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Dose- and time-dependent effects of a novel (–)-hydroxycitric acid extract on body weight, hepatic and testicular lipid peroxidation, DNA fragmentation and histopathological data over a period of 90 days

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Abstract

(–)-Hydroxycitric acid (HCA), a natural extract from the dried fruit rind of *Garcinia cambogia* (family Guttiferae), is a popular supplement for weight management. The dried fruit rind has been used for centuries as a condiment in Southeastern Asia to make food more filling and satisfying. A significant number of studies highlight the efficacy of Super CitriMax (HCA-SX, a novel 60% calcium-potassium salt of HCA derived from *Garcinia cambogia*) in weight management. These studies also demonstrate that HCA-SX promotes fat oxidation, inhibits ATP-citrate lyase (a building block for fat synthesis), and lowers the level of leptin in obese subjects. Acute oral, acute dermal, primary dermal irritation and primary eye irritation toxicity studies have demonstrated the safety of HCA-SX. However, no long-term safety of HCA-SX or any other (–)-hydroxycitric acid extract has been previously assessed. In this study, we have evaluated the dose- and time-dependent effects of HCA-SX in Sprague-Dawley rats on body weight, hepatic and testicular lipid peroxidation, DNA fragmentation, liver and testis weight, expressed as such and as a % of body weight and brain weight, and histopathological changes over a period of 90 days. The animals were treated with 0, 0.2, 2.0 and 5.0% HCA-SX as feed intake and the animals were sacrificed on 30, 60 or 90 days of treatment. The feed and water intake were assessed and correlated with the reduction in body weight. HCA-SX supplementation demonstrated a reduction in body weight in both male and female rats over a period of 90 days as compared to the corresponding control animals. An advancing age-induced marginal increase in hepatic lipid peroxidation was observed in both male and female rats as compared to the corresponding control animals. However, no such difference in hepatic DNA fragmentation and testicular lipid peroxidation and DNA fragmentation was observed. Furthermore, liver and testis weight, expressed as such and as a percentage of body weight and brain weight, at 30, 60 and 90 days of treatment, exhibited no significant difference between the four groups. Taken together, these results indicate that treatment of HCA-SX over a period of 90 days results in a reduction in body weight, but did not cause any changes in hepatic and testicular lipid peroxidation, DNA fragmentation, or histopathological changes. (Mol Cell Biochem **254**: 339–346, 2003)

Key words: *Garcinia cambogia*, (–)-Hydroxycitric acid, 90-day toxicity study, body weight, liver weight, testis weight, lipid peroxidation, DNA fragmentation, histopathology

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Introduction

Obesity is an epidemic in the United States, with approximately 40 million Americans classified as obese [1]. A sedentary lifestyle, unrestricted caloric intake, high carbohydrate and fat diets, minimal or no exercise and excessive alcohol intake have been identified as root causes of obesity. Obesity is a major challenge to health professionals [2]. Although, a number of natural weight management ingredients are available in the market, the safety and/or efficacy of these ingredients are either in question or inadequately supported by scientific research. Ephedra, a popular weight loss ingredient, may cause a number of adverse effects, including rapid heart rate, elevated blood pressure and death in very rare cases. Ephedra, as well as guarana and kola nuts, which are natural sources of caffeine, are central nervous system stimulants [3].

The dried fruit rind of *Garcinia cambogia* (family Guttiferae), also known as Malabar tamarind, is a unique source of (–)-hydroxycitric acid (HCA), which exhibits a distinct sour taste and has been used for centuries in Southeastern Asia to make meals more filling [4, 5]. No harmful effects have been associated with the consumption of *Garcinia cambogia.*

HCA has been demonstrated 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. HCA is known to cause a number of beneficial effects, including a reduction in food intake, decreased appetite, increased fat oxidation and reduction in sweet cravings, especially during the hyperlipogenic nutritional condition produced by high carbohydrate feeding [6, 7].

Recent studies in our laboratory have demonstrated that oral supplementation of Super CitriMax (HCA-SX) is highly bioavailable in human plasma as demonstrated by a gas chromatography-mass spectrometric technique [8]. HCA-SX remained in the blood for more than 4–9 h after oral ingestion. HCA-SX exhibited superior bioavailability on an empty stomach. In another study, we demonstrated that HCA-SX enhances serotonin availability in isolated rat brain cortex by acting as a mild serotonin receptor re-uptake inhibitor (SRRI), without demonstrating a stimulatory effect on the central nervous system [9].

