

Effects of a natural extract of (–)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX plus niacin-bound chromium and *Gymnema sylvestre* extract on weight loss

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Aim: The efficacy of optimal doses of highly bioavailable (–)-hydroxycitric acid (HCA-SX) alone and in combination with niacin-bound chromium (NBC) and a standardized *Gymnema sylvestre* extract (GSE) on weight loss in moderately obese subjects was evaluated by monitoring changes in body weight, body mass index (BMI), appetite, lipid profiles, serum leptin and excretion of urinary fat metabolites. HCA-SX has been shown to reduce appetite, inhibit fat synthesis and decrease body weight without stimulating the central nervous system. NBC has demonstrated its ability to maintain healthy insulin levels, while GSE has been shown to regulate weight loss and blood sugar levels.

Methods: A randomized, double-blind, placebo-controlled human study was conducted in Elluru, India for 8 weeks in 60 moderately obese subjects (ages 21–50, BMI >26 kg/m²). Subjects were randomly divided into three groups.

Group A was administered HCA-SX 4667 mg, group B was administered a combination of HCA-SX 4667 mg, NBC 4 mg and GSE 400 mg, while group C was given placebo daily in three equally divided doses 30–60 min before meals. All subjects received a 2000 kcal diet/day and participated in supervised walking.

Results: At the end of 8 weeks, body weight and BMI decreased by 5–6% in both groups A and B. Food intake, total cholesterol, low-density lipoproteins, triglycerides and serum leptin levels were significantly reduced in both groups, while high-density lipoprotein levels and excretion of urinary fat metabolites increased in both groups. A marginal or non-significant effect was observed in all parameters in group C.

Conclusion: The present study shows that optimal doses of HCA-SX and, to a greater degree, the combination of HCA-SX, NBC and GSE can serve as an effective and safe weight-loss formula that can facilitate a reduction in excess body weight and BMI, while promoting healthy blood lipid levels.

Keywords: (–)-hydroxycitric acid (*Garcinia cambogia*), appetite suppression, body mass index, *Gymnema sylvestre* extract, niacin-bound chromium, serum leptin, total cholesterol

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Introduction

Obesity is a complex, multifactorial and chronic condition characterized by excess body fat resulting from

an imbalance between energy expenditure and caloric intake [1,2]. More than half of USA adults are

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overweight (61%), having a body mass index (BMI) greater than 25 kg/m², while more than a quarter (26%) of USA adults are obese, having a BMI of greater than 30 kg/m² [3]. Low levels of physical activity, sedentary lifestyles, stress, depression and consumption of high-fat and fast foods are responsible for unwanted weight gain [4–9]. Recent studies have shown that approximately a third of the variance in adult body weights result from genetic influences [1]. Leptin, an adipocyte- and placenta-derived circulating protein, regulates the magnitude of fat stores in the body leading to obesity [10]. Gastrointestinal peptides, neurotransmitters and adipose tissue may also have an aetiological role in obesity [11]. Obesity and adipose tissue expansion increase the risk of hypertension, type 2 diabetes, arthritis, elevated cholesterol, cancer and serious hormonal imbalances in women, leading to sterility [12]. Low caloric diets with or without exercise can help with temporary weight loss; however, diet and exercise alone have not proven successful for long-term solutions in weight management. In addition, supplementation with drugs that suppress appetite, reduce food intake, increase energy expenditure and affect nutrient partitioning or metabolism have potential efficacy but is unfortunately accompanied by adverse side effects – some life threatening [13].

(-)-Hydroxycitric acid (HCA), an extract from the dried fruit rind of *Garcinia cambogia*, has been reported to cause weight loss in humans without stimulating the central nervous system [14]. HCA has been demonstrated to reduce food intake in animals, suggesting its role in the treatment of obesity and has been demonstrated to increase the availability of serotonin in isolated rat brain cortex [15–23]. HCA is a competitive inhibitor of ATP citrate lyase, an extra-mitochondrial enzyme involved in the initial steps of *de novo* lipogenesis. Consequently, HCA reduces the transformation of citrate into acetyl coenzyme A, a step necessary for the formation of fatty acids in the liver. In addition, there is increased production of hepatic glycogen in the presence of HCA, which may activate glucoreceptors, leading to a sensation of fullness and reduced appetite [17,20].

Niacin-bound chromium (NBC) plays an important role in regulating appetite and energy production. A human study involving African-American women who were administered 600 µg of elemental chromium as NBC in two divided doses, a moderate diet and exercise regimen for 2 months resulted in weight and fat loss and sparing of muscle and body composition with no significant adverse effects [24]. Grant *et al.* [25] reported significant weight loss in young obese women consuming 400 µg of NBC per day for 8 weeks with exercise. This

study also demonstrated an improved insulin response to an oral glucose load. In another animal study, rats were fed huge amounts of NBC for over a year and demonstrated no evidence of toxicity [26].

