

GRAS Notice (GRN) No. 500

<http://www.fda.gov/Food/IngredientsPackagingLabeling/GRAS/NoticeInventory/default.htm>

ORIGINAL SUBMISSION

GRN 000500



Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-200)
College Park, MD 20740-3835

January 30, 2014

ATTN: Dr. Antonia Mattia, PhD

Our Reference: GRAS Notification for Slimaluma™, a hydroethanolic extract of *Caralluma fimbriata*

Dear Dr. Mattia,

AIBMR Life Sciences, Inc. has been retained as an agent by GE Nutrients ("the Notifier") to submit this GRAS notification on Slimaluma™, a hydroethanolic extract prepared from the dried aerial part of *Caralluma fimbriata*, intended for use as an ingredient in specified food. The extract is standardized to 25–29% pregnane glycosides, and batch analyses indicate that the ingredient is made consistently and meets all product specifications. Because of its GRAS status as outlined in this notification, Slimaluma™ is considered exempt from the requirement of pre-market approval, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act.

The GRAS determination has been made based on scientific procedures. The basis for the determination relies on animal safety studies that were performed on Slimaluma™, including an Ames, chromosomal aberration, 6-month repeated oral toxicity study, and developmental toxicity study. These studies were published in the *International Journal of Toxicology*. Additionally, 50 million servings worth of the ingredient have been sold in the US without reportable adverse events. Published clinical studies and the historical consumption of *C. fimbriata* are also detailed.

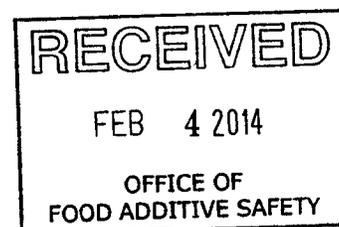
Please find enclosed one copy of the notification: *Notice to US Food and Drug Administration that the use of Slimaluma™, a Hydroethanolic Extract Prepared from Caralluma fimbriata, is Generally Recognized as Safe*. Also enclosed is an electronic copy of the notification, and copies of all references cited in the notification. As stated in the exemption claim, the data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of GE Nutrients, or will be sent to FDA upon request. Please do not hesitate to contact us with any questions.

Yours sincerely,

(b) (6)

John R. Endres
Chief Scientific Officer
john@aibmr.com

4117 SOUTH MERIDIAN
PUYALLUP, WA 98373
(253) 286-2888 PH
(253) 286-2451
WWW.AIBMR.COM



000002



Division of Biotechnology and GRAS Notice Review
Office of Food Additive Safety-CFSAN
U.S. Food and Drug Administration
5100 Paint Branch Parkway (HFS-200)
College Park, MD 20740-3835

January 30, 2014

ATTN: Dr. Antonia Mattia, PhD

Our Reference: GRAS Notification for Slimaluma™, a hydroethanolic extract of *Caralluma fimbriata*

Dear Dr. Mattia,

AIBMR Life Sciences, Inc. has been retained as an agent by GE Nutrients (“the Notifier”) to submit this GRAS notification on Slimaluma™, a hydroethanolic extract prepared from the dried aerial part of *Caralluma fimbriata*, intended for use as an ingredient in specified food. The extract is standardized to 25–29% pregnane glycosides, and batch analyses indicate that the ingredient is made consistently and meets all product specifications. Because of its GRAS status as outlined in this notification, Slimaluma™ is considered exempt from the requirement of pre-market approval, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act.

The GRAS determination has been made based on scientific procedures. The basis for the determination relies on animal safety studies that were performed on Slimaluma™, including an Ames, chromosomal aberration, 6-month repeated oral toxicity study, and developmental toxicity study. These studies were published in the *International Journal of Toxicology*. Additionally, 50 million servings worth of the ingredient have been sold in the US without reportable adverse events. Published clinical studies and the historical consumption of *C. fimbriata* are also detailed.

Please find enclosed one copy of the notification: *Notice to US Food and Drug Administration that the use of Slimaluma™, a Hydroethanolic Extract Prepared from Caralluma fimbriata, is Generally Recognized as Safe*. Also enclosed is an electronic copy of the notification, and copies of all references cited in the notification. As stated in the exemption claim, the data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of GE Nutrients, or will be sent to FDA upon request. Please do not hesitate to contact us with any questions.

Yours sincerely,

(b) (6)

John R. Endres
Chief Scientific Officer
john@aibmr.com

**4117 SOUTH MERIDIAN
PUYALLUP, WA 98373
(253) 286-2888 PH
(253) 286-2451
WWW.AIBMR.COM**

000003

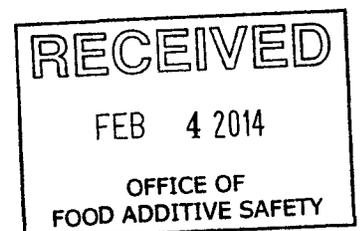
GRN 000500

**Notice to US Food and Drug Administration
that the use of Slimaluma™,
a hydroethanolic extract prepared from
Caralluma fimbriata,
is Generally Recognized as Safe**

Submitted by the Notifier:

GE Nutrients
920 East Orangethorpe Avenue, Suite B
Anaheim, CA 92801

January 28th, 2014



000004

**Notice to US Food and Drug Administration
that the use of Slimaluma™,
a hydroethanolic extract prepared from
Caralluma fimbriata,
is Generally Recognized as Safe**

Submitted by the Notifier:

GE Nutrients
920 East Orangethorpe Avenue, Suite B
Anaheim, CA 92801

January 28th, 2014

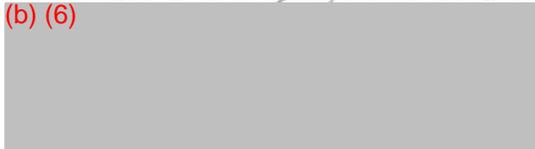
Table of Contents

GRAS Exemption Claim	3
Name and Address of the Notifier.....	3
Common or Usual Name	3
Conditions of Use	3
Basis for GRAS determination.....	4
Data/Information Availability Statement.....	4
Description and Characterization	4
Manufacturing and Production	5
Company Overview	5
Raw Materials.....	5
Manufacturing Overview	6
Specifications and Batch Analysis.....	7
Finished Product Specifications and Quality Control	7
Glycoside Content.....	9
Heavy Metal Analysis.....	9
Microbial Analysis	9
Residual Solvent Analysis.....	9
Residual Pesticide Analysis	9
Shelf-life Stability	9
Nutritional Information.....	11
Certification.....	12
Self-limiting Levels of Use	12
Safety Assessment of <i>Caralluma fimbriata</i>	12
Toxicology Studies.....	12
Ames (Bacterial Reverse Mutation) Assay.....	13
In Vitro Chromosomal Aberration Assay	13
Acute Oral Toxicity Study in Rats.....	14
13-Week Subchronic Oral Toxicity Study in Rats.....	14
6-Month Repeated Oral Toxicity Study in Rats	16
Developmental Toxicity Study in Rats.....	18
Clinical Studies	19
History of Consumption and <i>C. fimbriata</i> Products	20
Previous Sales and Reported Adverse Events	21
Intended Use and Estimated Exposure.....	21
Basis for the GRAS Determination.....	22
General Recognition.....	23
References	24

GRAS Exemption Claim

GE Nutrients has determined that Slimaluma™ is Generally Recognized as Safe (GRAS) for its intended use, consistent with section 201 (s) of the Federal Food, Drug and Cosmetic Act. The determination has been made based on scientific procedures, and therefore the use of Slimaluma™ for its intended purpose is exempt from the requirement of pre-market approval.

(b) (6)



1/30/2014

Mr. Jith Veeravalli
President and CEO
GE Nutrients

Date

Name and Address of the Notifier

Notifier

GE Nutrients
920 East Orangethorpe Avenue, Suite B
Anaheim, CA 92801

Agent of the Notifier

John R. Endres, ND
Chief Scientific Officer
AIBMR Life Sciences, Inc.
4117 S. Meridian
Puyallup, WA 98373
Tel: (253) 286-2888 x101; Fax: (253) 286-2451
john@aibmr.com

Common or Usual Name

Slimaluma™ is a hydroethanolic extract prepared from dried aerial parts of *Caralluma fimbriata*.

