

Safety and mechanism of appetite suppression by a novel hydroxycitric acid extract (HCA-SX)

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Abstract

A growing body of evidence demonstrates the efficacy of *Garcinia cambogia*-derived natural (–)-hydroxycitric acid (HCA) in weight management by curbing appetite and inhibiting body fat biosynthesis. However, the exact mechanism of action of this novel phytopharmaceutical has yet to be fully understood. In a previous study, we showed that in the rat brain cortex a novel HCA extract (HCA-SX, Super CitriMax™) increases the release/availability of radiolabeled 5-hydroxytryptamine or serotonin ($[^3\text{H}]\text{-5-HT}$), a neurotransmitter implicated in the regulation of eating behavior and appetite control. The aim of the present study was 2-fold: (a) to determine the effect of HCA-SX on 5-HT uptake in rat brain cortex *in vitro*; and (b) to evaluate the safety of HCA-SX *in vivo*. Isolated rat brain cortex slices were incubated in oxygenated Krebs solution for 20 min and transferred to buffer solutions containing $[^3\text{H}]\text{-5-HT}$ for different time intervals. In some experiments, tissues were exposed to HCA-SX (10 μM –1 mM) and the serotonin receptor reuptake inhibitors (SRRI) fluoxetine (100 μM) plus clomipramine (10 μM). Uptake of $[^3\text{H}]\text{-5-HT}$ was expressed as d.p.m./mg wet weight. A time-dependent uptake of $[^3\text{H}]\text{-5-HT}$ occurred in cortical slices reaching a maximum at 60 min. HCA-SX, and fluoxetine plus clomipramine inhibited the time-dependent uptake of $[^3\text{H}]\text{-5-HT}$. At 90 min, HCA-SX (300 μM) caused a 20% decrease, whereas fluoxetine plus clomipramine inhibited $[^3\text{H}]\text{-5-HT}$ uptake by 30%. In safety studies, acute oral toxicity, acute dermal toxicity, primary dermal irritation and primary eye irritation, were conducted in animals using various doses of HCA-SX. Results indicate that the LD_{50} of HCA-SX is greater than 5,000 mg/kg when administered once orally *via* gastric intubation to fasted male and female Albino rats. No gross toxicological findings were observed under the experimental conditions. Taken together, these *in vivo* toxicological studies demonstrate that HCA-SX is a safe, natural supplement under the conditions it was tested. Furthermore, HCA-SX can inhibit $[^3\text{H}]\text{-5-HT}$ uptake (and also increase 5-HT availability) in isolated rat brain cortical slices in a manner similar to that of SRRI, and thus may prove beneficial in controlling appetite, as well as treatment of depression, insomnia, migraine headaches and other serotonin-deficient conditions. (Mol Cell Biochem 238: 89–103, 2002)

Key words: *Garcinia cambogia*, (–)-Hydroxycitric acid, fluoxetine, clomipramine, rat brain cortex, serotonin release, appetite, acute oral toxicity, acute dermal toxicity, primary dermal irritation, primary eye irritation

Introduction

(–)-Hydroxycitric acid (HCA), a popular dietary supplement for weight loss, is a natural extract from the dried fruit rind of *Garcinia cambogia* (family *Guttiferae*), a tree that is native to southeast Asia. The dried fruit rind is also known as *Mala-*

bar tamarind, which is extensively used in southern India for culinary purposes [1]. The fruit exhibits a distinctive sweet and sour taste, and has been used for centuries with no harmful effects in Asian countries for preparing culinary dishes. It has been observed that when the dried fruit rind is sprinkled on curries it makes the food more filling and satisfying [1, 2].

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HCA has been shown to be a competitive inhibitor of ATP-citrate lyase, the enzyme catalyzing the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA. This enzyme is important in maintaining the acetyl-CoA pool for fatty acid and cholesterol biosynthesis, particularly during the hyperlipogenic nutritional state produced by high carbohydrate feeding. One study has shown that HCA administered before meals causes weight loss in obese subjects [2] and improves energy metabolism. HCA reduces food consumption in humans by possibly diverting carbohydrates and fatty acids that would have become fat in the liver into hepatic glycogen. This metabolic change may send a signal to the brain that results in reduced food intake, curbs the appetite, and the desire for sweets is also eliminated [3, 4]. Research has shown that HCA-SX inhibits fat production and decreases body weight in animals and humans [5]. No toxicity, either acute or chronic at any reasonable level of consumption has been observed.

Recent studies have demonstrated that oral HCA supplementation (as Super CitriMax™, a calcium/potassium salt of 60% HCA, which is tasteless, odorless and highly water soluble, HCA-SX) is highly bioavailable in human plasma as demonstrated by a gas chromatography-mass spectrometric method [6]. HCA-SX remained in the blood for more than 4–9 h after oral ingestion. These investigators also demonstrated that eating a meal shortly after taking HCA-SX reduced food absorption by about 60% [6].

HCA-SX has been shown to increase the release/availability of [³H]-5-hydroxytryptamine (serotonin; HT) from rat brain cortical slices *in vitro* [7]. Since 5-HT has been implicated in the regulation of eating behavior and body weight regulation [8–10], it is reasonable to suggest that a mechanism of appetite suppression induced by HCA-SX could be mediated by this neurotransmitter. Indeed, stimulants of this monoamine reduced food intake and weight gain and increased energy expenditure in both experimental animals and humans [8].

The objective of the present study was 2-fold, (a) to further investigate the effects of HCA-SX on 5-HT metabolism by examining the action of this novel appetite suppressant on the uptake of this monoamine in isolated rat brain cortex *in vitro*, and (b) to determine the safety profile of HCA-SX. For the latter, we conducted acute oral toxicity, acute dermal toxicity, primary dermal irritation and primary eye irritation studies in *in vivo* models.

Materials and methods

Chemicals

A natural, highly water soluble, calcium/potassium salt of 60% HCA extract from *Garcinia cambogia* commercially known as Super CitriMax™ HCA-SXS (HCA-SX) was obtained from

InterHealth Nutraceuticals, Benicia, CA, USA. [³H]-5-HT was purchased from NEN Life Sciences, Boston, MA, USA. Fluoxetine and clomipramine were obtained from Sigma-Aldrich, Natick, MA, USA. All other chemicals and reagents were obtained from Sigma Chemicals (St. Louis, MO, USA) and were of analytical grade or the highest grade available.

Animals and treatment for 5-HT uptake study

Male Sprague–Dawley rats (weighing 150–200 g) were obtained from Charles River Breeding Laboratories (Portage, MI, USA). Animals were maintained and used in accordance with the current National Institute of Health Guidelines and the ARVO Resolution on the Use of Animals in Research.