A number of clinical studies have demonstrated the efficacy of HCA-SX in weight management in human volunteers [4, 5, 10–13]. Acute oral, acute dermal, primary dermal irritation and primary eye irritation toxicity studies demonstrated the safety of HCA-SX. However, to date, no long-term studies have been conducted on HCA-SX or any other (–)-hydroxycitric acid product [14]. In the present study, we have conducted a 90-day safety study using three different doses of HCA-SX in both male and female rats. Doses used were 0.2, 2.0 and 5.0% of feed intake. The 0.2% feed intake of HCA-SX equals 4.62 g as a 60% HCA extract, or 2,772 mg HCA per day, which is the recommended dosage for human consumption, based on previous trials. The 2.0 and 5.0% of feed intake represents 10 and 25 fold higher doses, respectively [15]. Furthermore, to evaluate the safety of HCA-SX, we extensively investigated the liver and testis tissues to determine any adverse effects. Since, weight loss has been shown to be associated with hypophagia, we monitored the food and water intake over the 90 days of treatment. Water and feed intake, and body weights of the animals were monitored routinely. Liver, testis and brain weights were determined on days 30, 60 and 90 of treatment. Furthermore, hepatic and testis weights were determined as a percent of body weight and brain weight. To determine the effects of HCA-SX on hepatic and testis tissues, we conducted hepatic and testicular lipid peroxidation, DNA fragmentation and histopathological evaluations.

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-600-SXS (HCA-SX, Lot #'s: 105033, 203022 and 201030) was obtained from InterHealth Nutraceuticals, Benicia, CA, USA. HCA-SX samples were stored in a dry, cool place at room temperature (18–25°C). 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

Male and female Sprague-Dawley rats (males weighing 251–320 g; females weighing 154–241 g) were obtained from Charles River Breeding Laboratories (Portage, MI, USA). The animals were given access to lab chow (Purina Certified Rodent Chow, #5002) and given access to filtered tap water, *ad libitum*. Animals were allowed to acclimate in a 10" × 7" × 7" stainless steel cage with compressed pine pellets (Gentle Touch Products, Norfolk, NE, USA) used as bedding in an environment of controlled temperature (65–79°F), 40–70% relative humidity and 12 h light/12 h dark cycle for 10 days prior to initiation of the study. Animals were maintained one per cage and used in accordance with the current National Institute of Health Guidelines and the ARVO Resolution on the Use of Animals in Research. An animal research protocol (ARC# 0598) was obtained from Creighton University Medical Center (Omaha, NE, USA). HCA-SX was dissolved

in water and administered by gavage using a feeding needle. HCA-SX was given at 0, 0.2, 2.0 and 5.0% of feed intake. Food and water consumption was measured twice or thrice weekly. Mortality/morbidity was evaluated once daily on weekdays, weekends and holidays. Clinical signs were evaluated once to twice daily. Body weights were taken on day 1, twice weekly thereafter, and before necropsy. Animals were sacrificed on 30, 60 and 90 days of treatment and the target organs were either processed immediately, preserved in 10% buffered formalin for histopathology, or stored at –80°C.

Lipid peroxidation

The formation of thiobarbituric acid-reactive substances in liver and testis tissues from control and treated animals was assessed as a determinant of lipid peroxidation according to the method of Buege and Aust [16] and as published by us previously [17]. In summary, 3 ml of 1% H_3PO_4 and 1 ml of 0.6% thiobarbituric acid in water was added to each 0.50 ml sample. Samples were mixed, heated for 45 min at 90°C, cooled, and extracted with n-butanol. Malondialdehyde was used as the standard [18]. Absorbance values of the organic phases were measured at 535 nm, and an extinction coefficient of $1.56 \times 10^5\ M^{-1}\,cm^{-1}$ was used.

DNA fragmentation

Frozen liver and testis samples were homogenized in lysis buffer (5 mM of Tris-HCl, 20 mM of ethylenediaminetetraacetic acid, 0.5% Triton X-100, pH 8.0). Homogenates were centrifuged at 27,000 × g for 20 min to separate intact chromatin in the pellets from fragmented DNA in the supernatant fractions. Pellets were resuspended in 0.5 N of perchloric acid, and 5.5 N of perchloric acid was added to supernatant fractions to reach a concentration of 0.5 N. Samples were heated at 90°C for 15 min and centrifuged at 1500 × g for 10 min to remove protein. Resulting supernatant fractions were reacted with diphenylamine for 16–20 h at room temperature. Absorbance was measured at 600 nm. DNA fragmentation in control samples is expressed as the percentage of total DNA appearing in the supernatant fraction. Treatment effects are reported as percentages of control fragmentation [19].

Histology

A 2–3 mm section of the respective tissue was collected at the time of sacrifice and preserved in 10% buffered formalin. Sections were sent to IDEXX (West Sacramento, CA, USA) for further processing, sectioning and PAS (Periodic Acid Schiff: a purple color specific for glycogen or H&E) staining [20].

Statistics

The data were analyzed using ANOVA and Scheffe's S method as the *post-hoc* test. All values are reported as mean ± S.D. from 5–7 samples. Statistical significance was set at $p < 0.05$.

