

TITLE

Physico-chemical properties of a novel (-)-hydroxycitric acid extract and its effect on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90 days

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(16 Page Article) Document Name APB3.PDF

Study Type

In vivo placebo controlled study on male and female Sprague-Dawley rats

Types of HCA referred to in Study

Extract of the rind of the fruit of *Garcinia Cambogia* stabilised as a calcium/potassium salt and known commercially as Super CitriMax.

Quantity of HCA used in this study:

Doses equalling 0.2, 2.0 and 5.0% of feed intake. 0.2% is equal to a human equivalent of 4.62g of Super CitriMax, which delivers 2,772mg of HCA.

This safety review study reported the following:

Effect of HCA on Body and selected organ weights

Body weight for both male and female rats was reduced by 11.2 to 18.1%. There was no difference between the organ weights of treated and control animals

Effect of HCA on Hepatic Lipid Peroxidation and DNA Fragmentation

HCA did not induce a significant increase in hepatic lipid peroxidation and had no effect on hepatic DNA fragmentation.

Effect of HCA on Haematology and Clinical Chemistry

More than 30 parameters were measured and there were no significant differences observed in any of the treatment groups as compared to the control groups.

Effect of HCA on Histopathology

There were minimal morphological changes which were not significant changes due to HCA treatment

Summary Conclusion and Relevancy to Our Product >>>

CONCLUSION OF STUDY

HCA is safe and efficacious in weight management under the conditions of use employed in the study. Some minor histopathological disturbances were observed, however similar disturbances were observed in the control groups so changes were not thought to be significant changes due to (-)-HCA treatment.

HOW THIS STUDY IS RELEVANT TO OUR PRODUCT

Our product contains 1400mg of Citrin K, a potassium salt of (-)-HCA, which is an extract of the rind of the fruit of *Garcinia Cambogia*. This delivers 700mg of (-)-HCA. We recommend three bottles daily, which would deliver 2,100mg of (-)-HCA.

This study employs three different levels of (-)-HCA treatment. The lowest level employed in this study is equivalent to 2,772mg of (-)-HCA. Our product contains 2,100mg, which is almost 24% lower than this.

The two other levels of (-)-HCA used in the study were 10 and 25 fold higher doses than the lowest level employed in the study. In human terms, this is equivalent to 27,720g and 69,300mg respectively.

Our daily recommended level is 25% less than the safe level as determined by the Burdock Group (Food and Chemical Toxicology 42 (2004) 1513 – 1529) and just 0.56% of the No Observable Adverse Effect Level. (NOEL)

Physico-chemical properties of a novel (–)-hydroxycitric acid extract and its effect on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90 days

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Received 26 August 2003; accepted 3 October 2003

Abstract

Garcinia cambogia-derived (–)-hydroxycitric acid (HCA) is a popular and natural supplement for weight management. HCA is a competitive inhibitor of the enzyme ATP citrate lyase, which catalyzes the conversion of citrate and coenzyme A to oxaloacetate and acetyl coenzyme A (acetyl CoA) in the cytosol. Acetyl CoA is used in the synthesis of fatty acids, cholesterol and triglycerides, and in the synthesis of acetylcholine in the central nervous system. Studies have demonstrated the efficacy of a novel 60% calcium-potassium salt of HCA derived from *Garcinia cambogia* (HCA-SX, Super CitriMax) in weight management. Results have shown that HCA-SX promotes fat oxidation, enhances serotonin release and availability in the brain cortex, normalizes lipid profiles, and lowers serum leptin levels in obese subjects. Acute oral, acute dermal, primary dermal irritation and primary eye irritation toxicity, as well as Ames bacterial reverse mutation studies and mouse lymphoma tests have demonstrated the safety of HCA-SX. However, no detailed long-term safety of HCA-SX or any other HCA extract has been previously assessed. We evaluated the dose- and time-dependent effects of HCA-SX in Sprague-Dawley rats on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry over a period of 90 days. Furthermore, a 90-day histopathological evaluation was conducted. The animals were treated with 0, 0.2, 2.0 and 5.0% HCA-SX of feed intake and were sacrificed on 30, 60 or 90 days of treatment. The body weight and selected organ weights were assessed and correlated as a % of body weight and brain weight at 90 days of treatment. A significant reduction in body weight was observed in treated rats as compared to control animals. An advancing age-induced marginal increase in hepatic lipid peroxidation was observed in both male and female rats, while no such difference in hepatic DNA fragmentation was observed as compared to the control animals. Furthermore, selected organ weights individually and as a % of body weight and brain weight at 90 days of treatment exhibited no significant difference between the groups. No difference was observed in hematology and clinical chemistry or the histopathological evaluation. Taken together, these results show that 90 day treatment of HCA-SX results in a reduction in body weight, and does not cause any changes in major organs or in hematology, clinical chemistry, and histopathology. (Mol Cell Biochem 260: 171–186, 2004)

