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Subchronic toxicity and mutagenicity/genotoxicity studies of *Irvingia gabonensis* extract (IGOB131)

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ABSTRACT

African Bush Mango from *Irvingia gabonensis* is a West African culinary fruit and the mucilage from this fruit seed is used to make traditional soups and sauces. Extract from the kernel (IGOB131) has been claimed for its health benefits. In the present investigations, potential adverse effects, if any, of IGOB131 were investigated in dose–response 90-day study and genotoxicity studies. In the subchronic study, Sprague Dawley rats (20/sex/group) were gavaged with *I. gabonensis* extract (IGOB131) at dose levels of 0, 100, 1000 and 2500 mg/kg body weight (bw)/day for 90-days. No treatment-related changes in clinical signs, functional observations, mortality, ophthalmologic observations, body weights, body weight gain or feed consumption were noted. Similarly, hematological, clinical chemistry, urine analysis parameters, and organ weights did not reveal any toxicologically significant treatment-related changes. No treatment-related macroscopic and microscopic abnormalities were noted at the end of treatment period. The mutagenicity as evaluated by Ames assay, *in vitro* and *in vivo* chromosomal aberration test and *in vivo* micronucleus assay did not reveal any genotoxicity of IGOB131. The results of subchronic toxicity study suggest the no-observed-adverse-effect level (NOAEL) for *I. gabonensis* extract (IGOB131) as $\geqslant$2500 mg/kg bw/day, the highest dose tested.

1. Introduction

Irvingia gabonensis is a commercial and indigenous culinary fruit tree of Africa. This plant is well known in Nigeria and in the West African region, particularly in Togo, Dahomey, Cote d'Ivoire and Sierra Leone where fruit pulp and kernels from this plant are commonly used as food (FAO, 2003). The fruit commonly known as *African Mango, Bush Mango* is similar to mango, is a large drupe, greenish yellow in color with fleshy fibrous pulp surrounding a large hard stone. The seeds of the fruits of *I. gabonensis* are considered as major resource, can be eaten raw or roasted and are used in food preparations (National Research Council, 2006). Kernels yield an important food additive popular in West and Central Africa (Tchoundjeu and Atangana, 2007). The mucilage from kernel is used to make traditional soups such as Ogbono as well as sauces. The mucilage is also used as a suspending and emulsifying agent and has been claimed to be better than acacia and tragacanth (Isimi et al., 2000). The nut is also used as a food thickening agent (Leakey et al., 2005) and is highly valued to prepare "ndo'o" or "draw soup" as well as groundnut or njansang (*Ricinodendron heudelotii*) (Nangue et al., 2011). It is also used in making particular types of cakes that are highly popular (Ayuk et al., 1999). The traditional soup prepared from kernel has been sold in the United States at several ethnic restaurants and as packaged food item. The USDA Agricultural Research Service has listed uses of *I. gabonensis* fruit (dika nut) as an edible fruit and kernels as a source of edible fats. Available evidence indicates that *I. gabonensis* seed extract provides health benefits, including weight loss (Egras et al., 2011) and antioxidant activity (Agbor et al., 2005).

In recent years, use of *I. gabonensis* seed extract as a dietary supplement has gained popularity in Western countries. The extract has been marketed in the United States as a dietary supplement under different names such as African Mango Extract, African Mango Diet, Futurebiotics® African Mango, *Irvingia gabonensis* Seed Extract, etc. It is also marketed in combination with other dietary

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FDA, food and drug administration; GGT, gamma-glutamyl transferase; GLDH, glutamate dehydrogenase; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; MNPCE, micronucleated polychromatic erythrocytes; NOAEL, no-observed-adverse-effect level; OECD, organization for economic co-operation and development; PCE, polychromatic erythrocytes.

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supplement. These products are regulated under the Dietary Supplement Health and Education Act (DSHEA, 1994) in the US. The recommended dose for management of obesity and lowering cholesterol levels is reported as 1.05 g of crude seed extract three times daily. A dose of 150 mg of a standardized seed extract (IGOB131) twice daily has also been recommended.