Results

Dose and time-dependent effects of HCA-SX on feed and water intake

Dose- and time-dependent effects of HCA-SX on feed and water intake are shown in Tables 1a and 1b, respectively. Both dose- and time-dependent effects of HCA-SX were observed on feed intake in both male and female rats. On day 90, feed intake was reduced by 13.7, 26.7 and 25.6% in male rats following supplementation of 0.2, 2.0 and 5.0% HCA-SX as feed intake, respectively, as compared to the corresponding control animals, while under these same conditions approximately 16.3, 19.6 and 22.8% reduction in feed intake were observed in female rats (Table 1a). No significant change in water intake in any of the groups of rats was observed at the doses of HCA-SX and time points used (Table 1b).

Dose and time-dependent effects of HCA-SX on body weight, liver weight and testis weight

The changes in body weights following supplementation of HCA-SX to the male and female rats are presented in Table 2a. Approximately 11.2, 12.4 and 15.8% reduction in body weights were observed in male rats following supplementation of 0.2, 2.0 and 5.0% HCA-SX as feed intake, respectively, as compared to the corresponding control animals, while under these same conditions approximately 11.7, 18.1 and 13.0% reduction in body weights were observed in female rats (Table 2a).

The weights of the right and both testis in male rats at 30, 60 and 90 days of treatment following supplementation of 0, 0.2, 2.0 and 5.0% HCA-SX are provided in Table 2b. In control animals, a small but not statistically significant increase in testis weight with increasing age was observed. The testis weights of the HCA-SX supplemented animals was similar to the control animals.