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Key words: *Garcinia cambogia*, (-)-Hydroxycitric acid, 90-day toxicity study, body and selected organ weights, hepatic lipid peroxidation and DNA damage, hematology and clinical chemistry, histopathology

Introduction

Obesity is one of the most prevalent public health problems in the United States [1]. According to the National Health and Nutrition Examination Survey, 'overweight' (body mass index (BMI) = 25.0–29.9 kg/m²) adults now represent 59.4% of the male and 50.7% of the female population in this country, totaling more than 97 million people [2]. The corresponding figures for 'obesity' (BMI ≥ 30) are about 19.5% for men and 25% for women, involving a total of almost 40 million people. 'Morbid obesity' or clinically severe obesity (BMI ≥ 40 or > 100 lbs over normal weight) affects more than 15 million Americans. It is important to emphasize that the lowest health-risk category is that of individuals whose BMIs range from 20–25, and the highest risk category is that of individuals whose BMIs exceed 40. The treatment of obesity and its primary co-morbidities costs the US healthcare system more than \$100 billion each year; in addition, consumers spend in excess of \$33 billion annually on weight-reduction products and services [3, 4]. Moreover, obesity is associated with an increased prevalence of socioeconomic hardship due to a higher rate of disability, early retirement, and widespread discrimination [3, 4]. A number of weight management supplements are available in the marketplace, which are not adequately backed by scientific research. Ephedra, a popular weight loss ingredient containing ephedrine as the active ingredient, may cause a number of adverse effects, including rapid heart rate, elevated blood pressure and death in rare cases [5]. Thus, identification of a safe and efficacious supplement for weight management is extremely essential for health professionals in treating obesity.

The dried fruit rind of *Garcinia cambogia* (family Guttiferae) is a novel source for (-)-hydroxycitric acid (HCA) and has been used for centuries as a condiment in Southeastern Asia to make food more filling and satisfying [6, 7]. HCA, the principal acid in the fruits of *Garcinia cambogia*, makes up 16% of the content of the dried fruit. *Garcinia cambogia* has been used in Indian folk medicines for gastrointestinal complaints and rheumatism for centuries. No toxic or harmful effects of *Garcinia cambogia* have been demonstrated in the literature.

HCA is a competitive inhibitor of ATP-citrate lyase, the enzyme responsible for fatty acid, cholesterol and triglyceride biosyntheses [6–12]. HCA has been demonstrated to cause a significant reduction in food intake, increased fat oxidation and reduction in sugar craving without stimulating the central nervous system [6, 7, 11–14].