The effect of HCA-SX on radiolabeled 5-HT (serotonin) uptake was measured as described by Kirksey and Slotkin [11] and Goodlet *et al.* [12] with some modifications. Briefly, Male Sprague–Dawley rats were sacrificed by asphyxiation and the whole brain was quickly dissected and placed in ice-cold saline solution (0.9% w/v NaCl). The cortex was carefully removed from ice-cold saline solution and cut into thin slices of about 15–30 mg weight using surgical blades and scissors. Although tissues obtained differed slightly in size, the results are presented as d.p.m./mg wet weight in order to normalize the data. Cortex slices were equilibrated in 2 ml of Krebs buffer solution at 37°C under an atmosphere of 95% O₂ and 5% CO₂ for 20 min. The Krebs buffer solution was composed of the following (millimolar); sodium chloride, 118; potassium chloride, 4.8; calcium chloride, 1.3; potassium dihydrogen phosphate, 1.2; sodium bicarbonate, 25; magnesium sulfate, 2.0; and dextrose, 10 (pH 7.4). After equilibration, cortex slices were incubated in Krebs buffer solution (gassed with 95% O₂ and 5% CO₂) at 37°C, containing 80 nM of [³H]-5-HT and pargyline (10⁻⁵ M). During incubation, two slices were removed at times 0, 5, 10, 20, 25, 30, 60, and 90 min and rinsed in 10 ml ice-cold Krebs buffer solution to stop uptake of [³H]-5-HT into neurons. Each slice was blotted dry with Whatman paper #1, weighed and digested in 1 ml NaOH (1 N) at 60°C for 20 min. Each sample was combined with 12 ml of aqueous scintillation cocktail (Ecolume; ICN Radiochemicals, CA, USA), and analyzed for radioactivity by liquid scintillation spectrometry. The amount of [³H]-5-HT present in samples was expressed as d.p.m./mg wet weight. Test drugs were present in the Krebs buffer solution during the pre-incubation and incubation periods.

Animals and treatment for acute oral toxicity study

The objective was to determine the acute oral median lethal dose and evaluate the potential systemic toxicity of HCA-SX when administered as a single dose to Albino rats. The protocol was designed and the study was conducted in compli-

ance with the Environmental Protection Agency Guidelines for Registering Industrial Chemicals in the US (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals Section 81-1), the Toxic Substances Control Act (TSCA) Health Effects Test Guidelines, 40 CFR 798.1175.

Male and female Albino rats (weighing 208–216 g at study initiation) were obtained from Charles River Breeding Laboratories (Portage, MI, USA) and were allowed free access to lab chow (Purina Certified Rodent Chow, No. 5002, St. Louis, MO, USA) and municipal water *ad libitum*. Animals were acclimated to laboratory conditions for a minimum of 7 days prior to initiation of dosing. The animal room was kept at a controlled temperature (69–76°F), humidity (30–82%), and light (12 h light/12 h dark).

The amount of HCA-SX administration was based on body weights taken just prior to dosing using a dose volume of 10 ml/kg. HCA-SX was administered orally via gastric intubation with a French rubber feeding tube (14-gauge), which was affixed to a 3-ml syringe. The rats were fasted approximately 18–20 h prior to dosing and returned to feeding 3–4 h after dosing. One group of five male and one group of five female rats were administered a single dose at a level of 5000 mg/kg. Control animals received the vehicle.

The rats were observed for mortality at approximately 1, 3 and 4 h post-treatment on day 0 and twice daily (morning and afternoon) thereafter for 14 days. For clinical observations, the rats were observed at approximately 1, 3 and 4 h post-treatment on day 0 and once daily thereafter for 14 days. Body weights were obtained and recorded on study days –1, 0 (initiation), 7 and 14 (study termination). Upon study termination, all rats were euthanized by carbon dioxide asphyxiation. At the terminal necropsy, the major organ systems of the cranial, thoracic and abdominal cavities were examined for all animals.

Animals and treatment for acute dermal toxicity study

The objectives of this study was to determine the median lethal dose, evaluate the potential systemic toxicity and evaluate the local irritative potential of HCA-SX when applied once to the skin of Albino rabbits. The protocol was designed and the study was conducted in compliance with the Environmental Protection Agency Guidelines for Registering Industrial Chemicals in the US (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals Section 81-2), the Toxic Substances Control Act (TSCA) Health Effects Test Guidelines 40 CFR 798.1100, and the Japanese Agricultural Chemicals Laws and Regulations Testing Guidelines for Toxicology Studies published by the Society of Agricultural Chemical Industry under the auspices of MAFF (Ministry of Agriculture, Forestry and Fisheries).

Male and female New Zealand white Albino rabbits (weighing 2227–2466 g at study initiation) were obtained from Hazleton Research Products (Denver, PA, USA) and allowed free access to lab chow (Purina Certified Rodent Chow, No. 5322, St. Louis, MO, USA) and municipal water *ad libitum*. Animals were acclimated to laboratory conditions for a minimum of 6 days prior to initiation of dosing. The animal room was kept at a controlled temperature (66–70°F), humidity (50–80%), and light (12 h light/12 h dark).

Individual doses of the HCA-SX were calculated based on body weight obtained just prior to dosing with a dose of 2000 mg/kg bodyweight. HCA-SX was applied directly to clipped, unabraded skin. On the day prior to dosing, the hair was removed from the backs of the rabbits using an Oster® small animal clipper. Individual doses of HCA-SX moistened with approximately 4.5 ml of deionized water, were applied to the dorsal skin and covered approximately 15% of the total body surface. Plastic restraint collars were applied and remained on the rabbits for the duration of the exposure (24 h). Upon completion of the exposure period, the collars and bandages were removed and the application sites were wiped with disposable paper towels moistened with tepid water. There was one group of five male Albino rabbits and one group of five female Albino rabbits that was dermally administered single doses (24 h, semi-occluded exposure) of HCA-SX at a dose level of 2000 mg/kg.

The rabbits were observed for mortality at approximately 1, 3 and 4 h post-treatment on day 0 and twice daily (morning and evening) thereafter for 14 days. For clinical observations, the rabbits were observed at approximately 1, 3 and 4 h post-treatment on day 0 and once daily thereafter for 14 days. For dermal observations, the application sites were examined for erythema, edema (Table 1) and other dermal findings beginning approximately 30–60 min after bandage removal and daily thereafter for 13 days. The rabbits were shaved to facilitate dermal observations on study days 3, 7, 10 and 14. The body weights were obtained and recorded on days 0 (initiation), 7 and 14 (study termination). The rabbits were euthanized by intravenous injection of sodium pentobarbital solution (150 mg/kg, i.v.) upon termination of the study. The major organ systems of the cranial, thoracic and abdominal cavities were examined for all animals.