Gymnema sylvestre extract (GSE) helps promote weight loss and controls blood sugar levels [27,28]. GSE-derived peptide gurmarin inhibits the sweet taste response in rats [28]. Preuss *et al.* [29] demonstrated a significant lowering of cholesterol with GSE ingestion in hypertensive rats that were fed a high sucrose diet, while the placebo group showed a significant increase in cholesterol levels. GSE administered (400 mg/day) to insulin-dependent diabetes mellitus patients for 10–12 months resulted in significant improvement with no adverse side effects [30].

The efficacy of HCA in weight management has been previously reported [31–34]. However, no systematic approach has been conducted so far to evaluate the efficacy and modulation of fat degradation, lipid profiles and leptin level. Commercial sources of HCA are typically available as calcium salts that are relatively insoluble in water, poorly absorbed and lack proven bioavailability studies. Furthermore, earlier studies used a dose of 1500 mg of HCA per day but did not provide a rationale for dose selection or timing for dose administration. In this study, we used a highly bioavailable calcium–potassium salt of HCA (HCA-SX) and the daily dose was extrapolated from earlier *in vivo* studies conducted by Sullivan *et al.* [18,19] and our recently conducted *ex vivo* study on serotonin release from isolated rat brain cortex [23,35]. Accordingly, the human equivalent dosage of HCA-SX used in the present study was calculated to be 2800 mg/day, as explained in *Methods and Procedures*, which is significantly greater than the 1500 mg/day typically recommended in dietary supplements [31]. HCA-SX bioavailability was found to be significantly higher in fasting individuals as compared with subjects consuming HCA-SX in conjunction with food [36]. Accordingly, HCA-SX was given to the study participants 30–60 min before each meal, which addresses the timing of dose. In addition, extensive safety studies have demonstrated the safety of HCA-SX with an LD₅₀ (rats) greater than 5 g/kg [35].

The present study examined the efficacy of HCA-SX alone and in combination with NBC and GSE given on an empty stomach 30–60 min before breakfast, lunch and dinner in 60 human volunteers. Effects of these supplements were investigated on body weight, BMI, appetite (as determined by weighing the remaining food), lipid profiles, serum leptin (a biomarker of obesity regulatory gene) and excretion of urinary fat metabolites (a biomarker of fat oxidation).

Methods and Procedures

Institutional Review Board approvals, IRB 01-001 (Elluru, India) and IRB 01-142 (Georgetown University Medical Center, Washington, DC, USA) were obtained. All subjects gave written consent prior to participation. The following six key factors were monitored in a randomized, double-blind, placebo-controlled study over a period of 8 weeks: (i) whether optimal doses of HCA-SX and the combination of HCA-SX, NBC and GSE (HCA-SX formula) produce a greater reduction in body weight than placebo; (ii) whether HCA-SX and HCA-SX formula produce a greater reduction in BMI than placebo; (iii) whether HCA-SX and HCA-SX formula have an inhibitory effect on appetite as compared with placebo; (iv) whether HCA-SX and HCA-SX formula produce a beneficial effect on lipid profile, including low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, very-low-density lipoprotein (VLDL) and total cholesterol, as compared with placebo; (v) whether HCA-SX and HCA-SX formula have an inhibitory effect on serum leptin levels compared to placebo; and (vi) whether HCA-SX and HCA-SX formula cause fat oxidation, as estimated by enhanced excretion of urinary fat metabolites, including malondialdehyde (MDA), acetaldehyde (ACT), formaldehyde (FA) and acetone (ACON), as compared with placebo. Advertisements were placed and overweight subjects who responded and met the inclusion criteria during a screening were scheduled for a baseline visit. The evaluation included a questionnaire, physical examination, electrocardiogram and screening blood data. Qualified subjects were then randomized into three groups with equal probability through a random number generator.

Subjects

The study was performed in Elluru, India. Each subject was obese, aged 21–50, with a BMI ranging from 29.9 to 55.5 kg/m². Additional inclusion criteria consisted of having a negative pregnancy test, possessing the ability to understand the risks and benefits of the protocol, willingness to participate in a 30 min supervised walking exercise programme (5 days a week), eat the vegetarian or non-vegetarian prescribed diets of approximately 2000 kcal/day, sign an informed consent form, complete a standard health questionnaire and participate in three clinic visits at 0, 4 and 8 weeks. Subjects were excluded if they were pregnant or nursing, presently taking other weight-loss medications, had a history of thyroid disease, cardiovascular disease or diabetes, suffered from intractable obesity, had defined weight limits or had

experienced any recent, unexplained weight loss or gain. Subjects were required to fast overnight, and blood and urine samples were obtained at each clinic visit.