A previous study demonstrated the bioavailability of a novel calcium, potassium salt of HCA (HCA-SX, commercially known as Super CitriMax) in human plasma using gas

chromatography-mass spectrometry [15]. HCA-SX remains in the blood for more than 4–9 h after oral ingestion and greater bioavailability of HCA-SX was observed when taken on an empty stomach [15]. Ohia *et al.* 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 [13, 14]. A broad spectrum of safety studies including acute oral, acute dermal, primary dermal irritation, and primary eye irritation toxicity have demonstrated the safety of HCA-SX [14]. Recently, we have completed Ames bacterial mutation study and mouse lymphoma tests on HCA-SX, which further demonstrated the safety of HCA-SX. A number of clinical studies have demonstrated the efficacy of HCA-SX in weight management in human volunteers [8, 16–20]. However, no systematic, long-term studies have been conducted on HCA or *Garcinia cambogia* to assess the effects on organ weights, hematology and clinical chemistry and histopathology.

We conducted a 90-day safety study using three different doses of HCA-SX in both male and female Sprague-Dawley rats. The doses of HCA-SX that were used were 0.2, 2.0 and 5.0% of feed intake with the 0.2% of feed intake of HCA-SX being equivalent to 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 represent 10- and 25-fold higher doses, respectively [20]. Body weights were monitored on 30, 60 and 90 days of treatment. Selected organs were weighed as such and expressed as % of body and brain weight at 90 days of treatment. Hematology and clinical chemistry, and hepatic lipid peroxidation and DNA fragmentation were conducted on 30, 60 and 90 days of treatment. Histopathological evaluations were conducted on the 90-day treatment groups on various organs.

Materials and methods

Chemicals

Unless otherwise stated all chemicals and reagents were obtained from Sigma Chemical Company (St. Louis, MO, USA) and were of analytical grade or the highest grade available.

Physico-chemical properties of (-)-hydroxycitric acid

A natural, highly water-soluble, calcium-potassium salt of 60% HCA extract from *Garcinia cambogia* and 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). A typical compositional analysis of Super CitriMax contains 60% (–)-hydroxycitric acid in its free form, 1.0% (–)-hydroxycitric acid in its lactone form, 10% calcium, 15% potassium, 0.5% sodium, 0.05% total phytosterols, 0.3% total protein, 4.5% moisture and 8.5% soluble dietary fiber (by difference). Super CitriMax also contains 0.1% magnesium, 0.03% iron, and trace amounts of manganese, copper, zinc, selenium, total fat and total sugar. Super CitriMax provides approximately 150 calories per 100 g.

Super CitriMax HCA-SXS was characterized by injecting a 20 µl solution of HCA-SX (sample concentration 1.6 mg/ml) in water (pH 2.1, adjusted with sulfuric acid) on a Shimadzu HPLC (Tokyo, Japan) equipped with LC-10AT pumps, SCL-10A system controller, SIL-10A auto injector, SPD-M10AVP detector (detector was set at 210 nm) and CLASS-M10A software, and a 5 µ Altima C18 column (250 mm × 4.6 mm) (Alltech Associates, Inc., Deerfield, IL, USA) at a flow rate of 1 ml/min in an isocratic mode using a mobile phase of 0.05 M sodium sulfate in water (pH 2.3, adjusted with sulfuric acid) at 25 ± 2°C. The retention time for HCA was noted at 4.78 min, which was reconfirmed by spiking with an authentic standard of HCA (Wako, Japan).

Ultraviolet spectra of HCA-SX were recorded on a Varian Cary 50 UV-VIS spectrometer (Mulgrave, Victoria, Australia). UV spectra (H₂O) exhibited a shoulder at 210 nm.

Infrared spectra of HCA-SX were recorded on a Perkin Elmer Spectrum BX FT-IR spectrometer (Norwalk, CN, USA). IR (KBr pellet, cm⁻¹) 3403.20 (OH), 1599.39 (asymmetric C = O stretching band), 1397 (symmetric C = O stretching band), 1295.93 (C-O stretch), 1099.74, 1061.98 (alcoholic C-O absorption), 905.99, 837.40 (C-C stretching), 627.11 (C-C bending).