Animals and treatment for primary dermal irritation study

The objective was to determine the irritative potential of HCA-SX following a single exposure to the skin of Albino rabbits. The protocol was designed and the study was conducted in general compliance with the Environmental Protection Agency Guidelines for Registering Industrial Chemicals in the US (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals Section 81-5); the Toxic

Table 1. Acute dermal toxicity study of HCA-SX in Albino rabbits: Scoring criteria for dermal reactions

Evaluation of dermal reactions*					
Value	Erythema and eschar formation	Computer designation	Value	Edema formation	Computer designation
0	No erythema	No erythema	0	No edema	No edema
1	Very slight erythema (barely perceptible, edges of area not well defined)	Very slight erythema	1	Very slight edema (barely perceptible, edges of area not well defined)	Very slight edema
2	Slight erythema (pale red in color and edges definable)	Slight erythema	2	Slight edema (edges of area well defined by definite raising)	Slight edema
3	Moderate to severe erythema (definite red in color and area well defined)	Moderate erythema	3	Moderate edema (raised approximately 1 mm)	Moderate edema
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)	Severe erythema	4	Severe edema (raised more than 1 mm and extending beyond area of exposure)	Severe edema

*Source: Draize *et al.* [13].

Substances Control Act (TSCA) Health Effects Test Guidelines, 40 CFR 798.4470; the Organization for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Section 404; and the Japanese Agricultural Chemicals Laws and Regulations Testing Guidelines for Toxicology Studies published by the Society of Agricultural Chemical Industry, under the auspices of MAFF (Ministry of Agriculture, Forestry and Fisheries).

Two male and four female Albino New Zealand White rabbits (weighing 3477–3958 g at study initiation) were obtained from Hazleton Research Products (Denver, PA, USA) and were allowed free access to lab chow (Purina Certified Rabbit Chow, No. 5322, St. Louis, MO, USA) and municipal water *ad libitum*. Animals were acclimated to laboratory conditions for a minimum of six days prior to initiation of dosing. The animal room was kept at a constant temperature (70–72°F), humidity (42–48%), and light (12 h light/12 h dark).

The route of HCA-SX administration was direct application to shaved intact skin. This route of administration is standard for assessment of local dermal irritative potential. HCA-SX was assessed on two male and four female rabbits. On the day prior to dosing, the hair was removed from the backs of the rabbits using as Oster® small animal clipper. The HCA-SX was moistened with approximately 0.5 ml deionized water and applied to an area of skin approximately 2.5 × 2.5 cm under a secured 2-ply gauze patch, that was overwrapped with a gauze binder and secured with Dermiform® tape. Plastic restraint collars were applied and remained on the animals for the duration of the exposure period. One intact site per rabbit was used and the dosage level was 500 mg/site. Each animal received a single, 4-h, semi-occluded exposure. At the end of 4 h, the collars and bandages were removed and the sites wiped with disposable towels moistened with deionized water.

The rabbits were observed twice daily (morning and afternoon) for mortality for the duration of the study. For dermal

observations, the application sites were observed for erythema, edema and other dermal findings at approximately 30–60 min and 24, 48 and 72 h after patch removal. Dermal irritation was graded in accordance with the method of Draize [13]. In order to facilitate dermal observations, the rabbits were shaved approximately 1 h prior to collecting the 72 h dermal scores. The Primary Dermal Index was calculated from the scores recorded at 30–60 min, 24, 48 and 72 h after patch removal. The mean scores for erythema and edema were calculated separately to the nearest tenth and added together. Based on this value, the Draize grading system (Table 2) was used to arrive at the primary dermal irritation descriptive rating.

Body weights were obtained and recorded on study day 0 (initiation) and at each rabbit's termination from the study. Upon termination of the study, the rabbits were euthanized by intravenous injection of sodium pentobarbital solution and discarded.

Animals and treatment for primary eye irritation study

The objective was to determine the irritative potential of HCA-SX following a single exposure to one eye of Albino rabbits. The protocol was designed and the study was conducted in compliance with the Environmental Protection Agency Guidelines for Registering Industrial Chemicals in the US (Pesticide Assessment Guidelines, Subdivision F, Hazard Evaluation: Human and Domestic Animals Section 81-4); the Toxic Substances Control Act (TSCA) Health Effects Test Guidelines, 40 CFR 798.4500; the Organization for Economic Cooperation and Development (OECD) Guideline for Testing of Chemicals, Section 405; and the Japanese Agricultural Chemicals Laws and Regulations Testing Guidelines for Toxicology Studies published by the Society of Agricultural Chemical Industry, under the auspices of MAFF (Ministry of Agriculture, Forestry and Fisheries).

Table 2. Primary dermal irritation study of HCA-SX Albino rabbits: Scoring criteria for dermal reactions

Evaluation of dermal reactions*			
Value	Erythema and eschar formation	Value	Edema formation
0	No erythema	0	No edema
1	Very slight erythema (barely perceptible, edges of area not well defined)	1	Very slight edema (barely perceptible, edges of area not well defined)
2	Slight erythema (pale red in color and edges definable)	2	Slight edema (edges of area well defined by definite raising)
3	Moderate to severe erythema (defined in color and area well defined)	3	Moderate edema (raised approximately 1 mm)
4	Severe erythema (beet or crimson red) to slight eschar formation (injuries in depth)	4	Severe edema (raised more than 1 mm and extending beyond area of exposure)
4	Total possible erythema score	4	Total possible edema score
8 Total possible primary irritation score			
Descriptive ratings			
Mean primary dermal irritation index			
Range of values	Descriptive ratings		
0	Non-irritating		
0.1–2.0	Slightly irritating		
2.1–5.0	Moderately irritating		
5.1–8.0	Severely irritating		

*Source: Draize 1965 [13].

Three male and three female New Zealand white Albino rabbits (weighing 2172–2974 g at study initiation), obtained from Hazleton Research Products (Denver, PA, USA), were allowed free access to lab chow (Purina Certified Rodent Chow, No. 5322, St. Louis, MO, USA) and municipal water *ad libitum*. Animals were acclimated to laboratory conditions for a minimum of six days prior to initiation of dosing. The animal room was kept at a constant temperature (66–71°F), humidity (46–86%), and light (12 h light/12 h dark).

The route of HCA-SX administration was direct conjunctival instillation. This route of administration is standard for assessment of local ocular irritative potential. HCA-SX was placed directly into the cupped lower conjunctival sac of the right (test) eye at a dose of 54 mg/right eye. The eyelid was held closed for approximately 1 sec after instillation. The left eye was manipulated in an identical manner to simulate the dosing of the right eye. There was one group of six rabbits that received a single, unwashed exposure.

