

Exhibit 2





## **Caralluma fimbriata**

A new dietary supplement in weight management strategies

# ***Caralluma fimbriata* extract – a new dietary ingredient as a supplement in weight management strategies.**

## **Introduction**

Excessive body weight is no longer regarded merely as a cosmetic problem. It is a causal factor in chronic conditions like diabetes, atherosclerosis, hypertension, bone disorders, endocrine dysfunction and other ailments.

In addition to physiological ailments, excessive body weight can pose challenges to mental well-being as well. Such patients are predisposed to lower self-esteem, clinical depression, anxiety neuroses and eating disorders.

According to the World Health Organisation (WHO), weight management accounts for tens of billions of dollars in direct healthcare costs worldwide. A panel of experts convened by WHO stated on 12 June 1997 that '*... its impact is so diverse and extreme that it should now be regarded as one of the greatest neglected public health problems of our time. It has an impact on health, which may well prove to be as great as that of smoking*' (World Health Organisation, 1997).

Weight management therefore has become a matter of high priority for health administrators throughout the developed world.

## **Weight management – Important Considerations.**

### *Lifestyle parameters.*

There are key lifestyle parameters that contribute to excessive weight-gain. In the developed world, the increasing preference for mass-produced food over traditional home cuisine, increased intake of sugar and fatty foods, stress and lack of aerobic activity, all contribute to unhealthy weight gain.

One crucial element in the weight management strategies, therefore, is to ensure that the patient adheres to a healthy diet and exercise program.

### *Pharmaceutical intervention*

It is not always possible for patients to strictly adhere to a prescribed diet and a sustained exercise program. Age, climatic conditions, availability of prescribed foods, geographical considerations and pre-existing medical conditions can prevent the patient from following a diet and exercise program over a prolonged period of time.

In such cases, pharmaceutical intervention using prescription drugs becomes necessary.

Some of these prescription drugs include:

#### *1) Thermogenetic drugs.*

As the term implies, such drugs are postulated to increase core body temperature slightly, which process increases the burning of deposited lipids in the body. Since the core body temperature is controlled in the brain and thyroid gland, such drugs are believed to act on these systems.

By its nature, thermogenesis is not free from risk. Invariably, thermogenesis results in the over-stimulation of vital functions including cardiac rhythm, blood pressure, neurotransmitter levels and endocrine function. Patients experience nervousness, anxiety, hypersensitivity to stimuli, insomnia and irregular heart beats.

#### 2) *Appetite suppressants*

The suppression of excessive hunger is another approach to weight management.

Conventional sympathomimetic drugs can work as appetite suppressants but they carry with them the risks associated with sympathetic stimulation. These adverse effects are similar to the thermogenic class of drugs and include cardiac disturbances, elevated blood pressure, anxiety, insomnia and hypersensitivity.

#### 3) *Lipase inhibition*

Lipase is an enzyme that enables the body to absorb fat. Inhibiting this enzyme, prevents the absorption of fat, which gets eliminated by the large intestine.

Patient compliance is a consideration with lipase inhibitors, with several gastrointestinal side-effects reported. Uncontrollable gas and oily or fatty stools have been reported. Other GI side-effects include increased bowel movements, oily spotting between bowel movements, bowel urgency, inability to control bowel movements and inhibited absorption of fat-soluble vitamins.

#### 4) *Kreb's cycle inhibition*

The Citric Acid Cycle or the Krebs Cycle is a critical pathway for the body because it generates the energy the body needs for its day-to-day activities.

The Krebs Cycle generates energy from glucose. When excess energy is generated it is stored by the body in the form of fat. When glucose rises, fat is formed in adipose (fat) tissue and in the liver through a process called *gluconeogenesis*.

In theory, inhibition of Krebs Cycle at selected points can inhibit the synthesis of fat by the body and accelerate the rate at which the body burns off stored fat.

### **Supplemental approaches to weight management**

India is home to well developed indigenous systems of medicine. The science of Ayurveda has been practiced in India since 4000 years.

There are several treatises on Ayurveda which are used even today in modern practice. Ayurveda and other systems of traditional medicine use modern methods of documentation and data preservation. Even so, traditional texts are believed to describe not more than 5,000 plants of medicinal value.