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 (the Associa-

tion for Research in Vision and Ophthalmology) 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 including adrenal glands, brain, epididymes, esophagus, eyes, heart, intestine, kidney, liver, lymph nodes, lungs, mammary glands, ovary (females only), pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes (males only), thymus gland, thyroid gland, trachea and urinary bladder, were weighed and either processed immediately or preserved in phosphate buffered 10% 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 [21] and as published by us previously [22]. In summary, 3 ml of 1% H₃PO₄ 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 [23]. Absorbance values of the organic phases were measured at 535 nm, and an extinction coefficient of 1.56 × 10⁵ 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 to 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 [24].

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 processing. The tissues were processed by standard histologic methods and embedded in paraffin. Slides were prepared and stained with hematoxylin and eosin.

Statistics

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

Results

Dose and time-dependent effects of HCA-SX on body weight and selected organ weights

The changes in body weights following supplementation of HCA-SX to the male and female rats are presented in Fig. 1. 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.

Selected organs, including adrenal glands, brain, heart, kidneys, liver, prostate and seminal vesicles, spleen, testes and thymus in male rats, and adrenal glands, brain, heart, kidneys,

liver, ovaries, spleen, thymus and uterus in female rats were weighed as such and expressed as % of body and brain weight on 90 days of treatment (Tables 1A and 1B). No significant differences were observed in any of the treatment groups as compared to the control groups.

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

Figure 2 demonstrates the dose- and time-dependent hepatic lipid peroxidation in liver samples isolated from male and female Sprague-Dawley rats. A time-dependent increase in hepatic lipid peroxidation was observed in all samples. However, HCA-SX administration did not induce a significant increase in hepatic lipid peroxidation in these animals. Under identical experimental conditions, HCA-SX treatment caused no effect on hepatic DNA fragmentation in male and female rats on 30, 60 and 90 days of treatment (data not shown).

Dose and time-dependent effects of HCA-SX on hematology and clinical chemistry in male and female Sprague-Dawley rats

Tables 3A–H and 4A–H demonstrate the hematology and clinical chemistry results from blood samples taken from male and female Sprague-Dawley rats treated with 0, 0.2, 2.0 and 5.0% HCA-SX, respectively. White blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular concentration, platelet count, reticulocyte count, segmented neutrophils, absolute banded neutrophils, lymphocyte, monocyte, eosinophils, basophils, total serum protein, total albumin, globulin, alkaline phosphatase, blood urea nitrogen, creatinine, aspartate aminotransferase, cholesterol, total bilirubin, glucose, calcium, chloride, phosphorous, sodium, potassium, iron, total iron binding capacity and iron/

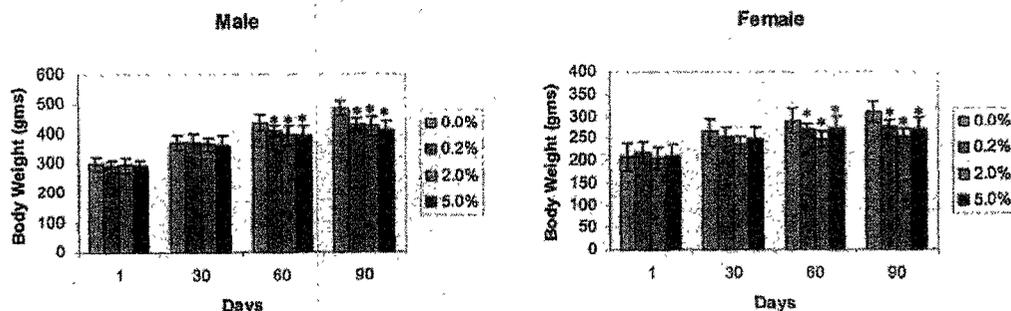


Fig. 1 Sprague-Dawley rats were individually treated with an oral, chronic dose of 0.2, 2.0 and 5.0% HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Body weights of the animals were taken on 30, 60 and 90 days of treatment. Each value represents the mean \pm S.D. of 5–7 animals. Significantly different from the control group (* $p < 0.05$).

total iron binding capacity parameters were determined in each sample. No significant differences were observed in any of the treatment groups as compared to the control groups.