Table 1a. Effect of HCA-SX on feed intake (g) in male and female rats

	Male				Female			
Days	0%HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
1–4	32.6 ± 5.98	41.4 ± 9.53	33.4 ± 7.23	39.2 ± 13.77	30.0 ± 8.94	38.2 ± 15.66	37.8 ± 11.95	27.8 ± 7.89
4–8	40.6 ± 11.59	45.8 ± 9.28	39.4 ± 12.58	53.4 ± 5.46	39.8 ± 9.50	47.2 ± 4.15	34.8 ± 8.58	42.0 ± 5.05
8–11	49.0 ± 11.51	44.6 ± 10.19	52.0 ± 16.69	59.4 ± 13.07	51.8 ± 10.87	51.6 ± 5.90	42.4 ± 10.43	52.2 ± 9.01
11–15	52.8 ± 11.54	51.8 ± 10.66	38.0 ± 10.27	59.2 ± 14.46	49.2 ± 9.04	52.4 ± 9.07	61.4 ± 8.35	62.6 ± 8.99
15–18	64.4 ± 7.50	64.2 ± 13.57	56.2 ± 11.76	55.6 ± 11.70	60.0 ± 11.42	53.6 ± 9.07	72.8 ± 6.83	61.2 ± 13.33
18–22	74.4 ± 9.24	58.0 ± 19.65	58.4 ± 12.97	69.4 ± 12.88	61.8 ± 9.12	62.4 ± 13.99	70.4 ± 9.21	63.4 ± 17.31
22–25	80.6 ± 14.24	60.8 ± 15.59	59.0 ± 10.22	63.4 ± 10.95	63.2 ± 10.43	66.2 ± 26.26	72.4 ± 13.39	66.0 ± 16.08
25–29	93.6 ± 6.27	77.2 ± 9.23	70.2 ± 14.50	65.0 ± 16.49	64.8 ± 14.13	67.0 ± 18.57	78.4 ± 15.06	69.2 ± 14.58
29–32	98.0 ± 11.90	71.8 ± 7.29	75.2 ± 14.82	76.0 ± 19.65	74.6 ± 13.52	77.8 ± 24.00	83.6 ± 18.45	73.2 ± 7.16
32–36	103.2 ± 14.70	78.0 ± 14.83	72.6 ± 16.85	76.0 ± 5.83	75.2 ± 10.92	73.4 ± 7.06	76.0 ± 11.90	76.6 ± 4.72
36–39	102.6 ± 9.37	88.0 ± 22.17	76.4 ± 11.61	75.4 ± 10.90	82.4 ± 15.98	77.6 ± 6.58	77.8 ± 11.92	77.2 ± 9.76
39–43	108.6 ± 7.02	86.8 ± 18.74	83.0 ± 13.30	80.6 ± 8.65	86.6 ± 13.70	77.4 ± 6.91	83.2 ± 16.12	75.4 ± 12.97
43–46	108.6 ± 15.09	97.6 ± 20.01	82.0 ± 16.67	83.6 ± 17.92	86.8 ± 12.91	79.8 ± 10.55	77.2 ± 11.26	75.2 ± 16.21
46–50	104.0 ± 10.98	102.4 ± 17.64	88.4 ± 16.82	82.2 ± 15.82*	83.2 ± 17.28	86.2 ± 4.15	79.6 ± 9.76	79.0 ± 6.28
50–53	98.2 ± 9.76	94.0 ± 16.91	90.0 ± 18.22	82.8 ± 7.56	92.4 ± 15.58	77.8 ± 6.30	79.8 ± 11.61	82.6 ± 15.77
53–57	106.4 ± 13.58	103.4 ± 11.93	76.4 ± 13.81*	80.0 ± 8.83*	94.4 ± 14.52	80.6 ± 9.15	81.8 ± 7.50	79.2 ± 5.02
57–60	102.2 ± 5.12	101.8 ± 13.50	83.6 ± 16.46*	77.2 ± 4.38*	95.4 ± 11.04	84.8 ± 18.07	86.4 ± 8.85	81.2 ± 6.69
60–64	102.6 ± 9.53	99.0 ± 7.55	80.8 ± 17.12*	78.4 ± 9.84*	97.4 ± 10.43	84.4 ± 18.50	92.0 ± 11.34	83.6 ± 10.74
64–67	98.8 ± 19.98	98.0 ± 7.62	88.2 ± 14.29	74.4 ± 13.96*	98.6 ± 11.61	82.8 ± 14.32	91.4 ± 13.74	87.6 ± 11.01
67–71	102.2 ± 17.84	103.4 ± 6.54	90.6 ± 13.89	72.4 ± 11.35*	96.2 ± 11.84	85.6 ± 17.95	87.4 ± 7.06	87.8 ± 11.37
71–74	105.8 ± 19.77	88.8 ± 8.41	84.4 ± 7.89*	76.8 ± 7.69*	95.6 ± 18.23	82.2 ± 10.69*	87.0 ± 6.71	86.6 ± 10.01
74–78	105.4 ± 17.99	92.0 ± 14.23*	86.4 ± 7.27*	79.2 ± 9.65*	97.0 ± 14.59	85.8 ± 6.46*	83.8 ± 4.27*	81.0 ± 7.42*
78–81	108.2 ± 10.94	97.2 ± 10.71	85.4 ± 14.06*	81.2 ± 9.65*	96.4 ± 11.15	85.5 ± 6.46*	80.8 ± 7.16*	78.8 ± 10.38*
81–85	100.2 ± 8.98	97.0 ± 15.62	92.4 ± 10.01	86.2 ± 8.07*	93.4 ± 13.30	82.8 ± 4.15*	79.2 ± 5.07*	78.4 ± 11.67*
85–88	104.6 ± 5.50	97.4 ± 13.67*	94.4 ± 12.99*	83.6 ± 4.34*	96.2 ± 12.13	84.6 ± 3.13*	78.0 ± 5.15*	75.2 ± 5.89*
88–90	112.4 ± 7.83	97.0 ± 13.56*	82.4 ± 6.84*	83.6 ± 5.90*	92.0 ± 14.76	77.0 ± 7.11*	74.0 ± 7.52*	71.0 ± 8.15*

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle. Each value represents the mean + S.D. of 5–7 animals. Significantly different from the control group (*$p < 0.05$).

Table 2c provides the data on liver and testis weights expressed as a percentage of body weight and brain weight of male rats at 30, 60 and 90 days of treatment. A comparison of the control samples with the three HCA-SX-supplement groups, indicates no significant difference between control and HCA-SX supplemented groups in liver and testis weights.

Dose and time-dependent effects of HCA-SX on hepatic and testicular lipid peroxidation

Dose- and time-dependent hepatic lipid peroxidation data in liver samples isolated from male and female Sprague-Dawley rats are shown in Table 3a. A time-dependent increase in hepatic lipid peroxidation was generally observed in all samples. However, HCA-SX administration did not induce a significant increase in hepatic lipid peroxidation in these animals. Dose- and time-dependent effects on testicular lipid peroxidation in male rats following supplementation of HCA-SX have been summarized in Table 3b. A small but non-significant increase in testicular lipid peroxidation was observed in the control group as well as the HCA-SX supplemented groups.

Dose and time-dependent effects of HCA-SX on hepatic and testicular DNA fragmentation

Dose- and time-dependent DNA fragmentation in liver samples isolated from male and female Sprague-Dawley rats is shown in Table 4a. HCA-SX treatment had no effect on hepatic DNA fragmentation in these animals. Dose- and time-dependent effects on testicular DNA fragmentation in male rats following supplementation of HCA-SX are summarized in Table 4b. As was observed in the liver, HCA-SX had no effect on testicular lipid peroxidation in these male rats.

Dose and time-dependent effects of HCA-SX on histopathology

Histopathological analyses were conducted on liver, brain and testis samples in all control and HCA-SX-treated animals. HCA-SX did not cause any morphological alterations in these samples (data not shown).