The rabbits were observed twice daily (morning and afternoon) for mortality for the duration of the study. For ocular observations, both eyes of the rabbits were examined for ocular abnormalities prior to study initiation. The pre-initiation examination included the use of sodium fluorescein and ultraviolet light for detection of corneal abnormalities. Rabbits assigned to the study had no pre-existing abnormalities. Following treatment, both eyes of the rabbits were examined macroscopically for ocular irritation using a handheld pen light in accordance with the method of Draize (Table 3) at approxi-

mately 1, 24, 48 and 72 h after dosing and on days 4, 7, 14, and 21 if irritation persisted. In addition, both eyes were further examined at 72 h and on days 7, 14 and 21 with sodium fluorescein and ultraviolet light.

Body weights were obtained and recorded on study day 0 (initiation) and at each animal's termination from study. Upon termination, the rabbits were euthanized by intravenous injection of sodium pentobarbital solution and discarded.

Statistics

Data from different experiments (control and test) were pooled and subjected to statistical analysis. Except where indicated otherwise, values given are arithmetic means \pm S.E.M. Significance of differences between control and test values were evaluated using analysis of variance (ANOVA) followed by Dunnett's test (GraphPad Software, San Diego, CA, USA). Differences with p values < 0.05 were accepted as statistically significant.

Results

The objectives of this study were to determine the effects of HCA-SX on 5-HT uptake in rat brain cortex *in vitro*, and to determine the safety profile of HCA-SX in *in vivo* models.

Table 3. Primary eye irritation study of HCA-SX in Albino rabbits: Scale for scoring ocular irritation^a

I. Cornea		
(A) Opacity-degree of density (area most dense taken for reading)		
No ulceration or opacity		0
Dulling of normal luster, details of iris clearly visible		1 *
Easily discernible translucent areas, details of iris slightly obscured		2 *
Nacreous areas, no details of iris visible, size of pupil barely discernible		3 *
Opaque cornea, iris not discernible through the opacity		4 *
(B) Area of cornea involved		
No ulceration or opacity		0
One quarter or less but not zero		1 *
Greater than one quarter, but less than half		2 *
Greater than half, but less than three quarters		3 *
Greater than three quarters, up to whole area		4 *
Score equals A × B × 5		Total maximum = 80
II. Iris		
(A) Values		
Normal		0
Markedly deepened rugae, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof), iris still reacting to light (sluggish reaction is positive)		1 *
No reaction to light, hemorrhage, gross destruction (any or all of these)		2 *
Score equals A × 5		Total maximum = 10
III. Conjunctivae		
(A) Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)		
Blood vessels normal		0
Some blood vessels definitely hyperemic (injected above) normal		1
Diffuse, deeper crimson color, individual vessels not easily discernible		2 *
Diffuse beefy red		3 *
(B) Chemosis: lids and/or nictitating membranes		
No swelling		0
Any swelling above normal (includes nictitating membrane)		1
Obvious swelling with partial eversion of lids		2 *
Swelling with lids about half closed		3 *
Swelling with lids more than half closed		4 *
(C) Discharge		
No discharge		0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals)		1
Discharge with moistening of the lids and hairs just adjacent to the lids		2
Discharge with moistening of the lids and hair, and considerable area around the eye		3
Score equals (A + B + C) × 2		Total maximum = 20
Total maximum Score Possible = 110		

^aDraize scale for scoring ocular lesions, as published in the guidelines in Subsection F. Hazard Evaluation: Human and Domestic Animals distributed in 1982 and the OECD Guidelines for Testing of Chemicals distributed by 1987.

*Starred figures indicate positive effect.

Effect of HCA-SX on [³H]-5-HT uptake

This study was conducted to determine the effects of HCA-SX on 5-HT uptake in rat brain cortex *in vitro*. As shown in Fig. 1, [³H]-5-HT was taken up into neuronal stores in rat cortical slices in a time-dependent manner reaching a maximum at approximately 90 min. After 40 min, HCA-SX (300 and 1 mM), and the 5-HT uptake inhibitors fluoxetine (100 μM) plus clomipramine (10 μM) decreased the time-dependent uptake of [³H]-5-HT. At 90 min, HCA-SX (300 μM) caused a 20% decrease in

[³H]-5-HT uptake, whereas fluoxetine plus clomipramine significantly inhibited ($p < 0.001$) [³H]-5-HT uptake by 30% (Fig. 2). At 90 min, significant inhibition of 5-HT uptake was produced by 300 μM HCA-SX ($p < 0.01$); however, no significant inhibition was observed following incubation with 1 mM HCA-SX (Fig. 2). Thus, kinetics studies were performed on HCA and fluoxetine plus clomipramine in order to determine optimal uptake at equilibrium. Uptake at equilibrium is a reflection of total uptake under our experimental conditions. Regardless of initial differences observed for the agents before

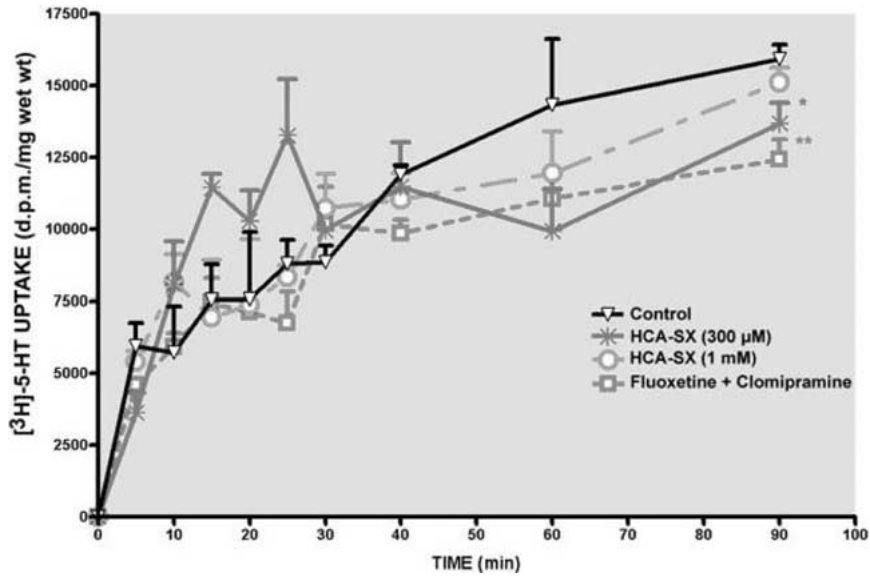


Fig. 1. Time-dependent uptake of [³H]-5-HT in isolated rat brain cortex: control and in the presence of (–)-hydroxycitric acid (HCA-SX, 300 μM and 1 mM) and fluoxetine (100 μM) plus clomipramine (10 μM). Each data point is the mean ± S.E.M. of 3–6 replicates. *p < 0.01; **p < 0.001 significantly different from untreated control.