Group A was given a daily dose of HCA-SX 4667 mg (60% HCA providing 2800 mg of HCA per day), group B was given a daily dose of a combination of HCA-SX 4667 mg, NBC 4 mg (providing 400 µg of elemental chromium) plus GSE 400 mg (providing 100 mg of gymnemic acid) and group C was given a placebo (microcrystalline cellulose) in three equally divided doses 30–60 min before breakfast, lunch and dinner for 8 weeks. All subjects included in the study were provided a daily vegetarian or non-vegetarian diet of 2000 kcal containing approximately 17% protein, 25% fat and 58% carbohydrate. The subjects also participated in a walking exercise programme for 30 min/day, 5 days a week, which was supervised by an exercise specialist. An individual diary was maintained for each subject.

Subjects were in touch with the physicians under the supervision of Dr C. V. S. Rao on a daily basis, and a detailed evaluation was performed at the beginning, week 4 and week 8 of treatment. Body weight, BMI, appetite, lipid profile, serum leptin levels and urinary fat metabolite levels were evaluated. Blood samples were drawn in the early morning (between 0600 hours and 0730 hours) to avoid diurnal variation. Appetite suppression was measured by weighing the remaining food after each meal.

Study Materials

A natural, highly bioavailable, water-soluble, tasteless and odourless calcium–potassium salt of 60% HCA extract from *G. cambogia* [commercially known as Super CitriMax (HCA-SX)], NBC [commercially known as ChromeMate (containing 10% elemental chromium)], and a standardized extract of GSE [commercially known as GYM-250 (containing 25% gymnemic acid)] were obtained from InterHealth Nutraceuticals (Benicia, CA, USA). Unless stated otherwise, all other chemicals and reagents were obtained from Sigma Chemical (St Louis, MO, USA) and were of analytical grade or the highest grade available.

Body weights of the subjects were measured using an Essae Digi (Model DS-410) digital weighing scale (Essae-Teraoka, Bangalore, Karnataka, India). Height was measured using a Benson Track and Field height scale. BMI was calculated by body weight in kilogram divided by square of height in meters. Appetite reduction was estimated by weighing the remaining food after each meal. Lipid profile, including HDL, LDL, VLDL and triglycerides, was photometrically determined using a

Diagnostica MERCK kit and Semi-automatic analyser MICROLAB-200 (E-Merck India, Mumbai, India). Radioimmunoassay (RIA) was used to determine serum leptin levels in the blood samples according to the manufacturer's instructions (Linco Research, St Louis, MO, USA). Excretion of urinary fat metabolites was measured using high pressure liquid chromatography (HPLC) in conjunction with gas chromatographic mass spectroscopy (GCMS), using a selective ion-monitoring technique as described by Shara *et al.* [37].

Dose Determination

Earlier animal studies by Sullivan *et al.* [18,19] indicate that higher levels of HCA than those typically recommended in dietary supplements for humans are required to produce significant weight-loss results. These authors demonstrated that rats (120–160 g) receiving trisodium salt of HCA at daily concentrations of 2.63, 1.32, 0.66 and 0.33 mmol/kg developed significant lipogenesis and weight loss at concentrations of 2.63 and 1.32 mmol/kg, whereas 0.66 and 0.33 mmol/kg produced insignificant results. These animals doses were extrapolated to human equivalency dose (HED) using the formula $HED = \text{animal dose} \times (\text{human body weight}/\text{animal body weight})^{1/3}$ [38]. Thus, 2.63 and 1.32 mmol/kg/day dose equals a human daily dose of 3002 and 5981 mg respectively. Taking an average of these two doses comes to a daily dose of approximately 4500 mg. Furthermore, a concentration-dependent study was conducted in our laboratories to evoke peak levels of serotonin release in rat brain cortex. A concentration of 300 μM HCA-SX exhibited maximum release of serotonin, beyond which no further increase of serotonin release was observed [23,35]. Considering a five-fold faster metabolism in

rats compared to humans, the 300 μM *ex vivo* dose extrapolates to a human dose of 4666.67 mg of HCA per day. Based on these two findings, we used a daily dose of 4666.7 mg of HCA-SX, providing 2800 mg free HCA in this study.

Statistical Analysis

The data set that was analysed in the first phase has 10 variables of interest. These variables are body weight, BMI, LDLs, HDLs, triglycerides, VLDLs, total cholesterol, serum leptin, excretion of urinary fat metabolites and remaining food [39–41].

Two-tailed Student's *t*-test with a level of 5% significance was performed in three groups for each of the variables to detect any significant differences. The groups administered were HCA-SX (group A), HCA-SX, NBC and GSE (group B) and placebo (group C). The number of subjects who completed the study in group A, B and C was 19, 18 and 16 respectively.

In each group, longitudinal data were collected for three time points denoted by initial (I), middle (M) and final (F) for the first nine variables and at eight time points for the last variable, which is 'remaining food'. There are no missing observations in this data set.