Table 1b. Effect of HCA-SX on water intake (ml/day) in male and female rats

	Male				Female			
Days	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
1–4	13.2 ± 3.03	16.0 ± 2.00	16.0 ± 2.83	14.6 ± 3.51	16.2 ± 1.92	14.0 ± 1.58	13.8 ± 1.92	13.4 ± 1.67
4–8	14.8 ± 4.02	14.8 ± 1.79	13.4 ± 2.19	13.8 ± 4.60	16.6 ± 3.13	15.2 ± 3.83	15.0 ± 2.45	14.0 ± 1.87
8–11	14.4 ± 3.65	15.2 ± 0.84	13.4 ± 2.79	13.0 ± 2.55	12.8 ± 3.56	14.6 ± 3.44	15.4 ± 1.82	14.8 ± 1.64
11–15	14.6 ± 3.21	14.8 ± 2.86	13.2 ± 3.42	15.2 ± 3.11	13.2 ± 1.10	15.8 ± 5.36	15.2 ± 1.92	16.0 ± 1.87
15–18	15.8 ± 2.05	15.0 ± 2.65	14.2 ± 2.95	11.04 ± 0.55	12.0 ± 2.35	16.6 ± 5.73	15.0 ± 1.87	15.2 ± 1.30
18–22	13.4 ± 1.67	14.6 ± 3.58	14.8 ± 3.27	13.8 ± 1.92	13.0 ± 2.83	13.6 ± 3.36	15.6 ± 1.82	16.0 ± 2.92
22–25	14.0 ± 2.00	14.8 ± 2.77	16.4 ± 4.56	16.6 ± 4.28	13.8 ± 2.68	14.4 ± 2.30	16.8 ± 3.03	14.6 ± 2.41
25–29	15.8 ± 2.77	15.4 ± 2.30	14.0 ± 4.36	14.8 ± 2.95	14.6 ± 2.61	15.6 ± 2.61	17.8 ± 2.59	15.0 ± 2.55
29–32	17.6 ± 1.34	15.6 ± 1.82	13.0 ± 2.45	16.0 ± 5.43	13.4 ± 2.41	16.4 ± 3.51	17.8 ± 3.49	15.8 ± 3.03
32–36	17.4 ± 2.19	15.4 ± 2.07	14.2 ± 2.59	18.6 ± 2.51	15.0 ± 3.94	17.2 ± 5.02	18.4 ± 2.41	15.4 ± 3.21
36–39	16.0 ± 3.87	15.8 ± 3.19	13.4 ± 1.82	17.8 ± 2.28	14.6 ± 3.05	18.6 ± 4.62	16.8 ± 3.11	16.4 ± 2.07
39–43	17.4 ± 3.97	15.8 ± 3.27	13.4 ± 2.97	17.6 ± 3.36	17.0 ± 4.64	19.2 ± 4.92	16.4 ± 2.07	17.2 ± 2.68
43–46	19.8 ± 2.49	16.6 ± 1.52	14.2 ± 6.02	17.0 ± 2.35	18.2 ± 3.63	16.6 ± 2.19	18.2 ± 2.77	16.4 ± 3.21
46–50	20.0 ± 3.00	15.4 ± 2.97	14.6 ± 5.03	16.4 ± 1.82	17.6 ± 2.51	17.0 ± 1.00	17.8 ± 3.03	18.2 ± 1.92
50–53	20.6 ± 3.58	16.0 ± 3.74	14.0 ± 5.52	17.0 ± 3.67	16.2 ± 2.28	17.8 ± 2.39	18.8 ± 1.64	17.4 ± 3.58
53–57	18.8 ± 2.86	14.4 ± 3.65	14.0 ± 5.52	17.4 ± 4.77	16.6 ± 2.88	17.4 ± 3.78	17.6 ± 4.04	18.8 ± 3.03
57–60	18.8 ± 3.11	15.6 ± 4.16	14.8 ± 5.02	16.0 ± 3.08	16.6 ± 4.16	17.2 ± 4.09	17.6 ± 3.29	18.8 ± 1.30
60–64	17.8 ± 4.38	17.0 ± 3.94	16.6 ± 3.91	13.6 ± 2.79	17.2 ± 3.19	18.6 ± 3.51	17.2 ± 2.77	18.6 ± 1.14
64–67	19.2 ± 3.42	18.4 ± 3.78	16.6 ± 4.10	12.8 ± 2.68	16.4 ± 2.19	19.8 ± 3.96	16.6 ± 2.79	17.8 ± 1.30
67–71	18.8 ± 4.60	19.2 ± 3.56	17.6 ± 3.78	15.6 ± 2.30	17.8 ± 1.79	18.4 ± 3.65	17.6 ± 3.44	18.0 ± 2.12
71–74	16.6 ± 3.21	18.2 ± 4.49	17.6 ± 3.29	15.2 ± 2.59	17.6 ± 3.21	18.2 ± 3.35	17.8 ± 3.42	18.2 ± 2.68
74–78	17.0 ± 3.32	18.2 ± 5.22	18.6 ± 3.29	16.4 ± 1.95	18.6 ± 1.52	20.8 ± 2.77	18.6 ± 3.05	18.8 ± 2.17
78–81	18.4 ± 2.41	18.4 ± 6.43	17.0 ± 2.12	16.8 ± 3.56	17.2 ± 1.92	17.4 ± 3.58	18.4 ± 2.88	18.8 ± 3.03
81–85	19.2 ± 2.39	15.2 ± 3.56	15.6 ± 1.82	16.2 ± 4.44	20.0 ± 2.55	17.6 ± 3.51	18.6 ± 3.36	17.6 ± 2.97
85–88	18.8 ± 3.49	17.0 ± 3.94	14.8 ± 2.77	17.0 ± 4.12	19.0 ± 2.35	17.6 ± 4.16	18.8 ± 3.27	18.0 ± 3.81
88–90	18.6 ± 4.62	17.2 ± 4.92	16.8 ± 4.44	18.4 ± 5.37	20.0 ± 1.58	19.0 ± 2.24	20.2 ± 3.49	19.2 ± 2.39