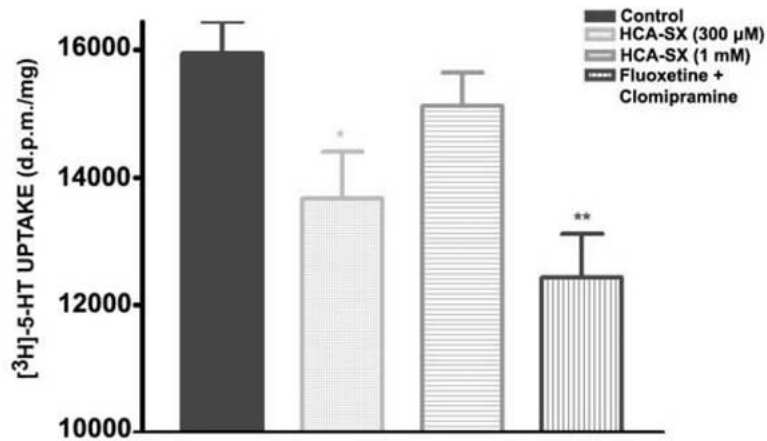


Fig. 2. [³H]-5-HT uptake in isolated rat brain cortex slices at 90 min control and in the presence of (–)-hydroxycitric acid (HCA-SX, 300 μM and 1 mM) and fluoxetine (100 μM) plus clomipramine (10 μM). Vertical bars represent mean ± S.E.M. Number of observations was 4 in each case. *p < 0.01; **p < 0.001 significantly different from untreated control.

90 min, only data obtained at or after 90 min is reflective of uptake at equilibrium.

HCA-SX safety studies

In this paper, we have conducted a number of *in vivo* studies to demonstrate the safety of HCA-SX. Acute oral toxicity, acute dermal toxicity, primary dermal irritation and primary eye irritation studies were conducted in animals to determine the safety profile of HCA-SX.

Table 4. Acute oral toxicity study of HCA-SX in Albino rats following a single oral dose of 5000 mg/kg: Summary of clinical findings: Total occurrence/number of animals

Group	Male	Female
	Days 0–14	Days 0–14
Acute		
Appeared normal	73/5	79/5
Scabbing dorsal head	12/1	–
Rales	–	5/2
Soft stool	–	1/1

See Materials and methods for details.

Table 5. Acute oral toxicity study of HCA-SX in Albino rats following a single oral dose of 5,000 mg/kg: Individual clinical observations

Observation	Sex	Hour post-dose			Day post-dose													
		1	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Appeared normal	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Male	P	P	P	P	P	—	—	—	—	—	—	—	—	—	—	—	—
	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	—	—	—	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	—	—	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	P	P	—	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Rales	Female	P	P	P	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Female	P	P	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Soft stool	Female	—	—	P	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Scabbing dorsal head	Male	—	—	—	—	—	P	P	P	P	P	P	P	P	P	P	P	P

Grade code: P = present; S = slight; M = moderate; V = severe; — = not seen at that interval. See Materials and methods for details.

Table 6. Acute oral toxicity study of HCA-SX in Albino rats following a single oral dose of 5000 mg/kg: Individual and mean \pm S.D. of body weights (g) on days post-treatment

Sex	–1 day	0 days	7 days	14 days	Sex	–1 day	0 days	7 days	14 days
Male	239	210	292	346	Female	232	211	240	260
Male	244	216	303	349	Female	232	208	237	240
Male	239	210	288	323	Female	245	215	245	258
Male	244	215	296	339	Female	234	213	244	261
Male	238	213	294	337	Female	230	211	249	256
Mean \pm S.D.	241 \pm 2.9	213 \pm 2.8	295 \pm 5.5	339 \pm 10.1		235 \pm 6.0	212 \pm 2.6	243 \pm 4.6	255 \pm 8.6

See Materials and methods for details.

Acute oral toxicity study

Mortality

No deaths were observed during the study from any of the doses of HCA-SX which were used.

Clinical observations (Tables 4 and 5)

All clinical findings were noted on the day of dosing, with the exception of scabbing of the dorsal head that was present for one male on day 3 and throughout the remainder of the study. Soft stool and rales were observed for one and two rats, respectively. There were no other clinical findings.

Body weights (Table 6)

There were no remarkable changes or differences observed in body weights under any of the experimental conditions.

Necropsy (Table 7)

There were no significant changes for all examined tissues at

terminal necropsy for any of the doses of HCA-SX which were used.

There were no toxicological gross findings for any examined tissues at the scheduled necropsy. The data indicates that the LD₅₀ of HCA-SX is greater than 5000 mg/kg when administered once orally via gastric intubation to fasted male and female Albino rats.

Table 7. Acute oral toxicity study of HCA-SX in Albino rats following a single oral dose of 5000 mg/kg: Gross necropsy observations incidence summary

Scheduled necropsy	Male	Female
Number of animals in dose group	5	5
Number of animals terminally euthanized	5	5
No significant changes observed	5	5
– all examined tissues		

See Materials and methods for details.

Acute dermal toxicity study

Mortality

There were no deaths during the study under the experimental conditions which were employed.

Table 8. Acute dermal toxicity study of HCA-SX in Albino rabbits following a single dose of 2000 mg/kg: Summary of clinical findings: Total occurrence/number of animals

	Male Days 0–14	Female Days 0–14
Appeared normal	82/5	85/5
Mucoid feces	3/1	–

See Materials and methods for details.

Clinical observations (Tables 8 and 9)

All animals appeared normal throughout the study with the exception of one male rabbit, which had mucoid feces on days 0–2. No other clinical findings were observed for animals receiving a dermal application of 2000 mg HCA-SX/kg.

Dermal observations (Table 10)

HCA-SX induced very slight to slight erythema on all rabbits. No edema was observed. Desquamation was noted on eight sites out of 10 animals by day 7. All treatment sites were stained yellow. All dermal irritation completely subsided by day 12 or earlier, and no other dermal findings were observed.

Body weights (Table 11)

No remarkable changes or differences in body weight occurred.