Many medicinal plants and vegetables of medicinal value used across India are not documented in the texts but are used nevertheless on a daily basis for various curative properties by native populations in India.

It is noteworthy that in native Indian populations, many ailments that afflict developed societies are unheard of. For example, type 2 diabetes is uncommon amongst tribals in India. The same is the case with hypertension, hypercholesterolemia and obesity.

In urban India, the incidence of type 2 diabetes, obesity and related ailments is on the rise, whereas rural Indian populations are largely unaffected by this rise.

This is due to two factors.

*i) Lifestyle*

Native populations have a lifestyle that is marked by frugal diets and vigorous aerobic activity. In many parts of interior India, walking is still the preferred mode of transport. Diets are largely millet based and any meat is used fresh. Preservatives are not used, minimal oil is used in cooking. Salt is used sparingly and more emphasis is placed on freshness and flavor.

Native Indians walk long distances, as part of their daily routines. Traditional farming methods are still used and these are dependent on hard physical effort.

*ii) Traditional / indigenous dietary supplements*

In addition to their healthy lifestyles, Indian native populations employ a wide range of dietary supplements which form part of their regular traditional diets.

Many herbs are used as supplements. Some in fact are used as vegetables and condiments and cooked along with daily food.

A typical example would be fenugreek (*Trigonella foenum-graecum*). Fenugreek is used in Indian cooking as a condiment, flavor enhancer and digestive. Roasted fenugreek seeds are used as a condiment, fenugreek leaves are used as vegetables and raw fenugreek seeds are given as digestives. Fenugreek has been well documented in Indian texts for its use as an anti-diabetic agent, which has been confirmed by modern-day clinical studies. Fenugreek has also been demonstrated to reduce LDL levels in blood, in controlled double-blind clinical trials.

Another example is bitter melon or karela (*Momordia charantia*). Bitter melon is used as a vegetable in Indian cooking. The bitter principles found in momordia are known to be useful in the management of elevated blood sugar. Diabetics in India are prescribed momordia as part of their daily diet, even by allopathic practitioners.

There are innumerable such examples of functional foods being used in Indian cuisine. The traditional Indian approach to health has always been holistic in nature, incorporating a healthy life style, stress management, functional foods and prescribed herbal remedies for specific ailments.

Tribal Indians use the same approach in health management. Use of locally available resources is central to this concept.

Accordingly, their daily diet reflects this approach, in that plants of medicinal value are treated as, and consumed as, vegetables.

*CARALLUMA FIMBRIATA - EDIBLE CACTUS.*

In keeping with the holistic approach, native populations of India consume several locally growing medicinal plants as part of their diets.

Edible, succulent cactii grow wild all over India and are part of the daily diets of several native populations. The *Caralluma* genus is one such genus of edible cacti, which includes several species, many of which grow across India.

*Caralluma fimbriata* is the most prevalent of these species and it flourishes in large parts of interior India. It grows wild in urban centers as well and is planted as a roadside shrub and as a boundary marker in gardens.

*Caralluma fimbriata* is essentially a vegetable of daily use in tribal India. It is eaten in several forms. It is cooked as a regular vegetable, with spices and salt, it is used in preserves like chutneys and pickles and it is even eaten raw.

**Botanical Description**

*Caralluma fimbriata* ( Roxbury) Family : Asclepiadaceae

**Synonym :** *Caralluma ascendens*

**Local names :**

Kullee mooliyan, kallimudayan ( tamil)

Karallamu ( Telegu)

Yugmaphallottama ( Sanskrit)

Ranshabar, makad shenguli, shindala makadi ( marathi)

This large group consists of tender succulents found wild in Africa, the Canary Islands, India, Arabia, southern Europe,

Ceylon, and Afghanistan. The plants of this group vary from thin, recumbent stems from ½ to 1½ inches thick to erect growing clumps up to 8 inches high. The spines that cover the angled stems are actually leaves. The star-shaped, fleshy flowers of these plants are some of the worst smelling of the succulent plants. Ordinarily borne in late summer, the foul-smelling blossoms are usually colored purple, black, yellow, tan, maroon, red, or dark brown. They are from ½ to 2 inches or more across and borne at the base of the plant. In the wild, these blossoms are pollinated by flies, which are greatly attracted to the plant.