To compare the differences at 5% level of significance, we have differences for 'I & M', 'M & F' and 'I & F' for the first nine variables. The p-value is reported in parenthesis for the tests 'I & M', 'I & F' and 'M & F' respectively. For remaining food, as data were collected at eight time points, there are 28 possible paired differences. Basic summary statistics and test for differences with respect to least-square means among the time points were conducted for each group on each variable at three time points.

Table 1 Effects of placebo (group C), (-)-hydroxycitric acid (HCA-SX) X alone (group A) and HCA-SX formula (group B) on body weight, body mass index (BMI) and serum leptin levels in human subjects

Group	Time point	Body weight (kg)	BMI (kg/m ²)	Serum leptin (ng/ml)
Placebo (group C)	I	80.44 ± 2.36 ^a	32.49 ± 0.57 ^a	33.73 ± 3.16 ^a
	M	79.16 ± 2.26 ^a	31.98 ± 0.54 ^b	31.88 ± 2.87 ^a
	F	78.84 ± 2.30 ^a	31.84 ± 0.54 ^b	33.04 ± 3.15 ^a
HCA-SX (group A)	I	91.74 ± 3.50 ^a	34.71 ± 1.22 ^a	30.22 ± 2.96 ^a
	M	89.39 ± 3.30 ^a	33.82 ± 1.15 ^b	25.05 ± 2.94 ^b
	F	87.21 ± 3.23 ^b	32.99 ± 1.13 ^c	18.37 ± 2.38 ^c
HCA-SX formula (group B)	I	92.41 ± 3.84 ^a	37.33 ± 1.70 ^a	42.31 ± 4.04 ^a
	M	89.67 ± 3.80 ^a	36.23 ± 1.69 ^b	31.69 ± 3.62 ^b
	F	86.72 ± 3.67 ^b	35.05 ± 1.62 ^c	23.55 ± 2.49 ^c

Data are presented as group mean ± s.e.m. Subjects were given (-)-hydroxycitric acid (HCA-SX) alone (group A), HCA-SX formula (group B) or placebo (group C) for 8 weeks. See *Methods and Procedures* for details. I, week 0; M, week 4; F, week 8. Values with non-identical superscripts are significantly different ($p < 0.05$).

Results

The present study evaluated the effect of supplementation with optimal doses of a highly bioavailable form of HCA (HCA-SX) alone or in combination with NBC and GSE on weight loss, BMI, appetite, lipid profiles, serum leptin levels and fat oxidation.

Table 1 summarizes the changes in body weight, BMI and serum leptin levels following supplementation of placebo (group C), HCA-SX (group A) and HCA-SX formula (group B) over the period of 8 weeks. There was a distinct change observed at the end of 4 and 8 weeks in both groups A and B. In group C, approximately 1.28 and 1.6 kg reductions in body weight were observed at the end of 4 and 8 weeks respectively. Under the same conditions, approximately 2.35 and 4.53 kg reductions in body weight were observed in group A and 2.74 and 5.69 kg reductions in body weight were observed in the group B, respectively, at the end of 4 and 8 weeks. Thus, at the end of 8 weeks, there were a 5 and 6.1% reduction in BMI observed in group A and group B respectively. There was a reduction of 2% BMI in group C at the end of 8 weeks. No significant changes were observed in serum leptin levels in group C, while both group A and group B exhibited a significant reduction. Approximately, 17.1 and 39.2% reductions in serum leptin levels were observed in group A and 25.1 and 44.3% reductions in serum leptin levels were observed in group B, respectively, at the end of 4 and 8 weeks. Approximately, 5.5 and 2.0% reductions in serum leptin levels were observed at the end of 4 and 8 weeks, respectively, in group C.

Table 2 summarizes the amount of remaining food over the period of 8 weeks for each group, which reflects a remarkable trend towards appetite suppression in both group A and group B. No changes were observed in the appetite of group C. Approximately, a 15.6 and 21.2% reduction in appetite was observed in group A and group B at the end of 8 weeks respectively.

Table 3 summarizes the changes in lipid profiles, including LDL, HDL, triglycerides, VLDL and total cholesterol, in groups A, B and C. There was some reduction in LDL and triglycerides in group A; however, the changes that were observed in group B were even more significant. Group B demonstrated a boost in HDL levels, while little effect was observed in group A. The overall total cholesterol level decreased significantly in both groups A and B. Approximately, 6.7 and 13.2% reductions in LDL levels were observed in group A, while under these same conditions, approximately 7.1 and 19% reductions in LDL levels were observed in group B at the end of 4 and 8 weeks respectively. A slight increase in LDL levels, however, was observed in group C at both time points. Approximately, 4.7 and 8.0% increases in HDL levels were observed in group A, while 11.8 and 22.0% increases in HDL levels were observed in group B, respectively, at the end of 4 and 8 weeks. No significant changes were observed in group C. Approximately, 2.9 and 5.9% reductions in triglyceride levels were observed in group A and 14.2 and 20.2% reductions in triglyceride levels were observed in group B, respectively, at the end of 4 and 8 weeks respectively. No significant changes were observed in group C. No significant changes were observed in the VLDL levels in any of the groups. Approximately, 3 and 7.2% reductions in total cholesterol levels were observed in group A and 1.2 and 9.5% reductions in total cholesterol were observed in group B at the end of 4 and 8 weeks respectively. No changes were observed in group C.