Effects of HCA-SX on histopathology

Histopathological analyses were conducted on tissues including adrenal glands, brain, epididymides, esophagus, eyes, heart, intestine, kidney, liver, lymph nodes, lungs, mammary glands, ovary (females only), pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes (males only), thymus gland, thyroid gland, trachea and urinary bladder, in all control and HCA-SX-treated animals at 90 days of treatment. Lesions were noted in a number of organs. With the exception of minimal changes in the gastric mucosa described below, the lesions were randomly distributed throughout all control and experimental groups and appear to be incidental, non-treatment related findings.

Scattered, small foci of hemorrhage were noted in the brain of some animals. This is considered to be most likely an agonal/anoxic change associated with euthanasia. Small cysts were noted in the pituitary gland of one animal.

Hemorrhage was noted within the trachea of some animals. A few animals had minimal tracheitis comprised primarily of lymphocytes and plasma cells.

Minimal multifocal myocarditis and associated myocardial degeneration were noted in a few animals. Some animals had multifocal peribronchial lymphoid nodules within the lung. Multifocal minimal pneumonic foci were also occasionally seen. Vascular basement membrane mineralization was noted in one animal.

Extramedullary hematopoiesis are not uncommon, incidental lesions of rats, are not considered to be treatment-related. Minimal swelling and vacuolation of hepatocytes was seen in some animals. Small foci of lymphoplasmacytic hepatitis were occasionally seen. The mucosa of the glandular stomach of one animal was severely atrophied and mineralized. Foci of glandular dilatation were noted in a number of animals.

Mineralization was noted in the ovary of one animal. And dilatation of the uterine lumen was occasionally seen. One animal had a focus of endometrial inflammation.

Testicular atrophy and aspermatogenesis was seen in one animal. No lesion was seen in any of the other organs examined.

Overall, these changes appear morphologically to be minimal and not significant changes due to HCA-SX treatment.

Discussion

In the present study, we investigated the dose- and time-dependent effects of a novel, bioavailable (-)-hydroxycitric acid

extract (HCA-SX, commercially known as Super CitriMax) on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90 days.

(-)-Hydroxycitric acid (HCA) is a naturally occurring organic acid found in the rind of the fruits from *Garcinia cambogia*, which grows extensively in Southeastern Asia. The fruit has been used for centuries in culinary dishes to make meals more filling [6, 7], HCA is believed to work by inhibiting lipogenesis and reduces the availability of acetyl-CoA, the building block for fat synthesis, by inhibiting ATP citrate lyase [6, 9-12]. 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 [15]. 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 reuptake inhibitor (SRRI), without stimulating the central nervous system [13, 14]. This is the first evidence linking the ability of HCA-SX to cause appetite suppression while increasing levels of serotonin [13, 14]. Acute safety studies, including acute oral, acute dermal, primary dermal irritation and primary eye irritation have been conducted on HCA-SX, which demonstrated the safety of HCA-SX [14]. Ames' bacterial reverse mutation studies and mouse lymphoma tests have recently been completed, which further demonstrated the safety of HCA-SX.

Five histidine-dependent strains of *Salmonella typhimurium* (TA98, TA100, TA1535, TA1537 and TA102) were used to evaluate the mutagenic potential of HCA-SX (up to 5000 µg/plate), both in the presence and absence of metabolic activation (\pm S9). No mutagenic potential of HCA-SX was observed.

The mutagenic potential of HCA-SX (up to the recommended dose level of 5000 µg/plate) was assessed in the mouse lymphoma assay using L5178Y mouse lymphoma cells, clone -3.7.2C (ATCC #CRL-9518, American Type Culture Collection, Virginia, USA). HCA-SX didn't induce mutagenic effects in the mammalian cell gene mutation test on L5178Y mouse lymphoma cells TK+/-, either with or without metabolic activation.