### History of Use

*Caralluma fimbriata* has been in use since centuries in India.

It is commonly used as a vegetable in several regions of India. It is eaten raw or cooked with spices, it is also used in pickles and chutneys. Indian tribals chew chunks of *Caralluma fimbriata* to suppress hunger when on a day's hunt. The cactus is used among the labor class in South India to suppress appetite and enhance endurance.

In the Kolli hills of South India, *Caralluma fimbriata* is a vegetable used daily. In the arid regions of Andhra Pradesh, *Caralluma fimbriata* is used in pickles and chutneys.

In Western India, *Caralluma fimbriata* is well known as a famine food, appetite suppressant and thirst-quencher. The green follicles are eaten, boiled and salted.

In Kerala, South India, *C. fimb* is used as a vegetable and appetite suppressant among tribal populations.

It also finds use today as an appetite suppressant and famine food during times of famine, in the semi-arid regions of India.

*Wealth of India*, the Indian Health Ministry's comprehensive compilation on medicinal plants, lists *Caralluma fimbriata* as a vegetable, used in curries, pickles or eaten raw. Exhibit 9

#### Safety profile

During its entire history of use, over centuries, on the Indian sub-continent, not a single adverse event has been reported on *Caralluma fimbriata*.

Several testimonials from botanical experts, noted Ayurvedic practitioners, University professors and botanists across India, have been attached, and testify to the safety and complete lack of toxicity of *Caralluma fimbriata*. Exhibit 8

These testimonials also attest to its use as in daily diets of local populations in the regions of India in which *Caralluma fimbriata* grows. The tribal community treats *Caralluma Fimbriata* as a food item for daily consumption. They believe that *Caralluma* is a unique herb which cures common health problems apart from its fantastic ability to suppress appetite and thirst. They eat a handful of *Caralluma* chunks during their hunting schedule which may last many days in a stretch. A bag full of *Caralluma* chunks are enough to cater the tribal group when they go for hunting; no need to carry food.

*Caralluma fimbriata* - Phytochemical constituents

The key phytochemical constituents are the **Pregnane Glycosides, Flavone Glycosides, Megastigmane Glycosides, Bitters principles, Saponins etc.**, The following are the specific active components:

Caratuberside A

Caratuberside B

Bouceroside I

Bouceroside II

Bouceroside III

Bouceroside IV

Bouceroside V

Bouceroside VI

Bouceroside VII

Bouceroside VIII

Bouceroside IX

Bouceroside X

Tomentogenin

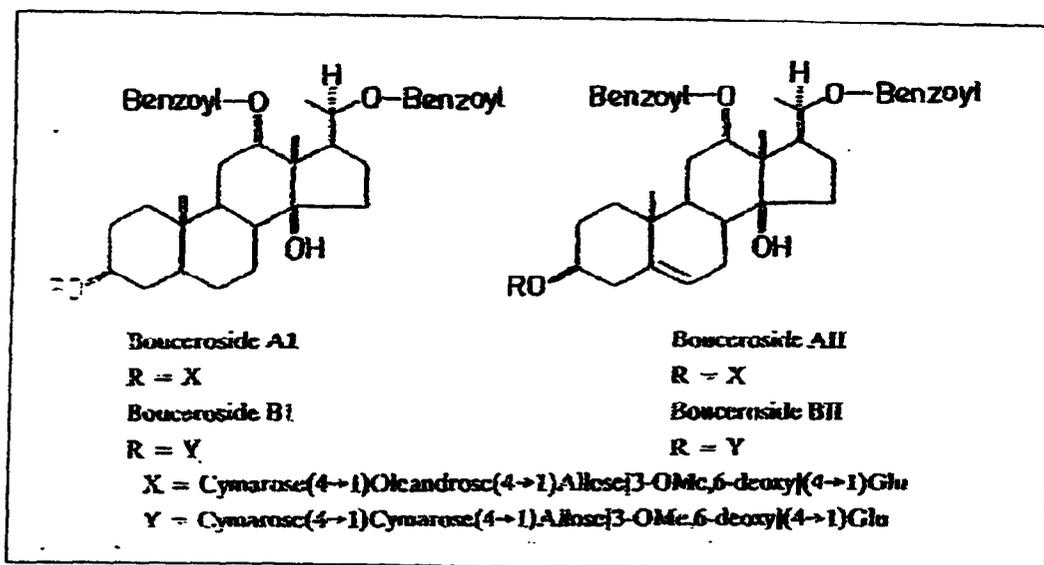
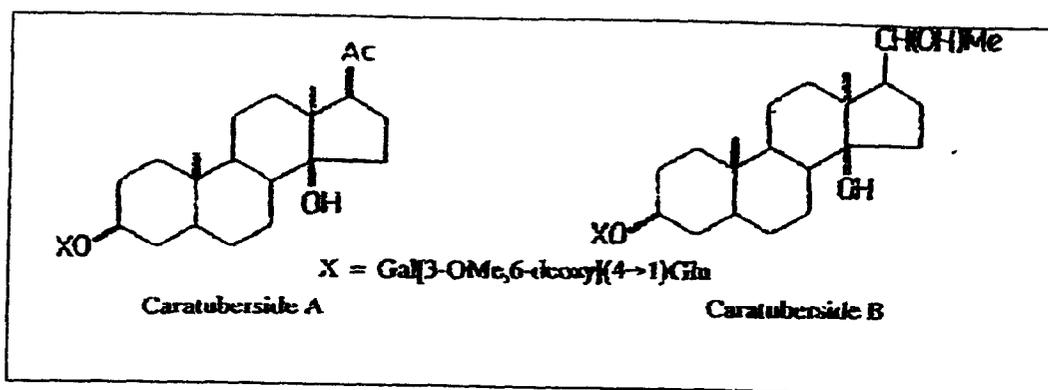
Sitosterol,