Conditions of Use

Slimaluma™ is intended for use at up to 1 g per day (up to 500 mg per serving) as an ingredient in foods for special dietary use in meal replacement products such as baked goods/bars, soups, and drinks/shakes. While adults are expected to be the primary consumers of the ingredient, there is no data to suggest a hazard to children who might consume it. Slimaluma™ is not intended for use in infant formula, meat, egg, catfish or any products that would require additional regulatory review by USDA.

Basis for GRAS determination

Scientific procedures are the basis for this GRAS determination.

Data/Information Availability Statement

The data and the information that serve as the basis for this GRAS determination will be available for review and copying at reasonable times at the office of GE Nutrients, 920 East Orangethorpe Avenue, Suite B, Anaheim, CA 92801; or will be sent to FDA upon request.

Description and Characterization

Slimaluma™ is a hydroethanolic extract prepared from the dried aerial parts of *Caralluma fimbriata*. The standardized extract contains 25–29% pregnane glycosides and is in the form of a brown powder.

Taxonomy and Background

The *Caralluma* R. Brown genus is comprised of several subgenera and species that are distributed across Africa (north, east and central), dry regions of tropical Asia and parts of the Mediterranean. This genus belongs to the *Asclepiadaceae* family, which is comprised of over 240 genera and includes around 3,400 species.¹⁻⁴

Caralluma fimbriata (Wallich) is synonymous with *Caralluma adscendens* var. *fimbriata* since it is classified as a *C. adscendens* variety, which further includes *C. adscendens* var. *adscendens*, *C. adscendens* var. *attenuate*, *C. adscendens* var. *carinata* and *C. adscendens* var. *geniculate*.³ Although these species are described as subgenera or varieties in the literature, *C. adscendens* is also designated as being synonymous with *C. attenuate* and *C. fimbriata*.^{1,5,6} The common Indian name for *C. fimbriata* is Cullee-moolayan¹ and this species is also referred to as the “Indian Hoodia”⁷ due to its appetite suppressing action.

Caralluma is an edible perennial succulent cactus-like botanical that grows in dry regions in the wild and is also frequently cultivated and grown in gardens and greenhouses.^{4,7} *C. fimbriata* is one of 13 *Caralluma* species (and 7 varieties) that are indigenous to India, with the majority of species native to peninsular or southern India where they have a long history of human use.^{4,8}

Various perennial *Caralluma* species are consumed daily as a vegetable source and they are also preserved by pickling and used to make chutneys.⁴ *Caralluma* is further reported in the literature as a famine food; for example, tribal people historically consumed *C. fimbriata* during long hunting excursions in order to suppress hunger.⁵ The various *Caralluma* species have also been used medicinally to alleviate stomach problems, pain, fever, migraines and inflammation, among others.^{7,6}

Chemical Composition

Botanicals in the *Asclepiadaceae* family are known to contain pregnane glycosides as part of their small molecule repertoire; these compounds are structurally diverse steroidal moieties and are recognized for their anorectic activity.⁹⁻¹³

The *Caralluma* genus is particularly rich in pregnane glycosides and a large number of these steroidal constituents and other phytochemicals, such as flavonoids, have been isolated, purified, characterized, and/or screened for biological activity in recent years.^{6, 7, 14-25} Basic pregnane glycosides identified in *Caralluma* spp. include caraumbellogenins, caraumbellosides, caratubersides, and boucerins, among many others.

Pharmacokinetic, Bioavailability and Metabolism

No investigation into the pharmacokinetics or bioavailability of *Caralluma* pregnane glycosides was found in the public domain. However, a pharmacokinetic and bioavailability study of another pregnane glycoside (P57) isolated from *H. gordonii* by methanol extract has been published.²⁶ Female Swiss mice were treated orally by gavage with 0.3 mL “enriched *H. gordonii* extract” that was equivalent to 25 mg P57/kg bw. A maximum serum concentration (C_{max}) of 2.89 $\mu\text{g}/\text{mL}$ was reached at 0.6 hours after treatment, and the authors stated that this corresponded to moderate gastrointestinal tract absorption. The degree of bioavailability was reported to be 47.5% and the half-lives of absorption and elimination were 0.13 h and 2.81 h, respectively. A plasma clearance rate of 1.09 L/h/kg was reported. In an earlier investigation the authors found that this pregnane glycoside exhibited high gastrointestinal degradation: a simulated gastric stability test indicated that the pregnane glycoside was rapidly broken down (hydrolyzed) to its aglycone derivative likely by bacterial and/or intestinal glycosidases. It is not unreasonable to expect a similar fate for ingested pregnane glycosides isolated from *C. fimbriata*.

Manufacturing and Production

Company Overview

GE Nutrients is a California based natural products company that specializes in natural botanical extracts designed to support the quality of human health and overall wellness through traditional Ayurvedic health solutions and rigorous scientific methodology. GE Nutrients is committed to providing high quality standardized herbal extracts that are free of contamination to consumers worldwide.

Raw Materials

The following raw materials are used in the manufacture of GE Nutrients' Slimaluma™:

- The main raw material used in the manufacture of Slimaluma™ is dried aerial parts of *C. fimbriata*.

- Potable water is used as one of the two solvents in the extraction process.
- Ethyl alcohol (90%, food-grade and CAS No. 64-17-5) is used as a solvent in the extraction process.
- Hexane (food-grade and CAS No. 110-54-3) is used in the manufacturing processes for the removal of resinous matter from the plant.
- Maltodextrin (USP-grade, CAS No. 9050-36-6) is an inactive ingredient used in the spray-drying process, as a stabilizing agent, and to achieve the standardized pregnane glycoside content. The ingredient is FDA GRAS, 21 CFR 184.1444 with no limitations other than current good manufacturing practices.

Manufacturing Overview

Slimaluma™ is a patented proprietary herbal extract manufactured for GE Nutrients by Green Chem™, an herbal extract and formulation company, located in Bangalore, India.

Manufacturing Facility

Green Chem™'s manufacturing facility was established in 1997 and employs US FDA validated analytical methods; Green Chem™ is an ISO 22000:2005 certified facility—Food Safety Management System, Certificate Reg. No. 99 510 00006/01. This facility is Scopes Organic Certified (ORG/SC/1010/001264) for production, processing and trading as set forth by requirements of India's National Program for Organic Product Standards.

Cultivated *C. fimbriata*

C. fimbriata seeds are initially cultivated in a greenhouse setting using manure-enriched soil and organic compost. Germinated seedlings are propagated to a height of 10 cm and transferred to individual polyethylene plant potting bags for 8–10 weeks with daily irrigation. At this stage plant branches have developed. Branches are cut and transferred to outdoor growing areas where they are grown for 6–8 months with irrigation at set intervals (3–4 days). Plants are also treated with a Neem oil natural insecticide every 3 months. Neem (*Azadirachta indica*) has a long history of human use²⁷ and Neem oil is widely used in cosmetics and is used as a natural organic insecticide.²⁸ The Neem oil specifications are as follows: azadirachtin NLT 1000 ppm, specific gravity 0.908–0.937, palmitic acid 13.6–16.2%, myristic acid 0.2–2.6%, stearic acid 14.4–24.1%, sulfur content 0.4–0.6%, saponification value 180–205 mg, iodine value 65–80 g, acid value NMT 15 mg, shelf-life of one year under proper storage conditions.

When plants reach a height of 10–15 cm, prior to its flowering stage, branches are harvested for the manufacturing of Slimaluma™. Additional branches are harvested every three months for the life span of the plant (2 years).

Brief Description of Manufacturing

Aerial parts of *C. fimbriata* are harvested before the flowering stage and dried outdoors in shaded cemented platform areas for 4–6 days until the moisture content is reduced to 10–15% (moisture is monitored by loss on drying using USP<731> method).