Table 9. Acute dermal toxicity study of HCA-SX in Albino rabbits following a single dose of 2000 mg/kg: Individual clinical observations

Observation	Sex	Hour post-dose			Day post-dose														
		1	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14	
Appeared normal	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Male	–	P	P	–	–	P	P	P	P	P	P	P	P	P	P	P	P	P
	Male	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
	Female	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Mucoid feces	Male	P	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–

Grade code: P = present; S = slight; M = moderate; V = severe; – = not seen at that interval. See Materials and methods section for details.

Table 10. Acute dermal toxicity study of HCA-SX in Albino rabbits following a single dose of 2000 mg/kg: Individual dermal observations

Study day	Erythema ⁺ /edema ⁺ /other findings									
	Male	Male	Male	Male	Male	Female	Female	Female	Female	Female
1	2/0/h	1/0/h	1/0/h	1/0/h	2/0/h	2/0/h	1/0/h	2/0/h	2/0/h	2/0/h
2	2/0/h	1/0/h	1/0/h	1/0/h	2/0/h	2/0/h	1/0/h	1/0/h	2/0/h	1/0/h
3	2/0	1/0	1/0	1/0	2/0	2/0	1/0	1/0	1/0	1/0
4	2/0	1/0	1/0	1/0	2/0	2/0	1/0	1/0	1/0	1/0
5	2/0	1/0	1/0	1/0	2/0	2/0	1/0	1/0	1/0	1/0
6	2/0/d	1/0	1/0/d	1/0	2/0/d	1/0/d	1/0	1/0	1/0/d	1/0
7	2/0/d	1/0/d	1/0/d	1/0/d	1/0/d	1/0/d	1/0/d	SNR	SNR	SNR
8	1/0/d	1/0/d	1/0	1/0	1/0/d	SNR	1/0	SNR	SNR	SNR
9	1/0/d	SNR	1/0	SNR	1/0/d	SNR	SNR	SNR	SNR	SNR
10	1/0	SNR	1/0	SNR	SNR	SNR	SNR	SNR	SNR	SNR
11	1/0	SNR	SNR	SNR	0/0/d	SNR	SNR	SNR	SNR	SNR
12	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR
13	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR
14	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR	SNR

+ = Refer to Draize scale for dermal scoring criteria; d = desquamation; SNR = scored, not remarkable; h = application site stained yellow. See Materials and methods section for details.

Table 11. Acute dermal toxicity study of HCA-SX in Albino rabbits: Individual and mean + S.D. body weights (g)

Sex	Day 0	Day 7	Day 14	Sex	Day 0	Day 7	Day 14
Male	2323	2835	2851	Female	2227	2322	2529
Male	2266	2622	2751	Female	2295	2557	2777
Male	2330	2656	2769	Female	2372	2742	2854
Male	2466	2953	3004	Female	2236	2655	2697
Male	2259	2623	2721	Female	2351	2674	2826
Mean ± S.D.	2329 ± 83.2	2738 ± 149.2	2819 ± 114.0		2296 ± 65.5	2590 ± 163.8	2737 ± 130.5

See Materials and methods for details.

Table 12. Acute dermal toxicity study of HCA-SX in Albino rabbits following a single dose of 2000 mg/kg: Summary of gross necropsy observations incidence

	Male	Female
Number of animals in dose group	5	5
Number of animals terminally euthanized	5	5
Application site – reddened	1	1
Kidneys – pale	0	1
Spleen – accessory	1	1
External surface – hair loss	1	0
All examined tissues – no significant changes observed	2	2

See Materials and methods for details.

Necropsy (Tables 12 and 13)

Reddened application sites were noted for two rabbits at the terminal necropsy. Single occurrences of pale kidneys, mottled lungs and hair loss were noted for one rabbit each. Two rabbits had accessory spleens, a common congenital abnormality. There were no other gross necropsy findings for all examined tissues at the terminal necropsy.

The data indicated that the LD₅₀ of HCA-SX is greater than 2000 mg/kg when it is applied once for 24 h to the shaved, intact skin of male and female Albino rabbits. There was no evidence of acute systemic toxicity among rabbits when 2000 mg/kg HCA-SX is dermally administered.

Primary dermal irritation study

Mortality

No deaths occurred during this study.

Dermal observations (Table 14)

HCA-SX induced very slight erythema on one animal and stained all treatment sites yellow. All erythema completely subsided by the end of day 1. No edema or other dermal findings were observed.

Body weights (Table 15)

No significant body weight changes occurred during the study period.

The Primary Irritation Index was calculated to be 0.0. HCA-SX received a descriptive rating classification (Table 2) of non-irritating.

Primary eye irritation study

Mortality

There were no deaths.

Ocular irritation (Tables 16 and 17)

Number with positive effect/Number treated

Group	Cornea	Iris	Conjunctiva	Total	Maximum average score (M.A.S.)
0.1 ml/ right eye, unwashed	0/6	6/6	6/6	6/6	15.0 at 1 h

None of the rabbits vocalized upon instillation of the test material (HCA-SX). The left (control) eyes were free of evidence of ocular irritation and other findings for the duration of the study. Individual and average ocular irritation scores for the treated eyes are presented in Table 16. Individual animal results (other findings), including sodium fluorescein examination results, are presented in Table 17. The scale for scoring ocular irritation and method of score calculation are presented in Table 3 with a total maximum possible score of 110.

A small area of inflammatory exudate with enlarged blood vessels was present at the apex of the lower conjunctival sac for three rabbits on day 7. This inflammatory exudate completely subsided by study termination (day 21) for two animals. One of the three rabbits also had inflammatory exudate on the nictitating membrane that was present on day 7 through study termination (day 21). The Maximum Average Score (M.A.S.) for HCA-SX was 15.0 at 1 h. The test material induced positive iridal and conjunctival reactions for all rabbits. There were no corneal reactions noted. Iridal irritation subsided by 48 h or earlier. With the exception of inflammatory exudate, conjunctival irritation completely subsided by study termination (day 21) or earlier for all animals.