Luteolin-4-neohesperidoside, and

Kaempferol-7-O-neohesperidoside.

The Pregnane glycosides are common to the *Caralluma* genus and various congeners are to be found in all *Caralluma* species, including *Caralluma fimbriata*.

Chemical structures of the Pregnane Glycosides are given below:



## CARALLUMA FIMBRIATA EXTRACT

***Caralluma fimbriata* Extract** is presented herewith for consideration as a New Dietary Ingredient, *for appetite suppression leading to weight-loss*, based upon its prolonged history of use in India and testimonials attached thereof, and based upon the results of two clinical trials and one acute toxicity study on Wistar rats.

### *Safety considerations*

Based on the long history of daily consumption of *Caralluma fimbriata* in India, in spite of which no adverse events have ever been reported, it was thought appropriate to develop and clinically test a hydro-alcoholic extract of *Caralluma fimbriata*.

### *Caralluma fimbriata* extract - Manufacturing Considerations

In its natural state, *Caralluma fimbriata* is either consumed raw or cooked with boiling water. It is also consumed as a dry powder. In addition, it is crushed, boiled and used in pickles and chutneys.

When developing extraction and manufacturing methodologies for herbs, the attempt is always to duplicate the use of that plant in its natural state, as much as possible, while bearing in mind the necessities of mass production.

An extract of the plant must be offered in an acceptable dosage form and in a reasonable dosage that ensures patient compliance, which at the same time, is equivalent to the daily consumed amount in the natural state.

Clinical Trial Number : 1

**USE OF CARALLUMA FIMBRIATA EXTRACT TO REDUCE WEIGHT.**

*Anura V Kurpad, Rebecca Raj, Amarnath L*

*Division of Nutrition, St John's Medical College and Hospital, Sarjapur Road, Bangalore 560 034 INDIA*

**ABSTRACT**

A double-blind, placebo-controlled, randomized clinical trial on *Caralluma fimbriata* extract was done on 50 human subjects. This study consisted of 50 obese patients, 25 on the active compound and 25 on placebo. The trial lasted for eight weeks. *Caralluma fimbriata* is an edible succulent cactus, that is native to India and well known amongst native Indian populations for its appetite suppressant properties. Subjects were tested for changes in key indicators of weight-loss, including anthropometry , body fat composition, BMI, net weight and systemic functions.