The dried plant material is mechanically pulverized using a hammer mill grinder and transferred to a stainless steel extractor where it is extracted with a water/ethanol solvent mixture. The extraction step is performed in a closed system for six hours at 75°C under mild pressure and repeated three times to ensure complete extraction of the herb's glucoside content. The extracts are combined, partially concentrated under reduced pressure and low temperature. The concentrated extract is washed with hexane to remove resinous matter and again concentrated to remove residual hexane. The extract is transferred to a spray dryer and spray-dried at an inlet temperature of 170–180°C and an outlet temperature of 80–90°C. The spray-dried extracted powder is transferred to a multi-mill and further ground to produce fine mesh particles that are then sifted to obtain uniform particle sizes. The extracted *C. fimbriata* powder is then transferred to a blender and mixed with maltodextrin to produce a uniform and homogenous lot that is standardized to contain 25–29% pregnane glycoside. At this stage, the product is heat sterilized and sieved, packed in food-grade double polyethylene bags and stored in blue high-density polyethylene drums. Twelve grams of dried *C. fimbriata* generates 1 g of Slimaluma™. The final product is subjected to quality control testing.

Specifications and Batch Analysis

Finished Product Specifications and Quality Control

The product specifications for Slimaluma™ that include physical characteristics, heavy metal, microbial, and pesticide analysis along with the methods for testing are listed in **Table 1**. Production consistency of Slimaluma™ batches along with product homogeneity is verified using various analytic assays. Green Chem™ conducts all of the listed analyses in house using General Analytical Methods (GAMs) that are either in accordance with United States Pharmacopeia (USP) procedures or are based on conventional/standard analytical laboratory practices. Every Slimaluma™ batch manufactured is subjected to all analyses and must conform to the specifications in order to establish reasonable batch consistency. As indicated in **Table 1**, three non-sequential batches of Slimaluma™ were shown to be reasonably consistent and met all of the product specifications.

Table 1. Slimaluma™ Batch Analysis with Product Specifications

Parameters and Analytical Methods ¹	Specification	CFE/11008 M.D. 4/2011	CFE/11055 M.D. 3/2012	CFE/12030 M.D. 10/2012
Physical Analysis				
Appearance and Color (Visual)	Brown Powder	Complies	Complies	Complies
Identification (HPTLC)	Positive	Complies	Complies	Complies
Solubility in water (Dissolution, GAM 3)	≥ 75%	97.7%	96.0%	96.2%
Moisture/Loss on Drying (USP <731>, IR, GAM 20)	≤ 10%	3.3%	3.1%	3.3%
Chemical Analysis				
Total Bitters (Gravimetric)	3–8%	6.8%	6.0%	6.1%
Total Saponin Glycosides (Gravimetric)	10–19%	11.5%	12.1%	12.0%
Total Pregnane Glycosides (HPLC)	25–29%	26.9%	27.2%	27.4%
Resinous Matter (Dissolution in Hexane)	≤ 1%	0.01%	0.02%	0.03%
Ash (USP <281>, GAM 17)	≤ 10%	4.7%	3.9%	4.0%
Particle size (USP <786>, Sieve Test, GAM 15)	98% min. passes through 20 mesh	100%	100%	100%
Heavy metal analysis (USP <231>, AAS, GAM 13)				
Lead	≤ 5 ppm	< 0.01 ppm	< 0.01 ppm	< 0.01 ppm
Cadmium	≤ 1 ppm	< 0.01 ppm	< 0.01 ppm	< 0.01 ppm
Arsenic	≤ 3 ppm	< 0.01 ppm	< 0.01 ppm	< 0.01 ppm
Mercury	≤ 1 ppm	< 0.001 ppm	< 0.001 ppm	< 0.001 ppm
Residual Pesticides: Organochlorine, Organophosphorous and Pyrethroid Insecticides (USP<561>, GC, GAM 39)				
Pesticides	Absent	Absent	Absent	Absent
Residual Solvent (USP <467>, GC with Headspace method, GAM 40)				
Ethanol	< 5000 ppm	Nil	Nil	Nil
Volatile Organic Compounds (USP <467>, GC with Headspace method, GAM 40)				
Chloroforms	Nil	Nil	Nil	Nil
1,4-Dioxane	Nil	Nil	Nil	Nil
Dichloromethane	Nil	Nil	Nil	Nil
Trichloroethylene	Nil	Nil	Nil	Nil
Hexane	Nil	Nil	Nil	Nil
Microbial analysis (USP <61>, GAM 28)				
Total Plate Count	≤ 1000 CFU/g	Complies	Complies	Complies
Yeast and Mold	≤ 100 CFU/g	Complies	Complies	Complies
Coliforms	Absent	Absent	Absent	Absent
<i>E. Coli</i>	Absent	Absent	Absent	Absent
<i>Salmonella sp.</i>	Absent	Absent	Absent	Absent
<i>Pseudomonas aeruginosa</i>	Absent	Absent	Absent	Absent
<i>Staphylococcus aureus</i>	Absent	Absent	Absent	Absent

¹In-house analytical methods and methods that are based on USP methods are employed.
M.D., manufacturing date; HPTLC, high-performance thin-layer chromatography; USP, United States Pharmacopeia;
GAM, general analytical method (in-house methods); AAS, atomic absorption spectroscopy;

Glycoside Content

Slimaluma™ is a standardized pregnane glycoside extract. Maltodextrin (approximately 9–10% by dry weight) is added to the final spray-dried extract to achieve a pregnane glycoside content of 25–29%. The batch analysis of three non-consecutive batches verified that the pregnane glycoside content is reasonably consistent between batches using an in-house high-performance thin-layer chromatography (HPTLC) method, Table 1. Bitters and saponin glycoside values for all batches were also reasonably similar and complied with product specifications.

Heavy Metal Analysis

Each batch of Slimaluma™ is subjected to heavy metal analysis by atomic absorption spectroscopy (AAS) using Green Chem™'s in-house method GAM 13 as per USP <231> to determine its lead, cadmium, arsenic and mercury content, as shown in Table 1. The analyses of three non-consecutive batches of Slimaluma™ verified that their heavy metal content was far below product specifications.

Microbial Analysis

Microbiological testing is performed on every batch of Slimaluma™ according to Green Chem™'s in-house method GAM 28 as per USP <61>, see Table 1. The analysis is conducted on every batch of Slimaluma™ to ensure that each batch meets microbial specifications. Analyses of three non-consecutive batches indicated acceptable levels of yeast, mold and aerobic bacteria. Furthermore, all three batches were free of *Escherichia coli* (*E. coli*), *Salmonella sp.*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Residual Solvent Analysis

Each batch of Slimaluma™ is tested for residual solvents using Green Chem™'s in-house method GAM 40 as per USP <476>. Solvents used in the manufacture of Slimaluma™ include ethyl alcohol (90%) and hexane. Three non-sequential batches of Slimaluma™ were analyzed using gas chromatography (GC) with headspace and verified the absence of these solvents in the final product, see Table 1. All batches were also free of other volatile organic compounds including chloroform, 1,4-dioxane, dichloromethane and trichloroethylene.

Residual Pesticide Analysis

Each batch of Slimaluma™ is analyzed for the presence of pesticide and insecticide residue according to Green Chem™'s in-house method GAM 39 as per USP <561> using GC with headspace. The analysis tests for the presence of organochlorine, organophosphorous and pyrethroid compounds, as shown in Table 1. Three non-consecutive batches of Slimaluma™ were analyzed and found to be free all tested compounds.

Shelf-life Stability

Slimaluma™ (batch number CFE/6021, manufactured July 2006) was subjected to a real time shelf-life stability assessment initiated July 16, 2006 and completed

January 1, 2012 (total of 66 months, 5.5 years). The batch was stored at 30 ± 2 °C and 65 ± 5 % relative humidity, and analyzed at set intervals for appearance, water solubility, loss on drying, total bitters, total saponin glycosides, total pregnane glycosides, and resinous matter. Microbial analysis was assessed at the beginning and end of the study. The outcome of this assessment is summarized in **Table 2**. The batch was also subjected to an accelerated shelf-life stability test (40 ± 2 °C and 75 ± 5 % relative humidity), **Table 2**. Microbial analysis was assessed at the beginning and end of the study.