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Each value represents the mean ± S.D. of 5–7 animals.

Discussion

This study primarily focused on the safety parameters on the long-term use of HCA-SX in weight management. (–)-Hydroxycitric acid (HCA) is a naturally occurring primary organic acid found in the fruit and rind of *Garcinia cambogia*, which grows extensively in Southeastern Asia. The fruit, also known as Malabar tamarind, has been used for centuries in culinary dishes to make meals more filling [4, 5]. HCA is believed to work by inhibiting lipogenesis, the process by which the body converts carbohydrates into fat, by inhibiting the enzyme ATP citrate lyase. HCA reduces the availability of acetyl-CoA, the building block for fat synthesis, by inhibiting this enzyme [6, 7, 21].

We have previously evaluated a novel calcium-potassium salt of 60% HCA known as Super CitriMax (HCA-SX), which is highly bioavailable in human volunteers [8]. Studies in our laboratories have shown that HCA-SX can increase the release of serotonin (5-HT) from rat brain cortical slices *in vitro* and act as a mild serotonin receptor re-uptake inhibitor (SRRI), without stimulating the central nervous system [9]. Previous studies have also demonstrated the beneficial effects of HCA-SX on body weight, body mass index (BMI), appetite suppression, lipid profiles, and fat oxidation in humans [22].

Acute safety studies, including acute oral, acute dermal, primary dermal irritation and primary eye irritation have also been conducted [9, 14]. However, this report represents the first chronic safety study of HCA.

It is important to note that physiological weight loss is also associated with hypophagia [23] as well as testicular atrophy. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), a well known environmental pollutant and a by-product in the manufacture of chlorinated hydrocarbons, and di-(2-ethylhexyl)phthalate (DEHP), widely used in the manufacture of plastics, are known to cause significant body weight reduction along with hepatic and testicular lipid peroxidation, DNA single strand breaks, hypophagia, testicular atrophy, and morphological changes [23–27]. To determine the safety of oral supplementation of HCA-SX for 90 consecutive days on hepatic and testicular tissues, feed and water intake and total liver and testis weight as such and as a percentage of body weight and brain weight were monitored. Furthermore, hepatic and testicular lipid peroxidation and DNA fragmentation, and his-