Enhanced excretion of urinary fat metabolites, including MDA, ACT, FA and ACON, was quantified as a biomarker of fat oxidation. Approximately, 35.6–106.4% increases in total urinary fat metabolites in group A and 56–134% increases in total urinary fat metabolites in group B were observed at the end of 8 weeks, respectively, as compared with the control sample. In group C, 6.2–21% increases were observed at the end of 8 weeks.

Table 2 Effects of placebo (group C), (-)-hydroxycitric acid (HCA-SX) alone (group A) and HCA-SX formula (group B) on appetite in human subjects

Group	Remaining food (g) on the plate as an index of appetite suppression							
	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7	Week 8
Placebo (group C)	110 ± 23.5	86.3 ± 18.4	79.4 ± 23.0	93.8 ± 37.3	91.3 ± 23.2	88.8 ± 33.5	91.9 ± 48.0	93.4 ± 35.2
HCA-SX (group A)	178 ± 49.9	257 ± 87.4	288 ± 41.6	242 ± 40.1	221 ± 48.3	285 ± 33.7	320 ± 46.4	352 ± 42.1
HCA-SX formula (group B)	208 ± 20.3	238 ± 32.1	268 ± 28.2	369 ± 43.5	391 ± 56.5	444 ± 55.7	422 ± 41.1	478 ± 40.0

Data are presented as group mean ± s.e.m. Subjects were given (-)-hydroxycitric acid (HCA-SX) alone (group A), HCA-SX formula (group B) or placebo (group C) for 8 weeks. See *Methods and Procedures* for details. In group A, only three of the possible 28 pairs of differences are significant. In group B, 17 of the possible 28 pairs of differences are significant, while in group C, none of the 28 possible differences is significant all at 5% level of significance.

Table 3 Effects of placebo (group C), (-)-hydroxycitric acid (HCA-SX) alone (group A) and HCA-SX formula (group B) on lipid profile in human subjects

Group	Time point	LDL (mg/dl)	HDL (mg/dl)	Triglycerides (mg/dl)	VLDL (mg/dl)	Total cholesterol (mg/dl)
Placebo (group C)	I	105.38 ± 5.44 ^a	27.31 ± 0.94 ^a	125.0 ± 12.59 ^a	25.0 ± 3.04 ^a	158.0 ± 5.11 ^a
	M	106.5 ± 6.25 ^a	27.75 ± 0.97 ^a	129.0 ± 13.12 ^a	26.50 ± 2.81 ^a	161.0 ± 4.96 ^a
	F	109.75 ± 6.55 ^a	26.13 ± 0.82 ^a	126.0 ± 11.97 ^a	24.13 ± 2.50 ^a	160.0 ± 6.30 ^a
HCA-SX (group A)	I	114.21 ± 5.23 ^a	30.05 ± 1.18 ^a	105.0 ± 9.96 ^a	22.26 ± 2.45 ^a	167.0 ± 6.20 ^a
	M	106.58 ± 4.72 ^b	31.47 ± 1.28 ^b	102.0 ± 7.98 ^a	23.84 ± 1.64 ^a	162.0 ± 5.11 ^a
	F	99.11 ± 4.70 ^c	32.42 ± 1.26 ^b	98.80 ± 7.38 ^a	23.05 ± 2.36 ^a	155.0 ± 5.25 ^b
HCA-SX formula (group B)	I	115.22 ± 4.04 ^a	29.72 ± 1.27 ^a	127.0 ± 13.09 ^a	24.17 ± 2.59 ^a	169.0 ± 5.19 ^a
	M	107.0 ± 4.10 ^b	33.22 ± 1.16 ^b	109.0 ± 12.55 ^b	26.56 ± 3.25 ^a	167.0 ± 5.71 ^a
	F	93.33 ± 4.54 ^c	36.28 ± 1.11 ^c	101.0 ± 9.44 ^b	23.78 ± 1.77 ^a	153.0 ± 5.14 ^b

HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very-low-density lipoprotein. Data are presented as group mean ± s.e.m. Subjects were given HCA-SX alone (group A), HCA-SX formula (group B) or placebo (group C) for 8 weeks. See *Methods and Procedures* for details. I, week 0; M, week 4; F, week 8. Values with non-identical superscripts are significantly different (p < 0.05).