**The following are the key observations made in this trial:**

**Statistically significant reductions were recorded in all key indicators of weight-loss.**

***Caralluma fimbriata* extract was well tolerated**

***Caralluma fimbriata* extract showed minimal adverse effects which were gastrointestinal and transient in nature.**

**Clinical Trial- Use of Caralluma fimbriata extract to reduce weight.**

**Conducted at: Division of Nutrition, St John's Medical College and Hospital, Sarjapur Road, Bangalore 560 034 INDIA**

**Under the supervision of :**

**Prof Anura V Kurpad, MD, PhD, Dean, Institute of Population Health and Clinical Research**

**Co-investigators:**

**Dr. Rebecca Raj, MSc, MPhil.**

**Dr Amamath L. MBBS, PGCD ( Human Nutrition)**

### **Introduction**

**A double-blind, placebo-controlled, randomized clinical trial on Caralluma fimbriata extract was done on 62 human subjects.**

**The trial was conducted under the purview of the Institutional Ethics Review Board of St Johns' National Academy of Health Sciences, Bangalore India.**

## **Patient Considerations**

**Subjects recruited 62**

**Dropouts -12**

**Subjects on placebo -25**

**Subjects on active medicine - 25**

**Duration of the project January -August 2003**

**Time points : Visit 1 (initial) Visit 2 (after 1 month) Visit 3 (after 2 months)**

### **Selection criteria**

**Obese patients, BMI > 26, adult, no pre-existing medication, both genders, normal health, normal lifestyle, no dietary restrictions.**

### **Exclusion criteria**

**Type 1 diabetes, essential hypertension, Type 2 diabetics on Metformin, endocrine dysfunction, arrhythmia, cardiac disorders, alcohol dependence or drug dependence.**

## Lifestyle counseling

No changes were advised in existing dietary patterns. All subjects were advised to walk for 30 mins, morning and evening. No other alterations were made to existing activities.

## Clinical tests

The following parameters were estimated :

### *Anthropometry*

Body weight in kg  
Waist circumference in cm  
Mid-arm-circumference in cm  
Hip circumference in cm

### *Fat Analysis*

Body fat was estimated by bio-impedance analysis. This test specifically determines the amount of fat in the body. It is a more relevant indicator of the efficacy of a weight-loss product. Weight measurements alone do not suffice, since weight loss may be due to loss of water, as has been observed during crash-diet programs.

*Blood chemistry and Systemic function tests*

**Fasting and post-prandial blood sugar**

**Lipid profile comprising total cholesterol, LDL, HDL, triglycerides**

**Renal function tests comprising serum creatinine and blood urea**

**Liver function tests comprising total protein, serum albumin, total bilirubin**

**conjugated bilirubin, AST and ALT and alkaline phosphatase**

**Blood picture comprising hemoglobin and gamma GT**

**These tests were done to ensure that *Caralluma fimbriata* extract does not have any adverse effects on vital systemic functions.**

*Cardiovascular function*

**Blood pressure, ECG**

**These tests were used to ascertain whether any sympathomimetic effects were being brought about by *Caralluma fimbriata* extract.**

**No changes were observed in the ECG, between all three time points, for any subject.**

*Appetite suppression*

Visual analog scales were used for the following parameters:

Thoughts of food

Fullness

Urge to eat

Hunger

Caralluma fimbriata extract is an appetite suppressant. These visual analog scales were specifically used to test this particular property of Caralluma fimbriata extract.

Detailed data on all the above tests, on each subject, for each time point, are presented in Exhibit 6

**Adverse Effects of Caralluma fimbriata extract**

The adverse effects observed were gastrointestinal in nature. In both active and placebo groups, these effects were observed.

Nearly all subjects reported moderate acidity, constipation and flatulence. In all cases, these effects subsided within a week after commencement of the trial.

It must be noted that subjects on placebo also reported the same gastro-intestinal adverse effects.

Therefore, it seems evident that these adverse effects were not being caused by Caralluma fimbriata extract, but by the gelatin capsules themselves.

It is pertinent to mention that the Indian dietary system is largely vegetarian in nature, especially in South Indian populations, who formed the subject population of this study. The gelatin used in hard capsules is derived largely from animal bones. Digestive systems accustomed to vegetarian diets will react adversely to

gelatin in the diet, at least for a few days. This is what was observed during the trial. All gastrointestinal effects subsided in a week after commencement and thereafter no further complaints occurred

**Conclusion of the trial**

Clinical Trial Number : 2

Caralluma Fimbriata Extract has been tested clinically by Western Geriatric Research Institute, Los Angeles, California, USA.  
The summary is given below:

**Caralluma Fimbriata in the treatment of Obesity**

*Ronald M. Lawrance and Suneeta Choudhary*

*Western Geriatric Research Institute, Los Angeles, California, USA*

**ABSTRACT**

This study consisted of 26 overweight patients, 19 on active compound, Caralluma Fimbriata Extract, and 7 on placebo, who were followed for four weeks.

