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Safety assessment of McB-E60 (extract of a *Momordica* sp.): Subchronic toxicity study in rats

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ABSTRACT

Momordica charantia plant is consumed as a foodstuff in some south Asian curries while its extract preparations have been traditionally used for lowering blood glucose levels in patients with diabetes mellitus. Nutritional Health Institute Laboratories (NHIL), LLC, Florida informed that it patented a new plant McB, as an interhybrid of three plants of *Momordica* genus. The objective of the present study was to investigate potential adverse effects, if any, of *McB-E60* (extract of a *Momordica* sp.) in rats following subchronic administration. Sprague-Dawley rats (10/sex/group) were administered via oral gavage 0 (control), 250, 500 and 1000 mg/kg body weight (bw)/day of *McB-E60* for 90 days. Additional 28-day recovery groups were maintained at control and high dose levels. No mortality or significant and adverse changes in clinical signs, neurological signs, body weight gain or feed intake were noted. No toxicologically significant changes in hematology, clinical chemistry, urinalysis and organ weights were noted. Gross and microscopic pathology examinations did not reveal treatment-related abnormalities. Any changes noted were incidental and within historical control ranges. Based on the results of this study, the No-Observed-Effect Level (NOEL) for *McB-E60* (extract of a *Momordica* sp.) was determined as greater than 1000 mg/kg bw/day, the highest dose tested.

1. Introduction

Approximately 40% of Americans use complementary and alternative medicine (CAM) for health promotion and the treatment of illness [10]. Of these users, 2 and 3 million specifically use CAM to lower blood sugar levels and to treat various stages of diabetes, despite limited studies of their safety and efficacy [2]. An estimated 79 million adult Americans have pre-diabetes, a condition that is preventable and treatable if recognized early [1]. Individuals with pre-diabetes have glycated hemoglobin (HgA1c) and blood sugar levels that are below the clinical threshold to be classified as type 2 diabetes but are higher than normal [4]. Pre-diabetics, if not treated appropriately, are at risk for becoming diabetic and developing cardiovascular disease [9,13].

Momordica charantia, also known as Bitter melon, is a widely used traditional remedy for hyperglycemia. A member of the cucerbitaceae family, Bitter melon is a perennial climber characterized by warty-fruit like gourds or cucumbers. It is a commonly consumed vegetable found throughout the sub-tropical world (China, India, Thailand, East Africa, The Caribbean, Central and South America) and is known by various names, such as balsam pear, bitter gourd, cundeamor, goo-fah, karela, etc [11,6]. The fruit, as well as the whole plant is believed to possess anti-diabetic, anti-viral, anti-bacterial and anticancer properties and has been scientifically evaluated in the recent past [5]. The immature fruits of *M. charantia* can be prepared in many ways for food uses. Fruits, flowers, and young shoots are also used as a flavoring. The young shoots and leaves are sometimes cooked and eaten as leafy vegetables [7]. In recent years, processed bitter gourd in the form of capsules or tablets is commonly marketed as a dietary supplement under the brand names Gourdin, Karela, and Glucobetic in Canada, India, United Kingdom, the United States, and many Asian countries [7].

In several *in vitro* and animal model studies, the plant has been investigated for its mechanism of action as an anti-diabetic. Over 228 different compounds with possible medicinal properties, acting alone or in combination, have been isolated from bitter melon fruit, seeds, leaves, stems, pericaps, endosperm, callus tissues, and cotyledons [3]. Of the various compounds identified,

Abbreviations: ALKP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; FDA, food and drug administration; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume; NOEL, no-observed-effect level; OECD, organization for economic co-operation and development.

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charatin, polypeptide-p, vicine, momordin, and similar derivatives have been claimed to improve glycemic control [3]. The available information indicates that bitter melon has been traditionally used to treat high blood sugar and diabetes. For over 70 years, studies have appeared sporadically in the literature indicating the benefit of bitter melon in lowering blood sugar. In human studies, bitter melon juice, fruit, and dried powder have been investigated for hypoglycemic effect. Only a few randomized controlled trials of bitter melon have been conducted. In a prospective, randomized, double-blinded and placebo-controlled trial of bitter melon, a statistically significant decrease in HbA1c levels after 4 months of intervention, compared with a referent group receiving refined soybean oil was noted [14].

In an attempt to further develop improved varieties of *Momordica* species for use in humans to control blood sugar, several investigators have attempted to develop new varieties and strains of *Momordica* by inter species hybridization. NHIL has developed and patented a new plant variety which is a plant grown from seeds derived from a new interhybrid of three plants of *Momordica* genus. This newly derived plant hybrid of genus *Momordica* (McB interhybrid) has been stabilized and asexually reproduced. The McB interhybrid has been described as an interhybrid derived from a cross of *Momordica charantia*, commonly known as bitter melon, *Momordica balsamina*, and a previously unnamed *Momordica* species native to Ecuador. In particular the McB interhybrid is a tetra-cross pollinated hybrid plant of the *Momordica* species. The extract (*McB-E60)* evaluated in the present study is derived exclusively from the leaves, petioles and stem (the fruits, flower and roots are not included) of the new interhybrid plant.

