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Full Length Research Paper

Toxicologic evaluation of a plant food concentrate

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The present study evaluated the mutagenic and toxicologic potential of a proprietary greens product sold under the trade names: All Greens®, Enriching Greens® Greengevity®. This is a plant food concentrate of berry and enriched greens extract. An *in vitro* bacterial reverse mutation assay, showed no toxic effects in any of the five tester strains used up to the highest dose group evaluated except for a weak mutation effect in tester strain TA 1537 at a concentration of 5,000 µg/plate without metabolic activation. No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed. In a 14-day feeding study of dietary levels of 0, 1.25, 2.5 and 5.0% in rats, there were no adverse clinical, body weight, food consumption or macroscopic changes associated with the administration of this product.

Key words: All greens, toxicology, Ames, 14-day dietary study.

INTRODUCTION

The health benefits of increasing natural fruits and vegetables in the diet are well documented. The reduction in oxidative stress derived from a balanced diet has been associated with a decreased risk of such chronic diseases as metabolic syndrome, malignancy and cardiovascular conditions among others (Mates, 1999). A commercially available plant food concentrate (All Greens® Enriching Greens® Greengevity®), containing extracts of berry (16.5%), greens and fruit powders (40.2%), herb extracts (6.3%) and digestive aids (37%) (Factors Group, Coquitlam, BC, Canada), intended for daily supplemental use and previously studied in a limited population of 15 male subjects (Ziccarelli and Basu, 2003), has been newly tested for its safety both in a bacterial reverse mutation assay (Ames test) and a 14-day feeding study in Sprague-Dawley rats under OECD guidelines.

These studies, conducted at Bioservice Scientific Laboratories (BSL) GmbH in Planegg, Germany (Ames) and Eurofins Product Safety Labs (14-day study), were in compliance with OECD Principles of Good Laboratory Practices (ENV/MC/CHEM (98) 17 OECD, Paris, 1998). The studies were conducted in conformance with the OECD Guidelines for Testing of Chemicals and Food Ingredients, Section 4, No. 471: *Bacterial Reverse Mutation Test*, and Part 407: *Repeated Dose 28-Day Oral Toxicity Study in Rodents* (2008) and US FDA Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, IV.C. 4 a. *Short-Term Toxicity Studies with Rodents* (2003). All work undertaken by the testing laboratory was in accordance with the most recent ‘’Guide for the Care and Use of Laboratory Animals’’, *(*NIH, 2011), operated under the surveillance of the Regierung von Oberbayern (German regulatory authority, BSL) and according to AAALAC standards and accreditation.

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MATERIALS AND METHODS

Bacterial reverse mutation test

The test item was investigated for its potential to induce gene mutations according to the plate incorporation test (experiment I) and the pre-incubation test (experiment II) (Ames et al., 1973; Maron and Ames, 1983) using *Salmonella typhimurium* strains TA 98, TA 100, TA 1535, TA 1537 and tester strain *E. coli* WP2 uvrA with and without metabolic activation in triplicate in the following concentrations: Experiment I: 31.6, 100, 316, 1000, 2500 and 5000 µg/plate. Experiment II**:** 3.16, 10.0, 31.6, 100, 316 and 1000 µg/plate. Controls (positive, sodium azide, 4-nitro-o-phenylene-diamine, methylmethanesulfonate, 2-aminoanthracene and negative, distilled water) were tested for validity of the assay. Data were evaluated for cytotoxicity (diminution of the background lawn or a reduction in the number of revertants), and mutagenicity (mutation factor = mean revertant value of test article/ mean revertants of control).

14-day dietary study

A 14-day dietary toxicity study was conducted in Hsd:SD® rats to determine the potential of the test product to produce toxicity. Forty healthy rats (20 males and 20 females) were selected for the test and equally distributed into four groups (5 males and 5 females per group). Dietary levels of 1.25, 2.5 and 5.0% of All Greens®, as well as a basal diet control (0%), were selected for the test. The test and control diets were presented to their respective groups on Day 0 of the study. Additional diet was provided as needed throughout the study to insure *ad libitum* feeding. The animals were observed daily for viability, signs of gross toxicity and behavioral changes and on Days 0, 7 and 14 for a battery of detailed observations. Body weights were recorded during the acclimation period including prior to test product introduction (Day 0), and on Days 3, 7, 11, and 14 prior to terminal sacrifice. Individual food consumption was also recorded to coincide with body weight measurements. Gross necropsies were performed on all animals. Male and female rats were evaluated separately. Mean and standard deviations were calculated for all body weight, mean daily body weight gain, mean daily food consumption and mean daily food efficiency. Data within groups was evaluated for homogeneity of variances and normality by Bartlett's test (Bartlett, 1937) (ANOVA, Dunnett, 1964, 1980) in Provantis™ version 8.4.2.0, Tables and Statistics version 8.4.2.0, Instem LSS, Staffordshire UK; INSTAT Biostatistics, Graph Pad Software, San Diego, CA.