The conclusions are excellent and the major observations are given below:

**The substance, Caralluma Fimbriata Extract showed a statistically significant reduction in bodyweight**

**Caralluma Fimbriata Extract was well tolerated**

**Caralluma Fimbriata Extract has shown long safety record with little, if any, side effects**

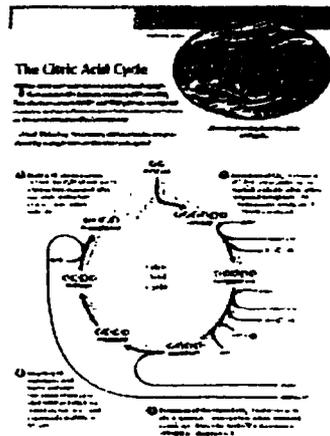
The details of this clinical study are given in Exhibit 7

Caralluma fimbriata extract - Proposed mechanism of action

*Kreb's Cycle* or the *Citric Acid Cycle*, takes place in the mitochondria, which are found in the cells of most living beings. Mitochondria are known as the body's "powerhouse" because they generate energy for the body from the food we eat.

The body obtains energy when carbohydrates, especially glucose molecules are broken down through a process called catabolism. This process is coupled with the synthesis of a high-energy molecule called *ATP (adenosine tri-phosphate)* which provide chemical energy to the body, much like a car battery which converts chemicals to electricity.

Glucose is broken down into a compound called *pyruvic acid*, which enters the mitochondria. Pyruvic acid in turn is broken down to *acetic acid* and ultimately, it is converted to *acetyl co-enzyme A* and *citric acid*, hence the name Citric Acid Cycle.



During this cycle, ATP is formed. The Krebs Cycle is a critical pathway for the body because it generates ATP, which in turn generates the energy the body needs for its day-to-day activities.

How is fat formed?

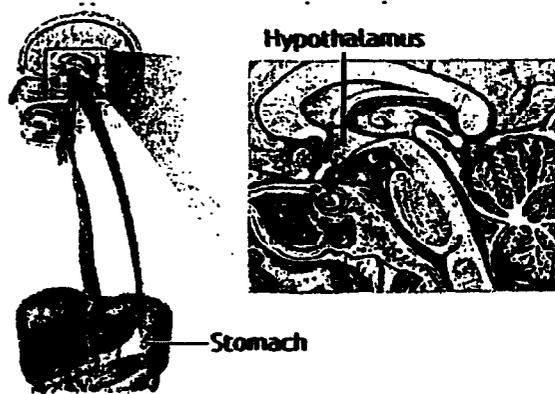
The Krebs Cycle described above, generates energy from glucose. What happens when too much energy is generated? This is stored by the body in the form of fat. When glucose rises, fat is formed in adipose (fat) tissue and in the liver through a process called *gluconeogenesis*.

Fat is formed after the Krebs Cycle and is synthesized in the form of fatty acids. The basic building block of fatty acids is *Acetyl Coenzyme A*, which is mentioned above.

Now, for the formation of AcetylCoA, a vital enzyme called *citrate lyase* is required. If this enzyme is blocked, then fat cannot be made by the body.

*Caralluma fimbriata* - mode of action.

*Caralluma fimbriata* contains pregnane glycosides which are believed to block the activity of citrate lyase. By blocking this enzyme, *Caralluma fimbriata* blocks the formation of fat by the body.



Further, *Caralluma fimbriata* also blocks another enzyme called Malonyl Coenzyme A. By blocking this enzyme, fat formation is further blocked and the body is forced to burn its fat reserves.

This accelerates the rate of fat loss by the body.

This accelerates the rate of

### Appetite Suppression

*Caralluma* is believed to act in another way as well. Controlled clinical trials on *Caralluma fimbriata* extract clearly demonstrate its ability to suppress appetite.

Therefore, *Caralluma fimbriata* is believed to have an activity on the appetite control mechanism of the brain.