It has been hypothesized that the potential health benefits of *I. gabonensis* are related to its ability to inhibit adipogenesis by down-regulating peroxisome proliferator-activated receptor gamma (PPAR-gamma) which is responsible for the differentiation of adipocytes. In a recent *in vitro* study using an experimental model (murine 3T3-L1 adipocytes), Oben et al. (2008a) reported that *I. gabonensis* seed extract may inhibit adipogenesis through modulation of PPAR gamma and glycerol-3 phosphate dehydrogenase in addition to changes in leptin and adiponectin. In a randomized, double-blind, placebo controlled clinical study, administration of *I. gabonensis* seed extract (IGOB131) at a dose of 300 mg/day to 52 volunteers for 10 weeks resulted in both weight reduction (body weight, body fat, waist size) and an improvement in metabolic parameters associated with insulin resistance (Ngondi et al., 2009). In an earlier randomized, double-blind study, Ngondi et al. (2005) also reported that administration of *I. gabonensis* extract (1.05 g three time a day for one month) to 28 subjects for one month resulted in decreased body weight by 5.26 ± 2.37%, while the placebo group revealed a decrease of 1.32 ± 0.41%. In yet another, randomized, double-blind, placebo-controlled design involving 72 obese or overweight participants (45.8% male; 54.2% female; ages 21–44; mean age = 29.3; mean BMI > 26 kg/m; divided in three groups; $n = 24$), administration of combination of *Cissus quadrangularis/Irvingia gabonensis* extract (500 mg/day) for 10 weeks did not cause any treatment-related adverse effects (Oben et al., 2008b).

Given the uses of *I. gabonensis* seed extract in foods and as a dietary supplement, adverse effects, if any, were investigated in a long-term repeat dose toxicity study and in genotoxicity studies. In the repeat dose subchronic toxicity study, a detailed assessment of the toxic potentials of a well standardized extract of *I. gabonensis* (IGOB131) prepared from kernel of the plant when administered daily for 90-days via oral gavage to Sprague Dawley rats was undertaken. The potential genotoxic effects of IGOB131 were investigated in bacterial reverse mutation assay (Ames test) using *Salmonella typhimurium* strains, *in vitro* mammalian chromosomal aberration assay in CHO-K1 cell line, *in vivo* chromosomal aberration study in rats, and *in vivo* mammalian erythrocyte micronucleus test in rats.

2. Materials and methods

2.1. Subchronic study

2.1.1. Study design

The study was performed in accordance with (A) FDA Redbook (2000a): Chapter IV.C.4.a Subchronic Toxicity Studies with Rodents, (B) Organization for Economic Co-operation and Development (OECD) Principles of Good Laboratory Practices (1997), (C) The standard operating procedures at Anthem Biosciences & Bioneeds and as per the mutually agreed study plan with the Sponsor, and (D) The recommendation of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines for laboratory animal facility published in the gazette of India, December 15th 1998 (CPCSEA, 1998). Standard safety precautions were observed during the course of study. The study protocol number BIO-IAEC 218-10/04 was approved by the Institutional Animal Ethics Committee (IAEC) on 10th April 2010.

2.1.2. Test item

Standardized and well characterized *Irvingia gabonensis* extract (IGOB131) used in the present study was produced by Synthite Industries Ltd., Kerala, India at a ISO certified facility that follows the HACCP protocols. *I. gabonensis* extract (IGOB131®) is manufactured according to current good manufacturing practices (cGMP). The kernel (inner edible seed of nut or fruit stone) from the fruits of *I. gabonensis* plant are collected and the powder is prepared by grinding. The powder is subjected to cold press extractor (at 50 °C for 6 h) to remove oil by filtration. The material is dried under vacuum and subjected to a series of extractions with demineralized water. The water extracts are concentrated under vacuum. The concentrate is checked for total solids and spray dried followed by pulverization and sieving to conform to 80 mesh specifications. The preparation procedure assures a consistent and high-quality product.