RESULTS AND DISCUSSION

Bacterial reverse mutation test

The test substance was autoclaved for 20 min at 121°C that followed by ultrasound treatment for 15 min at 37°C and diluted in DMSO prior to treatment. Precipitation of the test item was observed in all tester strains used in experiments I and II at a concentration of 100 µg/plate and higher. No toxic effects of the test item, (historical mean spontaneous reversion frequency) were noted in the bacterial reverse mutation assay experiments I and II, with one exception: In experiment I, a weak toxic effect of All Greens® was only noted in tester strain TA 1537 at a any of the five tester strains used up to the highest dose group evaluated with and without metabolic activation in concentration of 5,000 µg/plate without metabolic activation as detected by a reduction in the number of revertant colonies down to a mutation factor of 0.3 and a mean revertant colony value of 3 (historical = 5-28). No biologically relevant increases in revertant colony numbers of any of the five tester strains were observed following treatment with the test substance at any concentration level, neither in the presence nor absence of metabolic activation in experiment I and II. The reference mutagens induced a distinct increase of revertant colonies indicating the validity of the experiments. As such, under the experimental conditions reported, All Greens® did not cause gene mutations by base pair changes or frameshifts in the genome of the tester strains used.

14-Day dietary study

In a 14-day *ad libitum* feeding study, the test product, as received and in the diet, was considered stable and to be both homogeneously distributed in the diets and at the targeted concentrations throughout the study. Diet preparations and neat test substance were not analyzed as part of this subacute dietary study and preparations were mixed as is, from the manufacturer, for both test and control diets. There were no test substance-related or other mortalities. There were no adverse clinical observations associated with the product. Mean body weights for male and female rats at 1.25, 2.5, and 5.0% were comparable with control values throughout the study (Figure 1). Overall (Days 0-14) and mean daily body weight gain for male and female rats at 1.25, 2.5, and 5.0% were generally comparable with control values with the following exception: Males at 2.5 and 5.0% had a significant increase in daily body weight gain for Days 7-11. Overall (Days 0-14) and mean daily food consumption for male and female rats at 1.25, 2.5, and 5.0% were comparable with control values throughout the study. Overall (Days 0-14) and mean food efficiency for male and female rats at 1.25, 2.5, and 5.0% were generally comparable with control values with the following exception: Males at 2.5% had a significant increase in mean food efficiency for Days 7-11. Significant changes from control in body weight gain and food efficiency in males were considered incidental and transient such that by the end of the study, no differences existed between control and test substance-administered group values. There were no macroscopic observations at necropsy associated with the *ad libitum* dietary intake of test product at the levels tested. The mean overall (Days 0-14) daily intake of test product in male rats fed dietary concentrations of 1.25, 2.5, and 5.0% was 0, 1099.0, 2190.6, and 4389.7 mg/kg/day, respectively. For the same dietary concentrations, the mean overall daily intake of test product in female rats was 0, 1090.8, 2272.0, and 4433.8 mg/kg/day, respectively. Therefore, the animals were considered to have received the