*Figure 1 : Showing the appetite center in the brain, the hypothalamus.*

When we eat, nerves from the stomach send a signal to the hypothalamus in the brain. This is the part of the brain that controls appetite. When the stomach is full, the hypothalamus signals the brain to stop eating. When a person is hungry, the hypothalamus sends a signal to the brain that food is needed.

Conversely, by interfering with the signal or by creating a signal on its own, *Caralluma fimbriata* seems to fool the brain into thinking that the stomach is full, even when the person has not eaten.

This is how appetite suppression occurs.

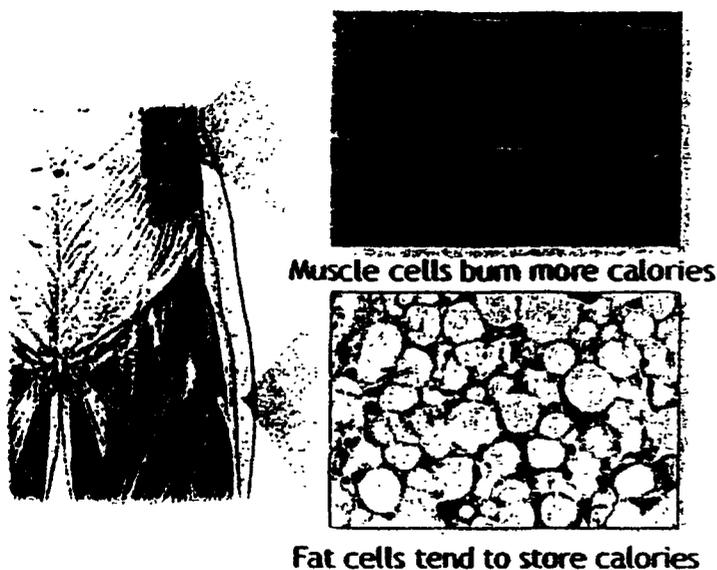
*Caralluma fimbriata* has been clinically demonstrated to suppress appetite and stop hunger pangs in patients. It is believed that the pregnane glycosides in *Caralluma fimbriata* inhibit the hunger sensory mechanism of the hypothalamus.

Generation of lean muscle mass.

The main reason why most weight-loss programs fail is that the patient always feels dull and tired after attaining weight loss. This makes the patient go back to his old eating habits and results in a rebound weight gain.

Patients on *Caralluma* however, report feeling more energetic and have gained lean muscle mass, while losing fat.

The reason for this is, that *Caralluma* not only inhibits fat synthesis as mentioned above, it also increases the burning of fat. This makes more energy available to the body and makes the patient more active and lively.



*Figure 2 : Fat cells v/s muscle cells.*

It is a well-known fact that muscle cells burn more calories than fat cells. So, when more energy is available to the body, muscle cells burn energy faster. The result is, fat cells shrink in size and muscle becomes stronger. Muscle cells are heavier than fat cells, but they are denser than fat cells. So, they occupy less space and the patient appears trim and compact, as compared to before.

Patients on Caralluma have clearly shown significant decrease in arm circumference and waist circumference, along with fat loss.

The reason for this is explained above.

Thus, by acting on different levels in the body's biochemical processes and brain function, Caralluma fimbriata works as an effective appetite suppressant and potent weight-loss agent.

**Caralluma Fimbriata Extract- Safety Pharmacology (LD50 study)**

The acute oral toxicity of Caralluma Fimbriata Extract was extensively studied by the Department of Pharmacology , St. John's Medical College, Bangalore, India.

**Dr.B.V.Venkataraman, Professor of Dept. of Pharmacology has given the following comments:**

The objective of this study was to determine the acute oral toxicity of Caralluma Fimbriata Extract following oral administration in male and female Wistar rats. For the sighting study a dose of 2 g/kg body weight was administered and for the main study a dose of 5 g/kg body weight was administered. All animals survived until the scheduled necroscopy at the end of the study period of 14 days.

Therefore Caralluma Fimbriata Extract did not produce signs of toxicity at very high doses of 5 g/kg and it could be classified in the classification of : LD50 more than 5 g/kg.

The details of this LD 50 study is given in Exhibit 5

References:

Various references from reported literature are given in Exhibit 9