This study primarily focused on the safety parameters on the long-term chronic use of HCA-SX. Our recent study has also demonstrated the beneficial effects of HCA-SX on body weight, body mass index (BMI), appetite suppression, lipid profiles, and fat oxidation in humans [20].

In this study, 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 intake represents 10- and 25-fold higher doses, respectively. Body weights were monitored on 30, 60 and 90 days of treatment. Selected organs, includ-

Table 1A. Effects of HCA-SX on body weight and vital organs of male Sprague-Dawley rats after 90 days of treatment

Organs	Male			
	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
Body weights	484.40 ± 24.97	430.00 ± 21.92	424.20 ± 33.76	408.00 ± 36.69
Adrenal glands (pair)	0.052 ± 0.006	0.044 ± 0.002	0.047 ± 0.006	0.004 ± 0.002
% Body wt	0.011 ± 0.001	0.010 ± 0.001	0.011 ± 0.001	0.011 ± 0.001
% Brain wt	2.55 ± 0.19	2.13 ± 0.12	2.31 ± 0.31	2.25 ± 0.17
Brain	2.05 ± 0.10	2.06 ± 0.07	1.996 ± 0.093	1.97 ± 0.16
% Body wt	0.42 ± 0.01	0.474 ± 0.011	0.474 ± 0.033	0.480 ± 0.014
Heart	1.35 ± 0.08	1.42 ± 0.11	1.18 ± 0.10	1.224 ± 0.074
% Body wt	0.28 ± 0.01	0.330 ± 0.017	0.280 ± 0.016	0.304 ± 0.030
% Brain wt	65.81 ± 1.41	68.36 ± 4.02	59.09 ± 6.27	62.38 ± 5.03
Kidneys (pair)	2.83 ± 0.28	2.84 ± 0.17	2.87 ± 0.12	2.72 ± 0.28
% Body wt	0.58 ± 0.04	0.662 ± 0.015	0.682 ± 0.031	0.666 ± 0.047
% Brain wt	138.13 ± 10.69	137.49 ± 3.87	144.18 ± 7.84	138.19 ± 13.03
Liver	13.98 ± 1.16	11.82 ± 0.56	12.09 ± 0.78	11.66 ± 1.23
% Body wt	2.88 ± 0.10	2.75 ± 0.09	2.856 ± 0.155	2.86 ± 0.25
% Brain wt	681.72 ± 29.59	572.85 ± 17.07	607.00 ± 52.29	592.52 ± 48.77
Prostate and Seminal Vesicles	3.55 ± 0.45	2.95 ± 0.26	3.88 ± 0.54	3.22 ± 0.45
% Body wt	0.73 ± 0.06	0.684 ± 0.031	0.916 ± 0.109	0.794 ± 0.054
% Brain wt	173.13 ± 16.20	142.81 ± 8.01	194.17 ± 23.47	163.30 ± 15.47
Spleen	0.79 ± 0.07	0.710 ± 0.089	0.624 ± 0.068	0.610 ± 0.075
% Body wt	0.160 ± 0.007	0.162 ± 0.013	0.144 ± 0.009	0.148 ± 0.019
% Brain wt	38.42 ± 1.87	34.38 ± 3.27	31.26 ± 3.06	31.07 ± 3.85
Testes (pair)	3.01 ± 0.33	2.92 ± 0.19	2.93 ± 0.28	2.99 ± 0.27
% Body wt	0.619 ± 0.035	0.682 ± 0.049	0.694 ± 0.063	0.732 ± 0.028
% Brain wt	146.66 ± 9.72	141.53 ± 8.75	146.75 ± 13.08	151.67 ± 5.51
Thymus	0.394 ± 0.039	0.316 ± 0.033	0.410 ± 0.067	0.441 ± 0.056
% Body wt	0.081 ± 0.007	0.073 ± 0.006	0.094 ± 0.011	0.108 ± 0.017
% Brain wt	19.26 ± 1.70	15.28 ± 1.34	20.12 ± 3.53	22.43 ± 2.70