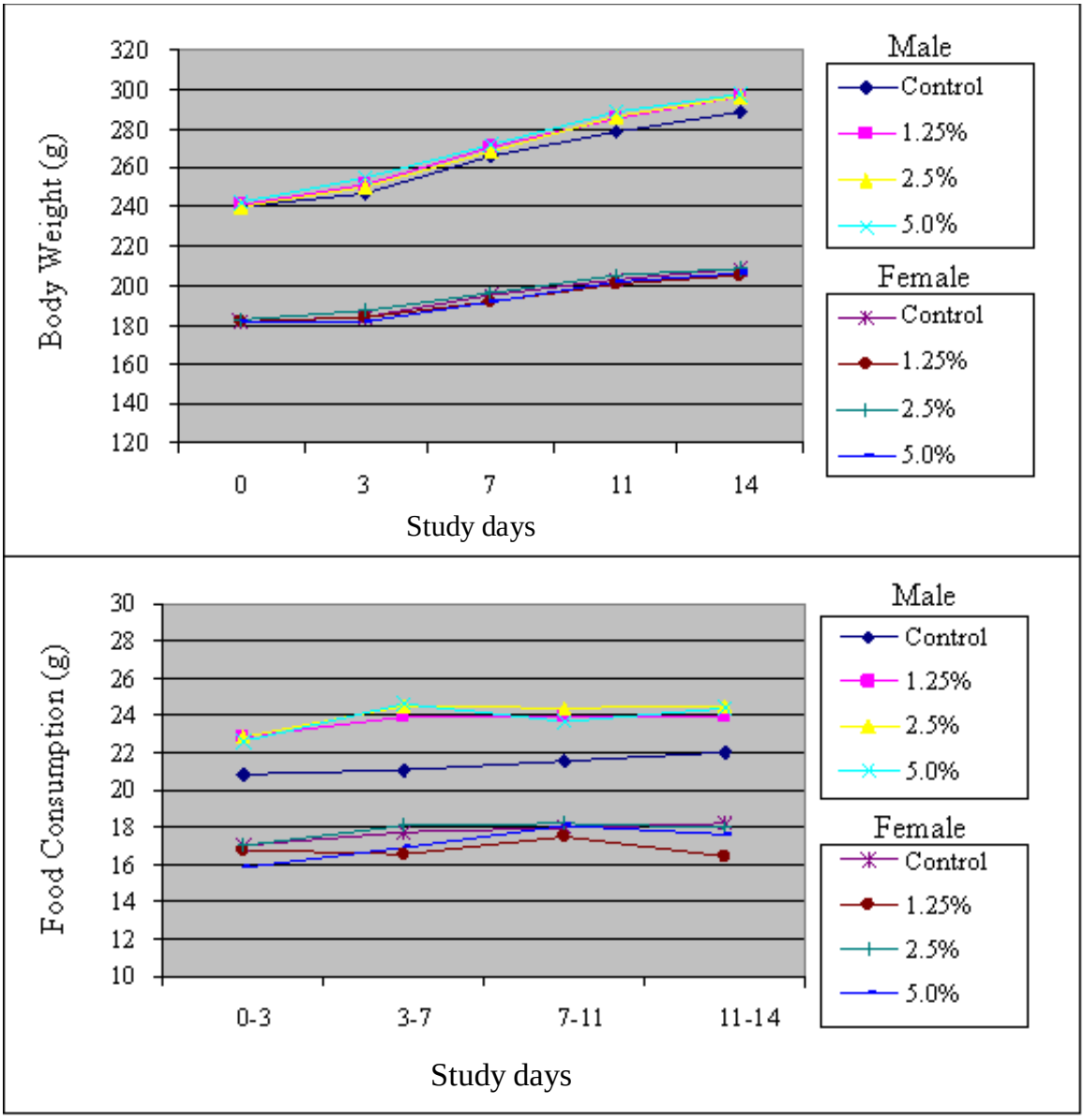

Figure 1. Mean body weight and food consumption.

targeted exposures with a no-adverse-effect level of 5% in the diet. Clinically, the recommended daily dose of test product is approximately 166.7 mg/kg (10 g per 60 kg human), making the highest dose tested in the present study over 26 times suggested intake.

In a previous report, a comparable plant food concentrate administered 8.5 g twice a day clinically to moderately hypercholesterolemic males for two weeks followed by a two week washout period (Ziccarelli and Basu, 2003) showed statistically significant reductions in total and LDL plasma cholesterol and LDL/HDL ratio, (but not HDL), which returned to baseline after cessation of administration. Zinc and copper-dependent erythrocyte superoxide dismutase, an indicator of superoxide radical removal, was elevated, indicative of the antioxidant potential of the test substance.

In the present study, the dietary administration of test product, a plant food concentrate, was well tolerated by rats up to a concentration of 5% in the diet. Dietary supplements over this level have the potential to adversely influence nutritional intake (Borzelleca, 1992). Based on the experimental conditions of this mutagenicity and 14-day test and the toxicological endpoints evaluated, these results indicate that test product did not cause gene mutations by base pair changes or frame shifts in the genome of the tester strains used. Neither did the sub-acute dietary administration of test product result in any toxicologic effects. Therefore, the use of

appropriate levels of test product (All Greens® Enriching Greens® Greengevity®) is considered safe. A study of longer duration is appropriate to extend these results.

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The test product used in the present study is a commercial grade product obtained from the producer (Factors Group of Nutritional Companies Inc, BC, Canada). All Greens® is a registered trademark of WN Pharmaceuticals Td, Enriching Greens® is the trade mark of Natural Factors Ltd and Greengevity® is the trade mark of BioClinic Naturals Inc., all in BC, Canada. Author Wood received financial support from Factors Group of Nutritional Companies, Inc. (Canada). R. Gahler owns the Factors Group of Companies, which retains an interest in the product.

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