Assessment of shelf-life stability at ambient conditions indicated that Slimaluma™ is stable for up to 66 months (5.5 years) when stored under normal storage conditions. That Slimaluma™ was further stable at elevated temperature for a period of 6 months also indicates that it will likely remain stable under these conditions for up to 5 years as indicated by the real time shelf-life test.

Table 2. Self-Life Stability testing of Slimaluma™

Ambient Storage Conditions							
30 ± 2°C, and 65 ± 5% Relative Humidity							
Parameter Specification	16 Jul. 2006	14 Oct. 2006	12 Jan. 2007	12 Apr. 2007	11 Jul. 2007	7 Jan. 2008	5 Jul. 2008
Appearance	Comply	Comply	Comply	Comply	Comply	Comply	Comply
Brown Powder							
Solubility in Water ≥ 75%	98.10%	98.0%	98.10%	98.20%	98.20%	98.10%	98.50%
Loss on Drying ≤ 10%	3.0%	3.3%	3.5%	3.2%	3.3%	3.0%	3.0%
Total Bitters ≥ 3%	6.40%	6.30%	6.20%	6.40%	6.10%	6.40%	6.30%
Saponin Glycosides ≥ 10%	18.70%	18.10%	18.70%	18.30%	18.50%	18.20%	18.70%
Pregnane Glycosides ≥ 25%	28.70%	28.50%	28.30%	28.50%	28.20%	28.40%	28.0%
Resinous Matter ≤ 1%	0.03%	0.02%	0.03%	0.02%	0.03%	0.02%	0.03%
Total Plate Count ≤ 1, 000 CFU/g	Comply	-	-	-	-	-	-
Yeast and Mold ≤ 100 CFU/g	Comply	-	-	-	-	-	-
Coliforms Absent	Absent	-	-	-	-	-	-
<i>E. Coli</i> Absent	Absent	-	-	-	-	-	-
<i>Salmonella sp.</i> Absent	Absent	-	-	-	-	-	-
<i>P. aeruginosa</i> Absent	Absent	-	-	-	-	-	-
<i>S. aureus</i> Absent	Absent	-	-	-	-	-	-

Ambient Storage Conditions Continue 30 ± 2°C, and 65 ± 5% Relative Humidity					Accelerated Storage Conditions 40 ± 2°C, and 75 ± 5% Relative Humidity		
Parameter Specification	5 Jul. 2009	5 Jul. 2010	5 Jul. 2011	1 Jan. 2012	16 Jul. 2006	14 Oct. 2006	12 Jan. 2007
Appearance Brown Powder	Comply	Comply	Comply	Comply	Comply	Comply	Comply
Solubility in Water ≥ 75%	98.50%	98.30%	98.10%	97.80%	98.50%	98.30%	98.10%
Loss on Drying ≤ 10%	3.2%	3.0%	3.1%	3.0%	3.2%	3.0%	3.1%
Total Bitters ≥ 3%	6.40%	6.30%	6.30%	6.10%	6.40%	6.30%	6.30%
Saponin Glycosides ≥ 10%	18.0%	18.70%	18.20%	18.10%	18.0%	18.70%	18.20%
Pregnane Glycosides ≥ 25%	28.20%	28.30%	28.20%	28.50%	28.20%	28.30%	28.20%
Resinous Matter ≤ 1%	0.02%	0.03%	0.03%	0.03%	0.02%	0.03%	0.03%
Total Plate Count ≤ 1000 CFU/g	-	-	-	Comply	Comply	-	Comply
Yeast and Mold ≤ 100 CFU/g	-	-	-	Comply	Comply	-	Comply
Coliforms Absent	-	-	-	Absent	Absent	-	Absent
<i>E. Coli</i> Absent	-	-	-	Absent	Absent	-	Absent
<i>Salmonella sp.</i> Absent	-	-	-	Absent	Absent	-	Absent
<i>P. aeruginosa</i> Absent	-	-	-	Absent	Absent	-	Absent
<i>S. aureus</i> Absent	-	-	-	Absent	Absent	-	Absent

Nutritional Information

The nutritional profile of one batch of Slimaluma™ (CFE/8020) is summarized in Table 3. ChromaDex™ completed the nutritional analysis according to procedures described by the Association of Analytical Communities (AOAC).

Table 3. Nutritional Value Assessment of Slimaluma™

Analytes	Methods	Amounts (per 100g)
Nutrient Analytes		
Total Calories/100 g	Calculated	361
Calories from Fat/100 g		1
Calories from Saturated Fat/100 g		1
Total Fat (%)	AOAC 996.06 (Modified)	0.14%
Saturated Fat (%)		0.07%
Monounsaturated Fat (%)		0.03%
Polyunsaturated Fat (%)		0.03%

Trans Fat (%)		0.01%
Cholesterol (mg/100 g)	AOAC 976.26 (Modified)	ND (RL 1.0)
Carbohydrates (%)	Calculated	86.5%
Dietary Fiber (%)	AOAC 991.43 (Modified)	1.5%
Total Sugar (%)	AOAC 977.20 (Modified)	1.01%
Fructose (%)		ND (RL 0.1)
Glucose (%)		ND (RL 0.1)
Sucrose (%)		1.01%
Maltose (%)		ND (RL 0.1)
Lactose (%)		ND (RL 0.1)
Protein (%)	AOAC 992.15, AACC 46-30	3.36%
Vitamin A (IU/100 g)	AOAC 2001.13; AACC 86-06	ND (RL 50)
Vitamin C (mg/100g)	AOAC 967.22, 984.26 (Modified)	ND (RL 1.00)
Metals		
Sodium (mg/100 g)	AOAC 975.03, 985.01, 990.08	206
Calcium (mg/100 g)		612
Iron (mg/100 g)		7.90
Contributing Analytes		
Moisture (g/100 g)	AOAC 934.06, 969.38, 977.21 (Modified)	3.55%
Ash (g/100 g)	AOAC 923.03	6.492%
Carotenoids		
Alpha-Carotene (IU/100 g)	AOAC 2005.07	ND (RL 0.5)
<i>cis</i> Beta-Carotene (IU/100 g)		ND (RL 0.5)
<i>trans</i> Beta-Carotene (IU/100 g)		ND (RL 0.5)
Total Beta-Carotene (IU/100 g)		0.00
Total Carotene (IU/100 g)		0.00

AOAC, Association of Analytical Communities; AACC, American Association of Clinical Chemists; ND, not detected; RL, reporting limit.

Certification

Slimaluma™ contains neither meat nor dairy and is certified as pareve by Star-K Kosher Certification. Slimaluma™ is further certified as Halal by the Islamic Food and Nutrition Council of America (IFANCA)—Certificate No.: GRE 4377.12007. IN

Self-limiting Levels of Use

There are no properties of Slimaluma™ that result in specific self-limiting levels of use.

Safety Assessment of *Caralluma fimbriata*

Toxicology Studies

Several toxicological evaluations using Slimaluma™ have been conducted in order to assess its safety.^{5, 29, 30} Summaries of these are provided below. The test article used in all of the studies was Slimaluma™, which is referred to as CFE (C. *fimbriata* extract). The Ames, chromosomal aberration, 6-month repeated oral

toxicity and developmental toxicity studies were published in the International Journal of Toxicology in June of 2013.³⁰

Ames (Bacterial Reverse Mutation) Assay

Study Description

The potential mutagenicity of CFE was evaluated in an Ames Salmonella test using a plate incorporation method and five strains of *Salmonella typhimurium* (TA97a, TA98, TA100, TA102, and TA1535).³⁰ The five *Salmonella* strains were exposed to 15–5000 µg CFE/plate in both the presence and absence of metabolic activation (rat liver S9) for 48 hours at 37°C. The study was completed twice to validate its outcome. The number of histidine revertant colonies was determined; the test article was considered mutagenic if a concentration-related and/or a reproducible increase in histidine revertant colonies was observed. CFE was considered non-mutagenic if no increase in histidine revertant colonies was observed. The study was conducted at INTOX (Pune, India), a GLP compliant facility, in accordance with Organization for Economic Co-operation and Development (OECD) 471 Guidelines for the Testing of Chemicals (Section 4, No. 471, adopted 21 July 1997).

