSHIBA Co., Ltd., Osaka, Country) included: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total bile acids (TBA), cholinesterase (CHE), total protein (TP), albumin (ALB), total bilirubin (TBIL), gamma-glutamyl transferase (GGT), glucose (GLU), total cholesterol (TC), creatinine (CREA), urea nitrogen (UREA), triglycerides (TG), phosphorous (P), sodium (Na), potassium (K), calcium (Ca), and chloride (CL). We used the K+/Na+/CL- Analyzer (CBS400, ZhuoYue Biotechnology Co., Ltd., Yan Tai, China) to assess Na, K and CL.

Table 1
Specifications of rice fiber.

Macronutrient	Value	Unit	Analytical method
TDF (dry wt. basis)[a]	>91	%	AOAC 991.43
Protein	<1.5	%	AOAC 984.13
Starch or sugars	<0.1	%	AOAC 996.11
Fat	<0.5	%	AOAC 954.02
Moisture	<4.0	%	AOAC 934.01
Ash	<4.0	%	AOAC 900.02

[a] TDF, total dietary fiber; cfu, colony forming units.

Table 2
Experimental design of 90-day rat subchronic toxicity study.

Group	Dose level	Number of animals (the total number is 99 animals)
1	Control group (0%)	26 (♀:13 + ♂:13)
2	2.5%	24 (♀:12 + ♂:12)
3	3.75%	25 (♀:12 + ♂:13)
4	5.0%	24 (♀:12 + ♂:12)

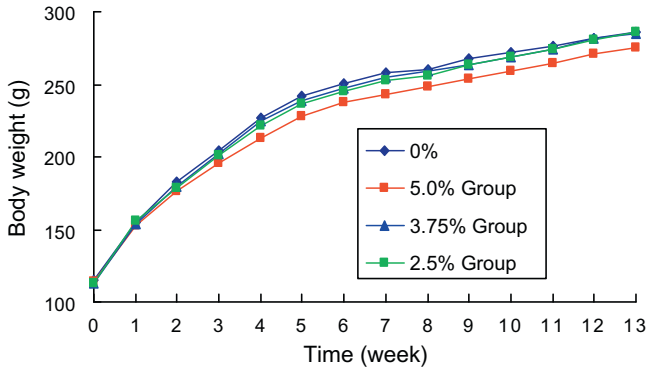

Fig. 1. The body weight change of female rats during administered rice hull fiber.

2.6. Pathology

2.6.1. Gross necropsy

At the end of treatment, all surviving animals were fasted overnight. The next day they were weighed before exsanguination and euthanized using chloral hydrate (300 mg/kg). We then conducted the external and internal gross pathological examination.

2.6.2. Organ weights

The liver, kidneys, adrenal glands, spleen, heart, uterus/epididymides, thymus, brain, testes/ovaries, and thyroid/parathyroid (post-fixed) organs were trimmed of any adherent tissue, as appropriate, and weighed wet as soon as possible. Paired organs were weighed together. Relative organ weights were calculated against fasting body weight.

2.6.3. Tissue collection

The adrenal glands, aorta, bone (femur), bone marrow (sternum), brain, cecum, colon, uterus, duodenum, epididymis, esophagus, eyes, harderian gland, heart, ileum, jejunum, kidneys, liver, lung, mandibular lymph nodes, mesenteric lymph

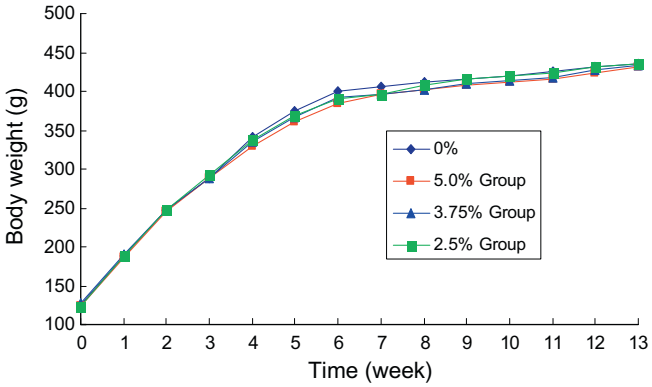

Fig. 2. The body weight change of male rats during administered rice hull fiber.