In group A, MDA, ACT, FA and ACON increased by 1.3-, 1.3-, 1.8- and 1.2-fold at the end of 4 weeks and 1.9-, 1.8-, 2.1- and 1.4-fold at the end of 8 weeks. In group B, MDA, ACT, FA and ACON increased by 1.3-, 1.4-, 1.6- and 1.3-fold at the end of 4 weeks and 2.3-, 1.9-, 2.0- and 1.6-fold at the end of 8 weeks. In group C, MDA, ACT, FA and ACON increased by 0.9-, 0.9-, 1.2- and 1.0-fold at the end of 4 weeks and 1.2-, 1.1-, 1.2- and 1.1-fold at the end of 8 weeks (table 4).

In summary, we observe that among the three groups, group B showed a significant difference among the least-square means for the three time points, which were 'I & M', 'I & F' and 'M & F' for the variables body weight, BMI, LDL, HDL, triglycerides, total cholesterol, serum leptin levels and excretion of urinary fat metabolites. It did not show any significant difference for the variable VLDL. It also showed the most number, which is 17, out of the 28 paired differences between the variable 'remaining food' as significant. Group A did show a

significant difference among the least-square means at the three time points for the variables body weight, BMI, serum leptin levels and excretion of urinary fat metabolites. For LDL and HDL, the 'I & M' and 'I & F' differences are significant but not the 'M & F' difference. For the variable VLDL, there are no significant paired differences. For total cholesterol, only the 'M & F' and 'I & F' differences are significant. It also showed only a few, which is three of the 28, paired differences between the variable 'remaining food' as significant. Group C showed some significant difference in body weight, BMI and HDL. For the remaining six variables, it did not show any significant change in the levels across the three time points. As for the remaining food variable, none of the 28 paired differences was significant. Thus, a statistically significant reduction in body weight, BMI, appetite, LDL, total cholesterol and triglyceride levels, an increase in HDL levels, a decrease in serum leptin levels and enhanced excretion of urinary fat metabolites were

Table 4 Effects of placebo (group C), (-)-hydroxycitric acid (HCA-SX) alone (group A) and HCA-SX formula (group B) on enhanced excretion of urinary fat metabolites (nmol/ml of urine)

Group	Time point	MDA	ACT	FA	ACON
Placebo (group C)	I	0.155 ± 0.014 ^a	1.277 ± 0.125 ^a	3.253 ± 0.190 ^a	18.09 ± 0.677 ^a
	M	0.146 ± 0.009 ^a	1.181 ± 0.220 ^a	3.789 ± 0.279 ^a	17.85 ± 0.881 ^a
	F	0.187 ± 0.011 ^b	1.437 ± 0.254 ^a	3.749 ± 0.240 ^a	19.20 ± 1.032 ^a
HCA-SX (group A)	I	0.138 ± 0.021 ^a	1.077 ± 0.066 ^a	2.98 ± 0.140 ^a	19.38 ± 1.054 ^a
	M	0.173 ± 0.021 ^b	1.363 ± 0.096 ^b	5.293 ± 0.406 ^b	22.54 ± 1.125 ^b
	F	0.26 ± 0.025 ^c	1.972 ± 0.126 ^c	6.152 ± 0.244 ^c	26.28 ± 1.216 ^c
HCA-SX formula (group B)	I	0.123 ± 0.030 ^a	0.93 ± 0.047 ^a	3.804 ± 0.234 ^a	19.35 ± 1.006 ^a
	M	0.159 ± 0.048 ^a	1.342 ± 0.101 ^b	6.099 ± 0.311 ^b	24.64 ± 0.904 ^b
	F	0.288 ± 0.058 ^b	1.764 ± 0.093 ^c	7.597 ± 0.225 ^c	30.18 ± 0.947 ^c

ACON, acetone; ACT, acetaldehyde; FA, formaldehyde; MDA, malondialdehyde. Data are presented as group mean ± s.e.m. Subjects were given HCA-SX alone (group A), HCA-SX formula (group B) or placebo (group C) for 8 weeks. See *Methods and Procedures* for details. I, week 0; M, week 4; F, week 8. Values with non-identical superscripts are significantly different (p < 0.05).

observed when taking HCA-SX alone and in combination with NBC and GSE. Supplementation with a combination of HCA-SX, NBC and GSE (group B) resulted in greater improvements than HCA-SX (group A) alone in all parameters evaluated.

Adverse Events

Fifty-three of the initial 60 subjects completed the study. During the 8-week study, no serious adverse effects were observed in any of the subjects. No patient was removed or dropped out of the study as a result of an adverse event caused by the treatment. In the HCA-SX group (group A), one incidence of leg cramps, two incidences of heartburn, four incidences of diarrhoea, four incidences of gas, one incidence of increased appetite, seven incidences of headaches, one incidence of stomach burn, one incidence of skin rash, one incidence of menstrual bleeding and two incidences of general weakness were reported. In the HCA-SX, NBC and GSE group (group B), two incidences of leg cramps, five incidences of mild diarrhoea, 10 incidences of mild gas and one incidence of headaches were observed. In the placebo group (group C), one incidence of leg cramps, two incidences of heartburn, nine incidences of diarrhoea, four incidences of gas, one incidence of increased appetite, one incidence of stomach burning, two incidences of irregular periods, two incidences of menstrual bleeding and one incidence of general weakness were reported. Taken together, the number of patients reporting adverse events in the supplemented groups was not significantly different from the placebo group.