Results

The revertant frequencies for all five *S. typhimurium* strains at all tested CFE concentrations (in the presence and absence of S9) compared to that of the vehicle control, and plate counts were within normal INTOX laboratory ranges. CFE had no mutagenic effect up to the maximum level tested (5000 µg/plate).

In Vitro Chromosomal Aberration Assay

Study Description

The potential clastogenic effect of CFE was assessed using an in vitro chromosomal aberration assay with human peripheral blood lymphocytes.³⁰ Lymphocytes were incubated for 3 hours at 37°C at three concentrations (500, 1500, and 5000 µg CFE/mL) with and without S9 and sampled at 24 hours. Standard positive controls were included and distilled water served as the negative control. Two hundred metaphases were evaluated for chromosomal aberrations and compared to the controls. The study was conducted at INTOX (Pune, India), a GLP compliant facility, in accordance with the OECD 473 Guidelines for the Testing of Chemicals (Section 4, No. 473, adopted 21 July 1997).

Results

The incidence/number of chromosomal aberrated cells (in the presence and absence of S9) at all CFE test concentrations did not differ significantly from that in the vehicle control group, and no dose-dependent response was reported. CFE did not induce structural chromosome aberrations up to 5000 µg CFE/mL. CFE was concluded as non-clastogenic in human peripheral blood lymphocytes.

Acute Oral Toxicity Study in Rats

Study Description

A 14-day acute oral toxicity study was conducted in Wistar rats (aged 3 months) at two CFE test concentrations, 2 and 5 g CFE/kg bw (formulated in distilled water (control/vehicle) at an administration volume of 2 mL/rat). Brief summaries of this study are available in the public domain (an abstract and a paragraph in a book chapter).^{5, 29} The study incorporated OECD Guidelines for the Testing of Chemicals (No. 420) but was not conducted according to GLP (St. John's Medical College, Bombay, India).

Laboratory Conditions and Tested Parameters

In the preliminary study (sighting study) two animals (one/sex) received a single dose of the test article at 2 g CFE/kg bw by oral gavage. After 48-hours two additional animals (one/sex) were treated to confirm/validate the outcome. The treated animals were then observed for 14 days. No mortalities occurred following the administration of 2 g CFE/kg bw. The dose was then increased to 5 g CFE/kg bw for the second study (main study) in which 20 rats (5/sex/group) received either a single dose of 5 g CFE/kg bw or the vehicle by oral gavage. The single administration of the test article was followed by a 14-day observation period. For both studies, animals were housed singly in Perspex cages in a room with 12-hour light-dark cycles, female rats were nulliparous and non-pregnant, and a one-week laboratory acclimation period preceded the study. Food and water were available *ad libitum*. After receiving the test article, animals were observed for signs of toxicity at set intervals. Daily observations included mortality, food/water intake and body weight changes. Urine and blood samples were collected on day 14 for analysis of the following parameters: urine and blood glucose, hemoglobin, white blood cells (WBCs), and differential count (percent lymphocytes). All animals were sacrificed (ether anesthesia) for gross pathological examinations and vital organs (brain, liver, kidneys, adrenals, spleen, heart, and lungs) were subjected to histological examination.

Results and Conclusion

No deaths were reported in either of the studies. No unusual behavioral changes or alterations to body weight or food/water intake were noted. Furthermore, no significant treatment-related hematological, gross pathological or histopathological findings were observed in the main study. The LD50 was concluded as greater than the highest dose tested (5 g/kg bw).

13-Week Subchronic Oral Toxicity Study in Rats

Study Description

A sub-chronic, 90-day repeated oral toxicity study was conducted with 100 Wistar rats (50/gender, aged 6–8 weeks). Brief summaries of this study are available in the public domain (as an abstract and a paragraph in a book chapter).^{5, 29} This study was conducted in accordance with OECD 408 Guidelines for the Testing of Chemicals (Section 4, No. 408, adopted 21 September 1998) at the Bombay College of Pharmacy, Mumbai, India. It was not GLP. Animals were

divided into to one of six treatment groups and the test article was formulated in distilled water at an administration volume of 2 mL/kg bw:

- Treatment groups: 0 (control), 90, 270, or 900 mg CFE/kg bw/d and 10 animals/sex/group; animals received the test article by oral gavage for 90 consecutive days.
- Satellite groups: 0 (control) and 900 mg CFE/kg bw/d and 5 animals/sex/group; animals received the test article by oral gavage for 90 consecutive days, followed by an additional 30-day recovery/observation period.

Laboratory Conditions and Tested Parameters

Animals were randomized and housed in polypropylene cages (five/cage), in a room set to maintain a temperature of 23–28 °C, a relative humidity of 50–70% and a 12-hour light/dark cycle. Food and water were available *ad libitum* and a 10-day laboratory acclimation period preceded the study. General cage-side and clinical observations were made during the treatment period and included body weights (on day 0 and weekly thereafter), daily food intake, mortality (twice daily), ophthalmological observations (day 0 and day 89), clinical observations (day 1 and weekly thereafter), and audio-visual reflex and grip strength tests (assessed during week 12). At the end of the treatment and recovery periods (treatment and satellite groups, respectively) animals were euthanized (chloroform anesthesia). Blood was collected for hematological and biochemical screening. Animals were subjected to necropsy examination to assess morphological and pathological changes, as well as organ weights (absolute and relative) were recorded.

Results and Conclusion

Four deaths occurred during the treatment period (90-days): one female in the 900 mg/kg group on day 78, one male in the 270 mg/kg group on day 78, one male in the 900 mg/kg on day 50 and one male in the 900 mg satellite group on day 18. Dramatic weight losses (30 g for the female rat and 33–100 g for male rats) and observed weakness preceded all deaths; however, no treatment-related histopathological abnormalities or tissue damage were observed upon necropsy, and the causes of death were not established. Several fluctuations in body weights and body weight gains were noted for all treated animals when compared to controls; however, overall final body weights were comparable to that of the control animals. Food consumption was comparable to the control group (with minor fluctuations in both directions) for females in all dose groups of the main study and for males in the 900 mg/kg satellite group. Females in the 900 mg/kg satellite group consumed significantly less than the control group during weeks 1 and 12 ($p < 0.05$), however no significant differences were seen during the remaining 15 weeks of the study/recovery period. Males in the main study consumed significantly ($p < 0.05$) less than controls in certain weeks throughout the study in all dose groups (consumption was significantly decreased compared to controls in 3 independent weeks for the 90 and 270 mg/kg groups, and in 7 independent weeks for the 900 mg/kg group). Subcutaneous fat loss as indicated by loose skin was reported in 20–50% of males in the 270 and 900 mg/kg groups and was in part attributed to the expected

action (anti-obesogenic effect) of CFE. These events were not seen in female rats or in any animals in the longer 6-month study described below. Sparse hair (shedding/hair loss) was reported for all males in the 270 and 900 mg/kg groups towards the end of the study (weeks 12–13); however hair shedding gradually reduced during the recovery period and was not seen in female rats or in the longer study described below; stress due to repeated oral gavage or grooming cannot be ruled out as a possible cause. All hematological and clinical chemistry parameters fell within the normal or expected ranges for laboratory values of Wistar rats. All organ weights and organ-to-body weight ratios compared to those recorded for the control animals.

Although four deaths occurred, the reason for the deaths could not be ascertained, as there were no other signs of morbidity. Histopathological examination did not reveal any abnormalities related to treatment. For surviving animals, general clinical observations, clinical chemistry and hematological markers, organ weights and histopathology were similar between the CFE treatment groups and the control group, indicating CFE to have low toxicity. The 90-day study was considered to be a sound rationale for conducting a longer, 6-month, repeated oral toxicity study conducted according to GLP.

6-Month Repeated Oral Toxicity Study in Rats

Study Description

A 6-month repeated oral toxicity study was conducted in 8-week old male and female Sprague-Dawley rats.³⁰ The study was conducted by INTOX (Pune, India) according to GLP, and in accordance with OECD 408 Guidelines for the Testing of Chemicals, Repeated Dose 90-day Oral Toxicity Study in Rodent, (Section 4, No. 408, 21 September 1998), OECD 452 Guidelines for the Testing of Chemicals, Chronic Toxicity Studies, (Section 4, No. 452, adopted 12 May 1981), and *US FDA Redbook 2000* standards, IV.C.4.a, Subchronic Toxicity Studies with Rodents.