Table 2a. Effect of HCA-SX on body weight in male and female rats

Day	Male				Female			
	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
1	299.8 ± 21.26	291.2 ± 17.91	294.0 ± 23.46	290.8 ± 16.50	208.8 ± 28.87	217.2 ± 23.38	206.2 ± 24.52	209.4 ± 27.37
6	313.0 ± 24.10	303.6 ± 14.24	302.2 ± 21.06	305.8 ± 24.32	221.4 ± 28.11	223.4 ± 24.22	212.0 ± 24.55	217.0 ± 29.00
13	330.2 ± 24.10	317.6 ± 17.70	324.6 ± 21.01	324.8 ± 32.38	240.6 ± 27.84	231.0 ± 22.68	217.8 ± 24.08	224.4 ± 30.97
20	341.4 ± 25.26	341.6 ± 24.85	337.6 ± 17.04	339.2 ± 36.85	247.6 ± 28.02	239.8 ± 22.55	224.0 ± 23.84	232.0 ± 30.95
25	359.8 ± 25.51	352.4 ± 27.39	350.2 ± 18.99	347.8 ± 41.03	255.6 ± 28.83	246.4 ± 21.55	229.0 ± 23.03*	239.0 ± 30.70
30	368.0 ± 23.91	367.0 ± 30.44	363.0 ± 20.82	359.4 ± 36.50	264.4 ± 30.19	252.8 ± 21.32	234.4 ± 20.74*	246.8 ± 28.73*
37	374.4 ± 24.37	379.6 ± 24.11	369.8 ± 24.11	366.2 ± 32.90	273.2 ± 29.84	257.8 ± 18.32	237.8 ± 18.90*	253.0 ± 28.61*
43	394.4 ± 24.63	387.2 ± 24.62	377.0 ± 29.77	372.0 ± 33.31*	280.4 ± 27.78	260.0 ± 18.61*	240.2 ± 17.25*	259.2 ± 27.89
48	404.2 ± 26.98	392.0 ± 24.75	381.0 ± 29.60*	377.2 ± 34.32*	283.4 ± 27.08	262.2 ± 17.56	242.8 ± 16.57*	265.8 ± 26.62*
54	417.2 ± 27.81	398.2 ± 22.75*	386.4 ± 29.91*	383.4 ± 36.27*	286.0 ± 27.76	265.2 ± 16.81*	245.4 ± 16.53*	270.2 ± 27.91*
60	435.8 ± 29.57	407.4 ± 17.81*	394.2 ± 30.75*	387.6 ± 37.80*	290.8 ± 28.63	267.4 ± 16.61*	247.4 ± 16.83*	272.2 ± 29.04*
66	442.8 ± 29.58	410.6 ± 18.06*	400.8 ± 33.24*	390.0 ± 37.20*	296.8 ± 25.32	268.6 ± 18.08*	249.6 ± 17.43	272.4 ± 28.48*
71	449.6 ± 27.81	421.2 ± 19.15*	406.2 ± 33.40*	394.8 ± 38.51*	301.0 ± 23.62	271.0 ± 17.71*	249.6 ± 17.01*	272.0 ± 29.50*
78	459.2 ± 28.08	424.4 ± 19.65*	411.8 ± 33.19*	400.6 ± 38.14*	303.6 ± 23.73	273.0 ± 17.33*	252.0 ± 17.72*	272.0 ± 29.15*
84	473.6 ± 25.13	427.6 ± 20.48*	419.8 ± 35.24*	404.0 ± 37.32*	307.0 ± 23.26	274.2 ± 16.93*	252.2 ± 16.72*	271.8 ± 29.26*
90	484.4 ± 24.97	430.0 ± 21.92*	424.2 ± 33.76*	408.0 ± 36.69*	310.6 ± 23.19	274.2 ± 18.32*	254.4 ± 15.96*	270.0 ± 29.08*

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Each value represents the mean ± S.D. of 5–7 animals. Significantly different from the control group (*$p < 0.05$).

Table 2b. Effect of HCA-SX on right and both testis in male rats

Days	0% HCA-SX		0.2% HCA-SX		2.0% HCA-SX		5.0% HCA-SX	
	Right	Both	Right	Both	Right	Both	Right	Both
30	1.33 ± 0.06	2.63 ± 0.12	1.34 ± 0.12	2.65 ± 0.23	1.35 ± 0.10	2.64 ± 0.16	1.37 ± 0.09	2.55 ± 0.26
60	1.39 ± 0.19	2.70 ± 0.23	1.37 ± 0.12	2.70 ± 0.23	1.33 ± 0.08	2.66 ± 0.13	1.38 ± 0.05	2.72 ± 0.19
90	1.46 ± 0.24	3.01 ± 0.33	1.47 ± 0.15	2.92 ± 0.19	1.43 ± 0.15	2.93 ± 0.28	1.43 ± 0.14	2.99 ± 0.27

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean ± S.D. of 5 –7 animals.

Table 2c. Effect of HCA-SX on liver and testis weight (expressed as a %) in male rats