Drop Outs

Seven subjects dropped out of this study. No subjects dropped out of the study as a result of an adverse event caused by the treatment. One subject dropped out of the HCA-SX group (group A) at the end of the twenty-first day of the study. This subject (initial body weight: 90 kg) reported a common headache on day 5. Two subjects dropped out of HCA-SX formula group (group B) at the end of the twelfth and twenty-eighth day. The first subject (initial body weight: 71 kg) reported regular leg cramps on day 3, while the second subject (94 kg) reported diarrhoea on the eleventh day. Four subjects dropped out of the placebo group (group C) at the end of the twelfth, thirty-sixth, forty-third and forty-eighth day. The first subject (109.5 kg) did not report any adverse events. The second subject (86 kg) did not report any adverse events, and at the end of the twenty-eighth day, his body weight was 84.5 kg. The third subject (78.5 kg) reported

gas on day 6 and diarrhoea on day 23, and at the end of 28 days, this subject gained approximately 1.0 kg (body weight: 79.5 kg). The fourth subject (106 kg) reported no adverse events during these 48 days, and at the end of 28 days, this subject lost approximately 1.5 kg (body weight: 104.5 kg).

Discussion

Obesity continues to be a major health problem in developed and developing countries. Obesity increases the risk of hypertension, type 2 diabetes, arthritis, elevated cholesterol, cancer and serious hormonal imbalances in women [42,43]. Obesity and its related metabolic and cardiovascular complications continue to present an escalating challenge to contemporary medicine. Obesity signifies chronic disequilibrium between food consumption and energy expenditure [3–6].

HCA, derived from the fruit rind of *G. cambogia*, exhibits a distinctive sour taste and has been used for culinary purposes in southern Asia for centuries to make meals more filling [44]. HCA is known to reduce appetite, inhibit fat synthesis and decrease body weight without stimulating the central nervous system. HCA does not cause nervousness, rapid heart rate, high blood pressure or insomnia, symptoms that are often associated with dietary stimulants such as ephedra (mahuang), caffeine or phenylpropanolamine [45].

HCA promotes weight reduction through suppressed *de novo* fatty acid synthesis [14,16,17]. One mechanism accounting for the beneficial effects of HCA may involve inhibition of ATP citrate lyase, which will eventually limit the availability of acetyl coenzyme A and lipid synthesis during carbohydrate feeding [14,16,17,44]. In our previous study and in the present study, we have demonstrated other important mechanisms including appetite suppression by HCA and serotonin release by rat brain cortex [23,35], regulatory roles in the lowering of lipid profiles and serum leptin levels and increased fat oxidation as demonstrated by enhanced excretion of urinary fat metabolites.

In examining some positive weight-loss studies, a randomized, placebo-controlled study on HCA-SX was conducted by Ramos *et al.* [31], involving 20 overweight adults, for a period of 8 weeks. It was demonstrated that 500 mg of HCA-SX taken three times per day before meals for 8 weeks resulted in 215% greater weight loss than those taking a placebo. This occurred without any side effects commonly associated with other dietary stimulants [45]. Of added importance, a significant reduction was observed in cholesterol and triglyceride levels.

Another study by Mattes and Bormann [32] demonstrated that a daily dose of 1.2 g of HCA along with a daily diet of 5020 kJ (1200 kcal) for 12 weeks resulted in a significant difference in weight loss (3.7 ± 3.1 vs. 2.4 ± 2.9 kg) compared to placebo. Westerterp-Plantenga and Kovacs [34] conducted a 6-week randomized, placebo-controlled, single-blind, cross-over study in 12 males and 12 females using a daily dose of 900 mg of HCA-SX for 2 weeks. They demonstrated that HCA-SX supplementation reduced 24-h energy intake in humans, while satiety was sustained.

In examining the 'negative studies', Heymsfield *et al.* [46] provided what became an accepted daily dose of 1.5 g of HCA along with a diet of 5020 kJ/day or 1200 kcal/day for 12 weeks and reported that no significant difference in weight loss was observed between the placebo and treatment groups. Several problems with the study, however, may be responsible for the negative results [46]. Heymsfield *et al.* [46] quantified the HCA content but did not assess the bioavailability of the HCA sample used in the study. Many HCA products are less than 50% soluble in water and poorly absorbed. Also, this low-calorie diet (5020 kJ/day) may have accounted for the substantial decreases in body weight of both treatment and placebo groups, blunting the ability of HCA to show curbed appetite and reduced food intake.