Animals were randomly divided into to one of 6 treatment groups in which the test article was formulated in distilled water at an administration volume of 5 mL/kg bw:

- Treatment groups: 0 (control), 100, 300, or 1000 mg CFE/kg bw/d and 20 animals/sex/group; animals received the test article by oral gavage for 6 months (180 days).
- Satellite groups: 0 (control) and 1000 mg CFE /kg bw/d and 10 animals/sex/group; animals received the test article by oral gavage for 6 months, followed by an additional 28-day recovery/observation period.

Laboratory Conditions and Tested Parameters

Animals were acclimated to the laboratory conditions at 8 weeks of age with 2 animals of the same sex/group housed per cage in a room set to maintain a temperature of 19–25 °C, a relative humidity of 30–70%, and a 12 hour light-dark cycle. Food and water were available *ad libitum*.

The following observations/recordings were made during the treatment period: daily cage-side clinical examinations; mortality and morbidity observations (twice daily); body weights (day 0 and weekly); food intake per cage (weekly); weekly clinical examinations and neurological assessment; ophthalmological observations in the control and high dose groups (prior to and after the treatment period); sensory, grip strength and motor activity assessments in the control and high dose groups after 22–23 weeks of treatment; clinical observations (day 1 and weekly thereafter); and hematological and biochemical screening (10 randomly selected animals/group at set intervals); clinical pathologically and urinalysis was also done on these 10 animals at the end of the treatment period.

At the end of the treatment and recovery periods (treatment and satellite groups, respectively), the animals were sacrificed (CO₂ anesthesia) for gross pathological evaluation. Animals were subjected to necropsy examination to assess for morphological and pathological changes. In addition, organ weights were recorded. Histopathological examinations were conducted on animals in the control and high-dose groups. Neurotoxicity and immunotoxicity screenings were also conducted to assess CFE's effect on the nervous and immune systems.

Results and Conclusion

No signs of treatment-related morbidity were reported for the study. A single death of a male in the 300 mg group occurred due to gavage error. Body weight and average daily food intake values were similar between groups. No significant clinical, neurological or ophthalmological abnormalities were observed during either the treatment or recovery periods. Isolated, non-dose related incidences of increased lacrimation, corneal opacity, diarrhea, nasal discharge, circling disorder and hypoactivity were reported in male rats only and these observations were regarded as incidental and unrelated to CFE treatments. Several hematological and clinical chemistry fluctuations were observed throughout the treatment period; however these biological differences were not regarded as toxicologically significant since there were no dose trends (fluctuations occurred across all groups) and all statistically significant differences remained within historical laboratory ranges.

Organ weights (absolute and relative) compared to that of the control group with one exception; males in the 300 mg group had a statistically significantly higher relative organ weight for the epididymides. This finding was not seen in any of the other male CFE dose groups and was not dose-dependent. Values were 0.30, 0.33, 0.34 and 0.33% for males of the 0, 100, 300 and 1000 mg groups, respectively, and 0.27 and 0.28% for males in the 0 and 1000 mg satellite groups. Gross pathological and histopathological examinations did not identify any treatment-related effects. Given the fact that no dose-response was seen and that no treatment related histopathological findings were reported, this finding was regarded as incidental and not related to administration of the test article. Necropsy findings were similar between treatment and control groups. Neurotoxicity and immunotoxicity screenings also did not reveal any CFE-related effects.

All fluctuations noted in the study remained within historical laboratory ranges and were not associated with dose-dependent trends. Overall, no target organs were identified. The NOEL of this study was concluded to be 1000 mg/kg bw/d.

Although several findings were noted in the shorter non-GLP 90-day study with Wistar rats, such as hair shedding, loose skin and four unexplained deaths, these were not seen in this longer GLP compliant 6-month study with Sprague-Dawley rats. In addition, no treatment-related toxicity was observed in the developmental study described below.

Developmental Toxicity Study in Rats

Study Description

A prenatal developmental toxicity assessment was conducted in female Sprague-Dawley rats.³⁰ This study was conducted at INTOX (Pune, India), according to GLP and in accordance with OECD 414 Guidelines for the Testing of Chemicals, Prenatal Development Toxicity (Section 4, No. 414 adopted 22 January 2001), *US FDA Redbook 2000* standards, IV.C.9.b, Guidelines of Developmental Toxicities Studies (July 2002).

Animals were randomly divided into to one of 4 treatment groups in which the test article was formulated in distilled water at an administration volume of 10 mL/kg bw:

- Treatment groups: 0 (control), 250, 500, or 1000 mg CFE/kg bw/d and 10 animals/sex/group; animals received the test article by oral gavage from gestation days 6–19.

Laboratory Conditions and Tested Parameters

Animals were acclimated for 5 days to the laboratory conditions in a room set to maintain a temperature of 19–25 °C, a relative humidity of 30–70% and a 12 hour light-dark cycle; food and water were available *ad libitum*. Male and female rats (13–14 weeks old) were mated and the females received the test article from gestation days 6–19.

Observations to detect morbidity, mortality, clinical abnormalities and food intake were made daily, and body weights were recorded at set intervals. On gestation day 20, the female rats were sacrificed (CO₂ anesthesia) for maternal, pregnancy, litter and fetal observations.

Results and Conclusions

Three deaths occurred during the study; two females (in the 250 and 500 mg groups) died due to gavage errors and the death of one additional female (in the 250 mg group) was preceded by nasal discharges and increased lacrimation; however, necropsy did not reveal any signs of treatment-related abnormalities.

Food intake was not altered, and body weights were similar between all groups. CFE did not alter uterus parameters, rate of pregnancy or miscarriages. Litter parameters were also similar between groups, and isolated instances of minor fetal malformations were regarded as incidental due to the lack of statistical

significance or dose-dependent trends. No skeletal or soft-tissue abnormalities were reported.

With the lack of toxicologically relevant incidences, CFE was considered non-teratogenic up to the highest dose tested, 1000 mg/kg bw/d.

Clinical Studies

Two published human studies are available in the public that utilized CFE as the test article.^{31, 32} A total of 42 subjects ingested 1 g of CFE per day (500 mg of CFE before each meal for a total of 2 meals) for 12 weeks and both studies reported that CFE was well tolerated by the adult participants and that no serious adverse effects were reported in any of the studies. Brief descriptions of these studies are provided below:

(1) Body Weight and Anthropometric Measurements of Individuals Receiving 1 g CFE/day for a 60-Day Period

Fifty adults (male and female, aged 28–83 years, BMI > 25 kg/m²) participated in a double-blinded, placebo-controlled study in which participants were divided into one of two groups, a treatment group receiving CFE at 1 g/day (n=25) or a placebo group (n=25) receiving 1 g of maltodextrin.³¹ CFE was prepared by Green ChemTM (Bangalore, India) and encapsulated in standard gelatin capsules at 500 mg CFE per capsule (2 capsules = 1 g CFE). All study subjects were provided with standard health advice on diet and physical activity targeted toward losing 5–10% of body weight over the study period.

Anthropometric measurements were collected at baseline, at 30-days and at the end of the study (day 60), and participants attended weekly clinic visits to receive their treatment capsules, report any adverse effects, and body weight measurements were recorded at that time. Blood was collected at baseline, day 30 and at the end of the study (day 60) to determine blood glucose levels, and to record lipid profile parameters (triglycerides, total cholesterol, LDL and HDL). Dietary, physical activity and appetite assessments were recorded at baseline, day 30 and at the end of the study.

Individuals in the CFE group exhibited statistically significant ($P < 0.05$) reduction in final body weights, BMI, waist and hip circumferences and percent body fat compared to the respective baseline values; these findings were not reported for the placebo group. With the exception of a significant ($P < 0.001$) reduction waist circumference, the remaining parameters did not differ with statistical significance between the two groups. No changes in any of the tested blood parameters were seen; CFE did not alter glucose or lipid profiles for individuals receiving CFE for a 60-day period. A significant ($P < 0.001$) reduction in perceived hunger was reported for the CFE group compared to placebo.