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 90 days of treatment. Each value represents the mean ± S.D. of 5-7 animals.

ing adrenal glands, brain, heart, kidneys, liver, prostate and seminal vesicles, spleen, testes and thymus in male rats, and adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, thymus and uterus in female rats were weighed as such and expressed as % of body and brain weight on 90 days of treatment. Hematology and clinical chemistry, and hepatic lipid peroxidation and DNA fragmentation were conducted on 30, 60 and 90 days of treatment. Histopathological evaluations were conducted on 90-day treatment groups on different organs including adrenal glands, brain, epididymes, esophagus, eyes, heart, intestine, kidney, liver, lymph nodes, lungs, mammary glands, ovary (females only), pancreas, pituitary, prostate, salivary glands, seminal vesicles, skin, spleen, stomach, testes (males only), thymus gland, thyroid gland, trachea and urinary bladder.

HCA-SX supplementation caused a significant reduction

in body weight in both male and female rats (Fig. 1). No significant changes in selected organ weights were observed in the treatment groups as compared to the control animals (Tables 1A and 1B). HCA-SX supplementation did not alter hepatic lipid peroxidation (Fig. 2) or DNA fragmentation (data not shown). Different doses of HCA-SX didn't cause any changes in hematology and clinical chemistry at 30, 60 and 90 days of treatment.

Three doses of HCA-SX didn't cause significant morphological changes in the organs tested in this study.

Scattered minimal or mild histologic lesions noted in all organs randomly distributed in all groups were considered to be incidental findings commonly seen in rats, or, in the case of hemorrhage, to be agonal or necropsy artifacts. The inflammatory lesions noted were consistent with mild subclinical infections, such as those caused by *Mycoplasma* sp.

Table 1B. Effects of HCA-SX on body weight and vital organs of female Sprague-Dawley rats after 90 days of treatment

Organs	Female			
	0% HCA-SX	0.2% HCA-SX	2.0% HCA-SX	5.0% HCA-SX
Body weights	310.60 ± 23.19	274.20 ± 20.99	254.40 ± 15.96	270.00 ± 17.89
Adrenal glands (pair)	0.068 ± 0.007	0.180 ± 0.252	0.064 ± 0.007	0.063 ± 0.009
% Body wt	0.022 ± 0.001	0.025 ± 0.003	0.023 ± 0.002	
% Brain wt	3.47 ± 0.16	3.45 ± 0.29	3.288 ± 0.282	3.224 ± 0.510
Brain	1.96 ± 0.16	1.90 ± 0.07	1.950 ± 0.086	1.966 ± 0.072
% Body wt	0.632 ± 0.026	0.666 ± 0.053	0.768 ± 0.024	0.722 ± 0.077
Heart	0.934 ± 0.055	0.866 ± 0.063	0.848 ± 0.044	0.860 ± 0.075
% Body wt	0.300 ± 0.017	0.302 ± 0.019	0.332 ± 0.023	0.317 ± 0.034
% Brain wt	47.84 ± 3.17	45.58 ± 2.80	43.56 ± 3.10	43.75 ± 3.57
Kidneys (pair)	1.92 ± 0.12	1.710 ± 0.095	1.65 ± 0.12	1.80 ± 0.12
% Body wt	0.622 ± 0.036	0.598 ± 0.022	0.648 ± 0.051	0.658 ± 0.051
% Brain wt	98.52 ± 8.17	90.10 ± 5.98	84.40 ± 4.42	91.59 ± 7.37
Liver	8.52 ± 0.59	7.52 ± 0.28	6.86 ± 0.35	6.86 ± 0.42
% Body wt	2.75 ± 0.18	2.63 ± 0.18	2.70 ± 0.20	2.51 ± 0.18
% Brain wt	436.74 ± 38.20	396.04 ± 11.37	351.87 ± 17.39	349.73 ± 28.08
Ovaries	0.09 ± 0.008	0.086 ± 0.008	0.096 ± 0.020	0.094 ± 0.009
% Body wt	0.032 ± 0.003	0.030 ± 0.002	0.038 ± 0.007	0.035 ± 0.004
% Brain wt	5.02 ± 0.55	4.51 ± 0.42	4.91 ± 0.85	4.79 ± 0.42
Spleen	0.494 ± 0.046	0.526 ± 0.036	0.484 ± 0.024	0.474 ± 0.046
% Body wt	0.154 ± 0.011	0.186 ± 0.018	0.190 ± 0.007	0.176 ± 0.015
% Brain wt	24.74 ± 2.22	27.66 ± 0.88	24.83 ± 1.15	24.08 ± 1.78
Thymus	0.316 ± 0.042	0.306 ± 0.027	0.238 ± 0.025	0.271 ± 0.046
% Body wt	0.101 ± 0.013	0.108 ± 0.011	0.093 ± 0.007	0.098 ± 0.013
% Brain wt	16.26 ± 2.70	16.11 ± 0.95	12.22 ± 1.20	13.79 ± 2.24
Uterus	0.571 ± 0.063	0.685 ± 0.053	0.602 ± 0.059	0.577 ± 0.105
% Body wt	0.184 ± 0.013	0.240 ± 0.028	0.237 ± 0.025	0.209 ± 0.029
% Brain wt	29.27 ± 3.37	36.04 ± 1.96	30.94 ± 3.31	29.31 ± 5.23