Table 13. Acute dermal toxicity study of HCA-SX in Albino rabbits: Individual gross description of organs

Sex	Organ findings	Grade	Organ – no significant findings	Sex	Organ findings	Grade	Organ – no significant findings
Male	None	NA	adrenal glands, application site, brain, intestine, epididymides, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus gland, thyroid glands, trachea, urinary bladder	Female	Kidneys gross: pale bilateral	P	adrenal glands, application site, brain, intestine, esophagus, eyes, gall bladder, heart, liver, lymph node, mesenteric, lungs, mammary gland, ovaries, pancreas, pituitary, salivary glands, skin, spleen, stomach, thymus gland, thyroid glands, trachea, urinary bladder, uterus
Male	Lungs gross: mottled all lobes	P	adrenal glands, application site, brain, intestine, epididymides, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, mammary gland, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus gland, thyroid glands, trachea, urinary bladder	Female	None	NA	adrenal glands, application site, brain, intestine, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, ovaries, pancreas, pituitary, salivary glands, skin, spleen, stomach, thymus gland, thyroid glands, trachea, urinary bladder, uterus
Male	Application site gross: reddened	P	adrenal glands, brain, intestine, epididymides, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus gland, thyroid glands, trachea, urinary bladder	Female	None	NA	adrenal glands, application site, brain, intestine, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, ovaries, pancreas, pituitary, salivary glands, skin, spleen, stomach, thymus gland, thyroid glands, trachea, urinary bladder, uterus
	Spleen gross: accessory two, 1 and 2 mm diameter	P	adrenal glands, brain, intestine, epididymides, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus gland, thyroid glands, trachea, urinary bladder				
Male	None	NA	adrenal glands, application site, brain, intestine, epididymides, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus gland, thyroid glands, trachea, urinary bladder	Female	Application site gross: reddened	P	adrenal glands, brain, intestine, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, ovaries, pancreas, pituitary, salivary glands, skin, spleen, stomach, thymus gland, thyroid glands, trachea, urinary bladder, uterus
Male	External surface gross: hair loss ventral abdominal	P	adrenal glands, application site, brain, intestine, epididymides, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes, thymus gland, thyroid glands, trachea, urinary bladder	Female	Spleen gross: accessory two, 2 mm in diameter	P	adrenal glands, application site, brain, intestine, esophagus, eyes, gall bladder, heart, kidneys, liver, lymph node, mesenteric, lungs, mammary gland, ovaries, pancreas, pituitary, salivary glands, skin, stomach, thymus gland, thyroid glands, trachea, urinary bladder, uterus

P = present; NA = none appeared. See Materials and methods for details.

Table 14. Primary dermal irritation study of HCA-SX in Albino rabbits: Individual dermal scores

Sex	Site	Erythema				Edema			
		1 h	24 h	48 h	72 h	1 h	24 h	48 h	72 h
Male	A	0	0	0	0	0	0	0	0
Male	A	0	0	0	0	0	0	0	0
Female	A	0	0	0	0	0	0	0	0
Female	A	1	0	0	0	0	0	0	0
Female	A	0	0	0	0	0	0	0	0
Female	A	0	0	0	0	0	0	0	0
Total		1	0	0	0	0	0	0	0

The dosage level was 0.5 g HCA-SX/site. *Dose site stained yellow. See Materials and methods for details.

PII calculated using test periods: 1, 24, 48, 72 h.

Primary irritation index: $(PII) = (1 + 0 + 0 + 0)/24 + (0 + 0 + 0 + 0)/24$

$$PII = (1/24) + (0/24) = 0.0 + 0.0$$

PII = 0.0 = non-irritating.

Table 15. Primary dermal irritation study of HCA-SX in Albino rabbits: Individual body weights (g)

Sex	Initiation Day 0	Terminal Day 3
Male	3952	3937
Male	3550	3465
Female	3738	3690
Female	3958	3754
Female	3477	3531
Female	3691	3629

See Materials and methods for details.

Body weights (Table 17)

There were no significant changes or differences observed in body weights during the study period.

Discussion

(-)-Hydroxycitric acid (HCA) is the natural primary organic acid found in the fruits and rinds of *Garcinia cambogia*. HCA works by inhibiting lipogenesis, the process by which the body converts carbohydrates into fat by temporarily inhibiting ATP

Table 16. Primary eye irritation study of HCA-SX in Albino rabbits: Individual ocular irritation scores

Sex	Tissue	Examination intervals							
		1 h	24 h	48 h	72 h	4 days	7 days	14 days	21 days
Male	Cornea (O-A)	0 0	0 0	0 0	0 0	0 0	0 0		
	Iris	1	1	0	0	0	0		
	Conjunctiva (R-C-D)	2 2 2	1 0 0	1 0 0	1 0 0	1 0 0	0 0 0		
Male	Cornea (O-A)	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	Iris	1	1	0	0	0	0	0	0
	Conjunctiva (R-C-D)	1 2 2	1 0 0	2 0 0	2 0 0	2 0 0	2 0 0	1 0 0	0 0 0
Male	Cornea (O-A)	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	Iris	1	1	0	0	0	0	0	0
	Conjunctiva (R-C-D)	1 2 1	1 0 0	2 0 0	2 0 0	2 0 0	1 0 0	0 0 0	0 0 0
Female	Cornea (O-A)	0 0	0 0	0 0	0 0	0 0	0 0	0 0	
	Iris	1	0	0	0	0	0	0	
	Conjunctiva (R-C-D)	2 1 2	1 0 0	1 0 0	1 0 0	1 0 0	1 0 0	0 0 0	
Female	Cornea (O-A)	0 0	0 0	0 0	0 0	0 0	0 0		
	Iris	1	1	0	0	0	0		
	Conjunctiva (R-C-D)	1 2 2	1 1 0	2 1 0	1 0 0	1 0 0	0 0 0		
Female	Cornea (O-A)	0 0	0 0	0 0	0 0	0 0	0 0	0 0	0 0
	Iris	1	0	0	0	0	0	0	0
	Conjunctiva (R-C-D)	1 2 2	1 1 0	2 0 0	2 0 0	2 0 0	2 0 0	0 0 0	0 0 0

Group: 54 mg/right eye, unwashed. *Fluorescein solution applied; O = opacity; A = area; R = redness; C = chemosis; D = discharge.

See Materials and methods for details.

Table 17. Primary eye irritation study of HCA-SX in Albino rabbits: Individual animal results – other findings

Sex	Initial body wt. (g) Day 0	Terminal body wt. (g) Day 0	Examination interval	Other findings	Sex	Initial body wt. (g) Day 0	Terminal body wt. (g) Day 0	Examination interval	Other findings
Male	2214	2449 (day 7)	1 h 24 h 48 h 72 h 4 days 7 days	b,s – – r(0%) – r(0%)	Female	2974	3423 (day 14)	1 h 24 h 48 h 72 h 4 days 7 days 14 days	b,s – – r(0%) – r(0%) r(0%)
Male	2239	2934 (day 21)	1 h 24 h 48 h 72 h 4 days 7 days 14 days 21 days	b,s – – r(0%) – e,r(0%) f,r(0%) r(0%)	Female	2346	2700 (day 7)	1 h 24 h 48 h 72 h 4 days 7 days	b,s – – r(0%) – r(0%)
Male	2172	2884 (day 21)	1 h 24 h 48 h 72 h 4 days 7 days 14 days 21 days	b,s – – r(0%) – g,r(0%) g,r(0%) r(0%)	Female	2334	2755 (day 21)	1 h 24 h 48 h 72 h 4 days 7 days 14 days 21 days	b,s – – r(0%) – e,f,r(0%) e,f,r(0%) f,r(0%)