Minor gastrointestinal track related adverse effects were reported, including bloating, flatulence, constipation and gastritis; these events were experienced by individuals in both the treatment (24%) and the placebo (20%) groups. Since the events occurred in both groups, it was not likely due to consumption of CFE. As such, CFE was well tolerated by subjects.

(2) Anthropometric Measurements of Individuals Receiving 1 g CFE/day for a 12-Week Period

A 12-week randomized, double-blind, placebo-controlled clinical trial with male and female adults (BMI > 25 kg/m²) indicated that participants (n=17) receiving 500 mg of CFE twice per day (1 g CFE/d) in combination with a calorie-restricted diet and physical activity exhibited favorable anthropometric outcomes beyond those experienced by participants in the placebo group (n=16) receiving maltodextrin (2 x 500 mg/d).³² Daily food intake (with a 500 kcal/d reduction) and physical activity for both groups were controlled and monitored.

Anthropometric measurements were collected at baseline, at 6-weeks and at the end of the study (12-weeks). Blood pressure and heart rates were collected at baseline, mid-way and at the end of the study. Biochemical analyses (fasting glucose, serum triglycerides, and total, LDL, and HDL cholesterol) were completed at baseline and at the end of the study and no significant differences were seen between groups at the end of the study.

Participants in both groups exhibited statistically significant ($P < 0.05$) reductions in final body weight, BMI, hip and waist circumferences and waist-to-hip ratios, and test meal palatability compared to baseline, however only waist-circumference (Difference: -3.847; 95% CI; -7.47 to 0.23) and waist-hip-ratio (Difference: -0.033; 95% CI; -0.06 to 0.00) were significantly different between the two groups.

Overall CFE was well tolerated; 2 participants in the CFE group experienced minor adverse events (skin rash and constipation) that subsided within two weeks of treatment termination (the author did not elaborate on this).

History of Consumption and *C. fimbriata* Products

According to the Wealth of India Dictionary of Raw Materials (Volume 3) *C. fimbriata* has a long history of human consumption particularly among the native and tribal population of peninsular India where the plant was historically harvested for use as a vegetable and also preserved and pickled.^{4, 7} Like many indigenous plants, this succulent cactus was likely incorporated into the daily diets of communities situated in rural areas. The plant is known as a famine food and an appetite suppressant since it was historically used during periods of food shortage and on hunting expeditions to reduce hunger.⁵

There are several dietary supplement products on the market that contain Slimaluma™ or other *C. fimbriata* extracts and the suggested daily intake ranges from 500 mg to up to 2000 mg taken prior to eating. Representations of such products are listed in **Table 4**.

Table 4. Representative List of *C. fimbriata* Products.

Company	Products	Level Per Serving and Per Day
NOW	Slimaluma PLUS 25% Pregnane Glycosides Serving Size: 2 Capsules Suggested Use: Take 2 capsules twice a day before meals.	500 mg CFE/ serving 1000 mg CFE/ day
Source Naturals	Slenderluma Slimaluma Serving Size: 1 Capsule Suggested Use: Take 1 capsule 2-3 times daily	500 mg CFE/ serving 1500 mg CFE/ day
Genesis Today	Caralluma Fimbriata Extract Serving Size: 2 Capsules Suggested Use: Take 2 capsules one (or more) a day before meals.	800 mg CFE/ serving ≥ 1600 mg CFE/ day
Solaray	Caralluma Fimbriata Ayurveda 25% Pregnane Glycosides Serving Size: 1 Capsule Serving Suggestion: Take one capsule daily with meals.	500 mg CFE/ serving 500 mg CFE/ day
BareNaturals	Pure Caralluma Fimbriata Serving Size: 2 Capsules Serving Suggestion: Take two capsules once (or more) a day before meals.	800 mg CFE/ serving ≥ 800 mg CFE/ day
Country Life	Caralluma Extracts Serving Size: 1 Capsule Serving Suggestion: Take one capsule before lunch and dinner.	500 mg CFE/ serving 1000 mg CFE/ day

CFE, *C. fimbriata* extracts (Slimaluma™)

Previous Sales and Reported Adverse Events

Slimaluma™ has been sold since 2005 around the world including the US, Canada, Mexico, Europe, India, South East Asia, Australia, Japan, Korea and China, among others. To date, bulk US sales of the raw material have reached over 50,000 kg, while bulk international sales are more than 40,000 kg for a total of more than 90,000 kg with no reportable adverse events.

To the best of our knowledge, FDA has not issued any letters regarding the concern for safety to companies that market products containing CFE. Additionally, a search of MedWatch (FDA’s adverse event reporting program) reveals no safety alerts or recalls for Slimaluma™, *C. fimbriata* or CFE; no warnings, recalls, or information related to the safety of the ingredient were found.

Intended Use and Estimated Exposure

Slimaluma™ is intended for use at up to 1 g per day (up to 500 mg per serving) as an ingredient in foods for special dietary use in meal replacement products such as baked goods/bars, soups, and drinks/shakes. While adults are expected to be the primary consumers of the ingredient, there is no data to suggest a hazard to children who might consume it. Slimaluma™ is not intended for use in

infant formula, meat, egg, catfish or any products that would require additional regulatory review by USDA.

Meal replacement products are generally used to replace one to two meals per day, as is discussed in the literature.³³⁻³⁶ Popular foods in the meal replacement market, such as Slim-Fast products, recommend replacement of up to two meals per day in their labeling. Thus, at a maximum addition level of 500 mg per serving, the estimated daily exposure to CFE is 500–1000 mg per day. If an individual were to consume an additional serving per day, the exposure would be on par with levels recommended for use as a dietary supplement; an exposure level at which no reportable adverse events have occurred after many years of sales, as discussed above.

Basis for the GRAS Determination

GE Nutrient's Slimaluma™, a hydroethanolic extract prepared from dried aerial parts of *Caralluma fimbriata*, has been the subject of a thorough safety assessment as described above. Batch analyses of Slimaluma™ show product consistency in meeting all product specifications.

As discussed in the Safety Assessment section above, a number of toxicological studies have been performed on this ingredient. An Ames, chromosomal aberration, 6-month repeated oral toxicity, and developmental toxicity studies have been published in a peer-reviewed academic journal specializing in toxicology. The Ames and chromosomal aberration tests concluded neither mutagenicity nor clastogenicity at CFE concentrations of 5000 mg of extract/plate and 5000 mg of extract/mL, respectively. An acute oral toxicity study in rats resulted in no mortality and no signs of unusual behavioral up to the highest dose group tested, 5000 mg/kg bw/d.

Furthermore, four deaths out of 100 animals occurred in a 90-day non-GLP repeated oral toxicity study conducted with Wistar rats (one male in the 270 mg/kg bw group, and one female and two males in the 900 mg/kg bw/d groups); deaths were preceded by dramatic weight-loss, however, the cause of death was not determined. No treatment related findings were seen upon histopathological examination. Towards the end of the study, males in the 270 and 900 mg groups exhibited hair shedding which was reversible. In surviving treated animals, general clinical observations, serum chemistry and hematology analyses, overall final body weights, organ weights, and histopathology were similar between the treatment groups and controls.

No treatment-related mortality occurred in the longer 6-month repeated oral toxicity study (GLP) in Sprague-Dawley rats (0, 100, 300 and 1000 mg/kg bw/d). All histopathological, urine, hematological and biochemical observations and parameters were comparable to those of the control animals and or remained within normal/historical laboratory ranges. No hair shedding or loose skin was observed in any of the treated rats and the NOEL for the study was the highest dose tested, 1000 mg/kg bw/d. A prenatal developmental toxicological study in Sprague-Dawley rats indicated no treatment-related maternal, pregnancy, developmental or fetal abnormalities up to the highest dose tested, 1000 mg/kg bw/d.

Lastly, two human studies, in which participants received a total of 1 g of Slimaluma™ per day for 12 weeks, suggested no treatment-related adverse events (events were similar among treatment and placebo groups), and the exposure to Slimaluma™ was considered well-tolerated.