Our present study was conducted using a highly bioavailable, water-soluble calcium-potassium salt of HCA (HCA-SX). Supplements were given on an empty stomach at least 30–60 min before meals to enhance bioavailability, which was previously demonstrated by Loe *et al.* [36]. This more efficacious dose was extrapolated from *ex vivo* and *in vivo* studies, as discussed in the *Methods and Procedures*, which is an increased dose of HCA compared to other studies. The available sources of HCA in the market today are a calcium salt that is poorly soluble in water, allowing for compromised absorption.

This study was designed to better determine the effects of HCA-SX, at a higher, more efficacious dose, on satiety. Subjects were administered a 2000 kcal or 8372 kJ diet/day, and all remaining food was weighed after each meal to determine HCA-SX's effect on appetite reduction. Results demonstrated a significant reduction in appetite following supplementation of HCA-SX alone or in combination with NBC and GSE.

A statistically significant reduction in body weight was observed between 'I' and 'M' but not between 'M' and 'F' in the placebo (group C), which signifies that a controlled diet and exercise initially result in weight reduction but eventually plateaus. This fact highlights the importance of a novel, efficacious safe supplement for weight management.

Perceived weight loss in both the HCA-SX (group A) and HCA-SX formula (group B) group is supported by the improvement in BMI, which implies a sparing of lean muscle and fat oxidation, as demonstrated by enhanced excretion of urinary fat metabolites (table 4). Muscle mass is denser and heavier, and thus, sparing lean muscle mass contributes to slow and steady fat loss that is preferable to most people.

Downregulation of obesity regulatory gene may be an additional mechanism of HCA's ability to reduce body weight and appetite. Leptin, a 167-amino acid protein hormone and a biomarker of the obesity regulatory gene, is synthesized and secreted by adipocytes and is present in the bloodstream and is directly related to body fat. Leptin binds to receptors in the brain and activates signals that inhibit food intake and increase energy expenditure [47–49]. Leptin resistance develops when receptor-binding activity is diminished and, as a result, plasma leptin levels increase, which in turn lose their ability to inhibit food intake and increase energy expenditure. Generally, plasma leptin levels are higher in overweight individuals than in normal individuals and higher in women than in men. Leptin has been shown to be able to modulate insulin secretion and action through these receptors [47]. In the present study, it was demonstrated that supplementation with HCA-SX alone and in combination with NBC and GSE significantly decreases serum leptin levels, which may play a significant role in downregulating the obesity regulatory gene.

The fat-degradation or fat-oxidation ability of HCA-SX was evaluated based on the excretion of urinary fat metabolites including MDA, ACT, FA and ACON. Enhanced β -oxidation of fat may be the prime sources of these four fat metabolites. Enhanced excretion of MDA was observed during increased oxidative stress [50]. In the same study, radiolabelled MDA administered to rats was found to be extensively metabolized to acetate and carbon dioxide. The enhanced excretion of urinary ACT identified in the present study may be due to the breakdown of MDA or as a result of fat oxidation/lipid peroxidation. The enhanced urinary excretion of ACON may occur in response to a consequence of enhanced β -oxidation, as reported earlier [51]. Metabolism of glycerol to FA has been reported in rat liver microsomes and is a result of the metabolism of triglycerides by adipose tissue and other tissues that possess the enzyme that activates glycerol, namely glycerol kinase [52]. High glycerol kinase levels are found in liver and brown tissues [52,53]. Other possible sources of FA might include the breakdown of MDA to acetate or ACT and a one-carbon fragment [50] and/or the cleavage

of a one-carbon fragment from acetoacetic acid with the formation of ACON. In the present study, the triglyceride levels were significantly reduced in subjects supplemented with either HCA-SX or HCA-SX, NBC and GSE, accompanied by significant increases in urinary excretion of FA, which suggests that these supplements may induce enhanced production of glycerol kinase in the adipose tissues.

Our earlier studies demonstrated that supplementation with NBC is bioavailable and helps in weight reduction and regulation of blood lipids in overweight and hypercholesterolemic subjects [24,54]. GSE has been demonstrated to promote weight loss by its ability to reduce sugar cravings and control blood sugar levels [27–30,55,56].

The current study demonstrates that supplementation with HCA-SX or HCA-SX, NBC and GSE significantly improves the levels of total cholesterol, LDL, HDL and triglycerides, which are primary risk factors of cardiovascular diseases.

Our present findings demonstrate that the present dose of HCA-SX alone or in combination with NBC and GSE, given 30–60 min before meals, is highly bioavailable, efficacious and safe as weight-management supplements. Furthermore, the present study demonstrated that HCA-SX alone or in combination with NBC and GSE can effectively cause fat degradation and beneficially regulate lipid profiles and serum leptin levels. The reduced weight loss, BMI, serum leptin levels, appetite, food intake and increased fat oxidation indicate that supplementation with HCA-SX alone and in combination with NBC and GSE is a novel therapeutic tool for weight management.

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