Group: 54 mg/right eye, unwashed; b = clear discharge; r = sodium fluorescein stain retention (0% of area); s = small amount of residual test material present in the eye; e = inflammatory exudate covering an area of approximately 4 × 2 mm containing enlarged blood vessels present at apex of lower conjunctival sac; f = inflammatory exudate covering an area of approximately 1 × 1 mm containing enlarged blood vessels present at apex of lower conjunctival sac; g = inflammatory exudate covering an area of approximately 5 × 3 mm containing enlarged blood vessels present at apex of lower conjunctival sac.

citrate lyase, the enzyme that converts excess glucose into fat. Furthermore, by inhibiting ATP citrate lyase, HCA reduces the availability of acetyl-CoA, the building block for fat synthesis. Earlier studies in humans have shown that HCA consumption (500 mg, t.i.d.) before meals for 8 weeks resulted in 215% greater weight loss than those taking a placebo without any adverse side effects [5]. Mattes and Bormann [2] conducted a placebo-controlled, double-blind study involving 89 moderately overweight female human subjects who ingested HCA (1200 mg/day) or placebo for 12 weeks and received a daily diet of 5020 kJ. A significant loss of body weight was observed as compared to the placebo group (3.7 ± 3.1 kg vs. 2.4 ± 2.9 kg) for subjects ingesting HCA.

We have recently shown that HCA (as Super CitriMax™, HCA-SX) can increase the release of 5-HT from rat brain cortex slices, *in vitro*, provided the first scientific evidence offering an explanation for the ability of HCA to suppress appetite [7]. In the present study, we now report that HCA-SX inhibited the time-dependent uptake of [³H]-5-HT, an action that was mimicked by the well-known serotonin receptor reuptake inhibitors (SRRIs), fluoxetine and clomipramine. Fluoxetine and clomipramine are potent selective inhibitors of [³H]-5-HT up-

take in neuronal tissue both *in vivo* and *in vitro* [14, 15]. For instance, Stauderman and Jones [16], demonstrated that fluoxetine inhibited accumulation of [³H]-5-HT into rat spinal serotonergic nerve terminals in a sodium-dependent manner. Taken together, these results support the view that HCA-SX increases the release/availability of [³H]-5-HT from neuronal serotonergic nerve terminals presumably via an effect on neuronal uptake of this monoamine.

Since increased brain levels of 5-HT are involved in regulation of sleep, mood changes and appetite suppression, the results strongly suggest that an effect on this monoamine could underlie the mechanism of appetite suppression and food intake induced by HCA-SX. Furthermore, HCA-SX may provide a therapeutic advantage by alleviating emotional issues of overweight people, including binge-eating and depression.

It has been demonstrated by GC-MS that HCA-SX is bioavailable in human plasma [6]. However, a major question remains whether HCA-SX can cross the blood–brain barrier. Recently, we have completed a human clinical study on HCA-SX, which demonstrated that HCA-SX supplementation over 8 weeks increases serum serotonin levels significantly in human volunteers (unpublished results). The other advantage

of HCA-SX is that many of the 'natural' diet products on the market, including Ma Huang (a natural source of ephedrine), or Kola Nut (a natural source of caffeine) and Guarana extract (a natural source of caffeine) are central nervous system stimulants known to cause serious adverse side effects, including increased heart rate, high blood pressure, upset stomach, severe headaches/migraines, vomiting, and others. HCA-SX has been in the market for more than 8 years with no such adverse side effects reported so far. In addition, high concentrations (20–30%) of HCA in the dried fruit rind of *Garcinia cambogia* have been consumed for centuries in the diets of South Asian people as a food condiment to make meals more 'filling' [1].

A number of safety studies have been conducted on HCA-SX. In the acute oral toxicity study, no deaths, remarkable body weight changes or gross necropsy findings were observed. Clinical findings were limited to soft stool and rales for one and two rats, respectively. A male rat with scabbing on the dorsal head was noted on days 3–14. All other animals appeared normal on day 1 and throughout the remainder of the study. There were no other clinical findings. These results demonstrate that the LD₅₀ of HCA-SX is greater than 5000 mg/kg when administered once orally via gastric intubation to fasted male and female albino rats.

In the dermal toxicity study, there were no deaths, HCA-SX-related clinical findings or remarkable body weight changes. The HCA-SX induced very slight to slight erythema with no edema. Desquamation was noted on eight sites. All dermal irritation completely subsided by day 12 or earlier. There were no other dermal findings. Reddened application sites were noted for two rabbits at the terminal necropsy. There were no other gross necropsy findings related to HCA-SX for all examined tissues at the terminal necropsy. The LD₅₀ of HCA-SX was found to be greater than 2,000 mg/kg when applied once for 24 h to the shaved, intact skin of male and female albino rabbits. There was no evidence of acute systemic toxicity among rabbits that were dermally administered HCA-SX at 2,000 mg/kg.

In the dermal irritation study, no deaths or significant body weight changes were observed during the study period. HCA-SX induced very slight erythema on one animal. No edema or other dermal findings were noted, and all irritation was reversible and completely subsided by the end of day 1. The Primary Irritation Index was calculated to be 0.0. Based on these observations, HCA-SX received a descriptive rating classification (Table 2) of non-irritating.

In the eye irritation study, no deaths or remarkable changes in body weights occurred during the study period. The results indicate that HCA-SX causes ocular irritation with production of inflammatory exudate in some animals. Positive iridal and conjunctival reactions were present in all animals, which subsided within 48 h. A total maximum score of 110 is possible. A score of 15 was obtained in the study, indicating mild irritation.

Taken together, this study had two major objectives (a) to determine the effect of HCA-SX on 5-HT uptake in the rat brain cortex *in vitro*, and (b) to assess the safety profile of HCA-SX. The *in vitro*, serotonin release and reuptake studies demonstrate that HCA-SX can inhibit [³H]-5-HT uptake (and increase 5-HT availability) in a manner similar to that of serotonin receptor reuptake inhibitors (SRRIs), and thus may prove beneficial in controlling appetite, as well as, in the treatment of depression, insomnia, migraine headaches, and other serotonin deficient conditions. However, fluoxetine plus clomipramine exhibited significantly higher potency as compared to HCA-SX. Acute oral toxicity, acute dermal toxicity, primary dermal irritation and primary eye irritation studies indicate that HCA-SX is a safe, natural supplement under the conditions it was tested.

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