Pivotal data for the GRAS determination include that the NOEL of the 6-month repeated-dose oral toxicity study was concluded as 1000 mg/kg bw/d, the highest dose group tested (suggesting that the true NOEL is likely higher than this limit dose); that no toxicologically relevant effects were observed in the developmental study up to 1000 mg/kg bw, the highest dose tested; that the results from the two clinical studies using 1 g per day for 12 weeks were without adverse events of concern; and that there is significant history of human consumption worldwide of CFE as a dietary supplement (50,000 kg, equivalent to 50 million 1000 mg servings, have been sold since 2005 in the US with no reportable adverse events). This data is corroborated by a history of human consumption of the *C. fimbriata* plant as a food. Together this data provides for the conclusion that the intended use of Slimaluma™ is reasonably certain to be safe under the conditions of its intended use.

General Recognition

The scientific studies that provide the basis of this GRAS determination, and information related to the historical consumption of *C. fimbriata* as well as additional scientific studies which corroborate the scientific safety data, are published and available in the public domain. The reference section of this notification cites these studies. This data provides ample evidence of consensus among qualified experts that there is reasonable certainty that consumption of Slimaluma™ for its intended use is reasonably certain to be safe. The general availability of this information satisfies the common knowledge component of this GRAS notification.

References

1. Gupta A and Sharma M. Caralluma R.Br (Asclepiadaceae). Reviews on Indian Medicinal Plants (Ca-Ce). New Delhi: Indian Council of Medical Research: 2007. 462.
2. Albers F and Meve U. Asclepiadaceae. Illustrated handbook of succulent plants: asclepiadaceae: 2002. 5-8.
3. Muller B and Albers F. Caralluma. Illustrated handbook of succulent plants: asclepiadaceae: 2002. 46-49.
4. Ambasta S. Caralluma R.Br. (Asclepiadaceae). The wealth of India: A dictionary of Indian raw materials & industrial products: raw materials, vol. 3: Ca-Ci. New Delhi: Publications & Information Directorate (CSIR): 1993. 266-267.
5. Venkatesh R and Rajendran R. Chapter 34. Role of caralluma fimbriata in weight management. Obesity: epidemiology, pathophysiology, and prevention. D Bagchi and H Preuss. Boca Raton: CRC Press: 2007. 443-449.
6. Avula B, Shukla YJ, et al. Quantitative determination of pregnanes from aerial parts of Caralluma species using HPLC-UV and identification by LC-ESI-TOF. *J AOAC Int.* 2011; 94: 1383-90.
7. Dutt HC, Singh S, et al. Pharmacological review of caralluma R.br. With special reference to appetite suppression and anti-obesity. *J Med Food.* 2012; 15: 108-19.
8. Rajaram K, Priya D, et al. In vitro regeneration of Caralluma fimbriata Wall. by organogenesis: a potent medicinal plant. *AJCS.* 2012; 6: 41-45.
9. Deepak D, Khare A, et al. Review Article Number 49: Plant Pregnanes. *Phytochemistry.* 1989; 28: 3255-3263.
10. Vajha M, Audipudi A, et al. Biochemical study of caralluma species to understand species homology. *International Journal of Applied Biology and Pharmaceutical Technology.* 2011; 2: 139-144.
11. van Heerden FR, Marthinus Horak R, et al. An appetite suppressant from Hoodia species. *Phytochemistry.* 2007; 68: 2545-53.
12. MacLean DB and Luo LG. Increased ATP content/production in the hypothalamus may be a signal for energy-sensing of satiety: studies of the anorectic mechanism of a plant steroidal glycoside. *Brain Res.* 2004; 1020: 1-11.
13. Shukla YJ, Pawar RS, et al. Pregnane glycosides from Hoodia gordonii. *Phytochemistry.* 2009; 70: 675-83.
14. Abdel-Sattar E, Ahmed AA, et al. Acylated pregnane glycosides from Caralluma russeliana. *Phytochemistry.* 2007; 68: 1459-63.
15. Abdel-Sattar E, Harraz FM, et al. Acylated pregnane glycosides from Caralluma tuberculata and their antiparasitic activity. *Phytochemistry.* 2008; 69: 2180-6.
16. Ahmad V. New pregnane glycosides from caralluma tuberculata. *J Nat Prod.* 1988; 51: 1092-1097.
17. Lin L, Lin L, et al. Pregnane glycosides from caralluma umbellata. *Phytochemistry.* 1994; 35: 1549-1553.
18. Halim AF and Khalil AT. Pregnane glycosides from Caralluma retropiciens. *Phytochemistry.* 1996; 42: 1135-9.

19. Qiu S, Cordell G, et al. Bisdesmosidic pregnane glycosides from *Caralluma lasiantha*. *Phytochemistry*. 1999; 50: 485-491.
20. Abdul-Aziz Al-Yahya M, Abdel-Sattar E, et al. Pregnane glycosides from *Caralluma russeliana*. *J Nat Prod*. 2000; 63: 1451-3.
21. Abdel-Sattar E, Abdul-Aziz Al-Yahya M, et al. Penicillosides A-C, C-15 oxypregnane glycosides from *Caralluma penicillata*. *Phytochemistry*. 2001; 57: 1213-7.
22. Abdel-Sattar E, Meselhy MR, et al. New oxypregnane glycosides from *Caralluma penicillata*. *Planta Med*. 2002; 68: 430-4.
23. Braca A, Bader A, et al. New pregnane glycosides from *Caralluma negevensis*. *Tetrahedron*. 2002; 58: 5837-5848.
24. Waheed A, Barker J, et al. Novel acylated steroidal glycosides from *Caralluma tuberculata* induce caspase-dependent apoptosis in cancer cells. *J Ethnopharmacol*. 2011; 137: 1189-96.
25. Kunert O, Rao VG, et al. Pregnane glycosides from *Caralluma adscendens* var. *fimbriata*. *Chem Biodivers*. 2008; 5: 239-50.
26. Madgula VL, Ashfaq MK, et al. Bioavailability, pharmacokinetics, and tissue distribution of the oxypregnane steroidal glycoside P57AS3 (P57) from *Hoodia gordonii* in mouse model. *Planta Med*. 2010; 76: 1582-6.
27. Kumar VS and Navaratnam V. Neem (*Azadirachta indica*): prehistory to contemporary medicinal uses to humankind. *Asian Pac J Trop Biomed*. 2013; 3: 505-14.
28. EPA. Cold pressed neem oil. PC Code 025006. U.S. Environmental Protection Agency, Office of Pesticide Programs. 2012. 1-21.
29. Jagtap A, Shirke S, et al. Toxicological evaluation of *caralluma fimbriata* extract in wistar rats [Abstract]. *AAPS J*. 2006; e.
30. Odendaal AY, Deshmukh NS, et al. Safety Assessment of a Hydroethanolic Extract of *Caralluma Fimbriata*. *Int J Toxicol*. 2013.
31. Kuriyan R, Raj T, et al. Effect of *Caralluma fimbriata* extract on appetite, food intake and anthropometry in adult Indian men and women. *Appetite*. 2007; 48: 338-44.
32. Astell K, Mathai M, et al. A pilot study investigating the effect of *Caralluma fimbriata* extract on the risk factors of metabolic syndrome in overweight and obese subjects: a randomised controlled clinical trial. *Complement Ther Med*. 2013.
33. Anderson JW, Luan J, et al. Structured weight-loss programs: meta-analysis of weight loss at 24 weeks and assessment of effects of intervention intensity. *Advances in Therapy*. 2004; 21: 61-75.
34. Phelan S, Nallari M, et al. What do physicians recommend to their overweight and obese patients? *J Am Board Fam Med*. 2009; 22: 115-22.
35. Ditschuneit HH. Do meal replacement drinks have a role in diabetes management? *Nestle Nutr Workshop Ser Clin Perform Programme*. 2006; 11: 171-9; discussion 179-81.
36. Heymsfield SB, van Mierlo CA, et al. Weight management using a meal replacement strategy: meta and pooling analysis from six studies. *Int J Obes Relat Metab Disord*. 2003; 27: 537-49.

Pages 000030-000315 have been removed in accordance with copyright laws. Please see the bibliography list of the references that have been removed from this request on pages 000028-000029.