Given the potential use of bitter melon (*M. charantia*) as a dietary supplement in possible support for maintaining blood glucose, the objective of this study was to investigate the long-term repeat dose toxicity of *McB-E60* (extract of a *Momordica* sp.). In the repeat dose subchronic toxicity study, a detailed assessment of the toxic potentials of a standardized extract when administered daily for 90-days via oral gavage to Sprague Dawley rats, was carried out.

2. Materials and methods

2.1. Study design

The 90-day subchronic toxicity study was performed in accordance with Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals, No. 408: Repeated Dose 90-day Oral Toxicity Study in Rodents; the U.S. FDA, CDER Guidance for Industry, Botanical Drug Products, June 2004; the ICH Harmonized Tripartite Guideline M3 (R2), Current Step 4 version dated 11 June, 2009; and the WHO Guidelines for Toxicity Investigation of Herbal Medicines, Research guidelines for evaluating the safety and efficacy of Herbal Medicines, 1993. The study was conducted in compliance with the principles of Good Laboratory Practice as set forth in OECD Series on Principles of Good Laboratory Practice and Compliance Monitoring, Number 1, 'OECD Principles on Good Laboratory Practice' ENV/MC/CHEM (98) 17 (as revised in 1997).

2.2. Test article

Standardized *McB-E60* (extract of a *Momordica* sp.) used in the present study was provided by Nutritional Health Institute Laboratories (Tallahassee, FL, USA) and was manufactured at Metaugus, Inc., GA30125, USA as production batch number 9012701 of year 2008. The product is a green colored clear liquid.

The product quality was defined by parameters such as ash, pH, protein, dietary fibre, gentisic acid, stigmasterol and total sterols. The product had a characteristic odor (a balanced herbal estery lightly ethanolic smell) and bitter taste. The concentrate was checked for heavy metals, microbial load and pesticide residues. The physical characteristics and chemical specifications of the product are presented in Table 1.

Table 1
Description and specifications of *McB-E60* (extract of a *Momordica sp.*).

Product Description		
It is a green clear crystalline liquid extract obtained from a new interhybrid of three plants of *Momordica* genus		
Parameter	Results	Method
pH	4.12	USP 791
Gentisic Acid	<0.01 ppm	Internal (HPLC) Method—KK123
Stigmasterol	0.01% (w/w)	Internal (GC) Method—KK329
Total sterols	0.0115% (w/w)	Internal (GC) Method—KK329
Dry Ashing	<1%	–
Residual ethanol	48,000 mg/kg	USP/NF 467
Total dietary fibre	<0.2% (w/w)	
Combustion proteins	1.41%	AOAC 990.03, 992.15
Heavy metals		
Total heavy metals	<0.02 ppm	
Cadmium	<0.002 ppm	AOAC 993.14 Mod.
Lead	<0.007 ppm	AOAC 993.14 Mod.
Mercury	<0.004 ppm	AOAC 993.14 Mod.
Arsenic	<0.007 ppm	AOAC 993.14 Mod.
Microbiological assays		
Yeast and moulds	<10 (est) CFU/g	BAM Chapt. 18
Total Aerobic plate count	<10 (est) CFU/g	AOAC 990.12
Total coliform	<10 (est) CFU/g	AOAC 991.14
Escherichia coli	<10 (est) CFU/g	AOAC 991.14
Staphylococcus aureus	<10 (est) CFU/g	AOAC 2003.07
Salmonella	Not Detected per 25 g	AOAC 2003.09
Other Contaminants		
Apart from above, the sample was analysed for several pesticides—organochlorine, pyrethroids, organonitrogen, organophosphorous, carbamates and for aflatoxins, to ensure that it was free from undesired levels of the same.		

Ref. (except for product description and ash content): Certificate of analysis—AR-15-KK-000614-01 dated January 12, 2015, of Eurofins Scientific, Inc., Supplement Analysis Center, CA for sample # 740-2014-00021037.

2.3. Animals

Healthy male and female Sprague-Dawley rats were procured from Reliance Life Sciences (Navi Mumbai, India) and were acclimatized to experimental room conditions for up to six days. Veterinary examinations were then conducted following which 100 rats (50 males and 50 females) were selected such that the weight variation within each sex of rats did not exceed ±20% of the mean weight for that sex (male rats 196.6 g; female rats 160.7 g). The selected rats were then assigned to control and treatment groups (10/sex/group) using random numbers generated with MS-Excel. The rats were group housed with up to two rats of similar sex per cage in sterilized solid bottom polypropylene cages with bedding of clean and sterilized paddy husk. Cages had facilities for food and water bottle, and were suspended on movable stainless steel racks. HVAC conditions in the experimental animal room were set to maintain 10–15 air changes per hour of 100% fresh and filtered air, conditioned with temperature between 19–25 °C and relative humidity 30–70%. The animal room was illuminated for 12 h light on each day while darkness was maintained for rest of the 12 h. The rats were fed *ad libitum* with Nutrilab rodent feed manufactured by Provimi Animal Nutrition India Pvt. Ltd. (Bangalore, India) and were provided with potable water filtered through 'Aquaguard' water filter having built-in ultra violet irradiation process.