Days	Liver			Testis	
	Group	% Body wt	% Brain wt	% Body wt	% Brain wt
30	0% HCA-SX	2.95 ± 0.23	640.70 ± 77.54	0.68 ± 0.05	1.52 ± 6.56
	0.2% HCA-SX	2.83 ± 0.20	573.56 ± 37.39	0.69 ± 0.06	151.21 ± 13.10
	2.0% HCA-SX	2.89 ± 0.25	568.64 ± 37.87	0.73 ± 0.06	152.42 ± 8.24
	5% HCA-SX	2.81 ± 0.16	624.47 ± 19.00	0.70 ± 0.09	156.83 ± 18.86
60	0.% HCA-SX	2.79 ± 0.22	672.13 ± 62.94	0.67 ± 0.06	153.40 ± 14.90
	0.2% HCA-SX	2.79 ± 0.17	592.14 ± 45.09	0.66 ± 0.07	151.14 ± 19.95
	2% HCA-SX	2.73 ± 0.28	568.59 ± 26.58	0.68 ± 0.08	147.54 ± 8.71
	5.0% HCA-SX	2.76 ± 0.27	608.65 ± 53.54	0.69 ± 0.09	148.14 ± 11.12
90	0% HCA-SX	2.88 ± 0.10	681.72 ± 29.59	0.62 ± 0.04	146.66 ± 9.72
	0.2% HCA-SX	2.75 ± 0.09	572.85 ± 17.07	0.68 ± 0.05	141.53 ± 8.75
	2.0% HCA-SX	2.86 ± 0.15	607.00 ± 52.29	0.66 ± 0.07	139.87 ± 15.22
	5.0% HCA-SX	2.86 ± 0.25	592.52 ± 48.77	0.68 ± 0.04	140.58 ± 10.36

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean ± S.D. of 5–7 animals.

topathological evaluations were determined. Three different doses of 0.2, 2.0 and 5.0% of feed intake were used over a period of 90 days. The 0.2% dose is equivalent to the daily recommended dosage in humans, while the 2.0 and 5.0% feed

Table 3a. Effect of HCA-SX on lipid peroxidation in the liver tissue of male and female rats

	Male				Female			
Day	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
30	6.34 ± 0.90	6.81 ± 0.80	6.67 ± 0.70	6.81 ± 0.80	4.83 ± 0.61	4.41 ± 0.80	5.87 ± 0.65	5.34 ± 0.63
60	6.91 ± 0.82	6.19 ± 0.92	6.86 ± 0.67	8.29 ± 1.03	4.74 ± 0.56	4.14 ± 0.32	4.98 ± 0.72	5.25 ± 0.47
90	7.55 ± 0.74	8.69 ± 0.59	7.19 ± 0.64	8.49 ± 0.81	5.18 ± 0.72	4.94 ± 0.58	5.92 ± 0.88	5.97 ± 0.48

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean ± S.D. of 5–7 animals.

Table 3b. Effect of HCA-SX on lipid peroxidation (nmol/mg protein) in testis of male rats

Days	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
30	0.28 ± 0.03	0.31 ± 0.05	0.30 ± 0.05	0.32 ± 0.05
60	0.34 ± 0.04	0.33 ± 0.05	0.36 ± 0.05	0.34 ± 0.04
90	0.33 ± 0.05	0.35 ± 0.03	0.32 ± 0.05	0.36 ± 0.05

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean ± S.D. of 5–7 animals.

Table 4a. Effect of HCA-SX on DNA fragmentation (% control) in liver tissues in male and female rats

	Male				Female			
Day	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
30	4.41 ± 0.60	4.47 ± 0.38	4.37 ± 0.26	4.59 ± 0.54	4.24 ± 0.28	3.99 ± 0.51	4.40 ± 0.54	4.38 ± 0.62
60	4.56 ± 0.62	4.73 ± 0.52	4.51 ± 0.50	4.71 ± 0.60	4.48 ± 0.39	4.36 ± 0.42	4.61 ± 0.50	4.41 ± 0.50
90	4.64 ± 0.49	4.69 ± 0.50	4.65 ± 0.42	4.64 ± 0.51	4.19 ± 0.36	4.31 ± 0.45	4.34 ± 0.37	4.59 ± 0.48

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Each value represents the mean ± S.D. of 5–7 animals.

Table 4b. Effect of HCA-SX on DNA fragmentation (% control) in testis of male rats

Days	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
30	2.26 ± 0.34	2.63 ± 0.41	2.58 ± 0.42	2.67 ± 0.53
60	2.59 ± 0.42	2.55 ± 0.32	2.63 ± 0.52	2.53 ± 0.44
90	2.49 ± 0.50	2.49 ± 0.46	2.58 ± 0.47	2.56 ± 0.45

Sprague-Dawley rats were individually treated with an oral, chronic dose of HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean ± S.D. of 5–7 animals.

intake represents 10 and 25 fold higher doses, respectively.

HCA-SX supplementation caused a significant reduction in body weight in both male and female rats. No significant changes in weights were observed in liver and testis of these animals. Liver and testis weight of male rats, expressed as a percentage of body weight and brain weight, also did not change. Feed intake was significantly reduced in HCA-SX-supplemented rats, demonstrating appetite suppression. None of the groups demonstrated any changes in water intake during the 90 days of treatment. HCA-SX supplementation did not alter hepatic and testicular lipid peroxidation or DNA fragmentation. Taken together, these results indicate that HCA-SX is safe and efficacious in weight management under the conditions employed in these studies. Future studies will need to focus on determining the mechanistic role of HCA-SX in weight management.

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