The product quality is defined by parameters such as loss on drying, pH, bulk density, albumin levels, and soluble fiber. All lots are subjected to IR-Scans to further ensure batch to batch consistency. Additionally, HPLC finger printing for the extract has been developed to test the consistency of the production. The physical characteristics and chemical specifications of the product are presented in Table 1. The extract (IGOB131) is a off-white, non-fibrous powder with slightly nutty odor and taste. The powder is partly soluble in water. The dry powder is standardized to contain 10% albumin with a fiber content of 15%. The product is routinely checked for contaminants such as heavy metals, pesticide residues and microbial to make sure that the product is suitable for human consumption.

2.1.3. Animals

Sprague–Dawley rats (Bioneeds, Laboratory Animals and Preclinical Services, Bangalore, India) aged 6–7 weeks, following examination by a veterinarian were used for the present study. The males and females were acclimatized for five and six days, respectively, to laboratory conditions. One hundred sixty rats were equally divided into 4 groups (20/sex/group). The average age at the time of treatment was 6–7 weeks and the average weight (±20% variation) of males and females was approximately 146 and 128 g, respectively. All animals were housed under standard laboratory conditions and in accordance with CPCSEA (1998) guidelines. The animals were fed with Nutrilab rodent feed (Tetragon Chemie Private Ltd., Bangalore, India) throughout the acclimatization and experimental period. All animals were provided with deep bore-well water purified using Aqua guard water filter (Eureka Forbes Ltd., Mumbai, India) in polypropylene water bottles with stainless steel sipper tubes.

2.1.4. Treatment

The rats divided into four groups were treated orally (gavage) once daily with *I. gabonensis* extract (IGOB131) preparation at dose levels of 0 (Group I-control), 100 (Group II-low dose), 1000 (Group III- mid dose), or 2500 (Group IV-high dose) mg/kg bw (dose volume 10 mL/kg) for 90 consecutive days. Given the historical uses and safety data for *I. gabonensis* kernel and related products, high dose was considered based on intended maximum consumption in humans and the preliminary dose range finding study of IGOB131 in rats which was conducted prior to the main study. It revealed no adverse toxic effects on rats. Distilled water was used as a vehicle for preparing formulation. Formulation was prepared freshly on daily basis before administration. The test item was stable in water even after 24 h at pH 3.0, 5.0, 7.0 and temperature of 82 °C.

Throughout the study period the animals were observed for clinical signs of toxicity and mortality/morbidity (daily), detailed clinical examination, body weight and food consumption (weekly), functional observation tests during week 13, ophthalmoscopy at pretest and during week 12, hematological, clinical chemistry were conducted on Days 15, 45 and 91, urinalysis, gross pathology and organ weights were recorded at termination. Histopathological examination was conducted on the specified list of tissues from all the groups as mentioned in FDA Redbook (2000a).

2.1.5. Parameters investigated

2.1.5.1. Clinical examination, body weight and feed consumption. All animals were subjected to detailed clinical examinations before initiation of the treatment and weekly thereafter (varied by ±1–2 days) during the study. Individual animal body weights were recorded at receipt, on the day of initiation of treatment and weekly thereafter (±2 days) during the course of study. Fasting body weights were recorded at terminal euthanasia. Individual animal feed consumption was recorded weekly (±2 days) except for the animals during interim blood collection. Group mean feed consumption was calculated.

2.1.5.2. Neurological/functional, ophthalmoloscopic examination. Neurological/functional examination was carried out during 13th week for control and high dose group animals. For neurological examinations cage-side observation included movements, respiration, skin and hair coat, salivation, lacrimation, urine staining, and fecal staining or diarrhea. Neurological observations on examination table included tactile response and response to tail pinch-unreceptive stimulus. Additional neurological measurements included locomotor activity, head shaking, grasping strength and equilibrium test. Functional observation record included visual response, auditory response, and response to proprioceptive stimulus (gait, landing foot splay, righting reflex). Ophthalmological examination was performed on all animals before the study begins and on control and high dose group animals during 12th week of the study.