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 90 days of treatment. Each value represents the mean ± S.D. of 5-7 animals.

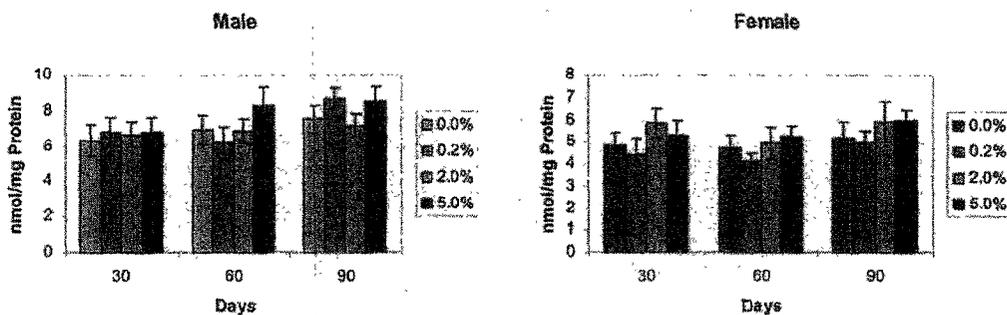


Fig. 2. Sprague-Dawley rats were individually treated with an oral, chronic dose of 0.2, 2.0 and 5.0% HCA-SX in water for 90 consecutive days. Control animals received the vehicle (water). Animals were sacrificed on 30, 60 and 90 days of treatment. Hepatic lipid peroxidation was measured as discussed in the Materials and methods section. Each value represents the mean ± S.D. of 5-7 animals.

Table 3A. Hematology and clinical chemistry results of 0% HCA-SX treated male Sprague-Dawley rats

		Male 0% HCA-SX																	
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticulocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin	
	Units	$\times 10^3/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fL	pg	%	$\times 10^3/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL	
30	Mean	12.16	8.49	15.32	45.74	53.40	17.88	33.60	1527.20	0.98	1473.60	0.00	11026.00	420.20	73.00	0.00	7.00	3.36	
	S.D.	0.35	0.14	0.16	0.35	0.61	0.12	0.42	57.66	0.04	59.61	0.00	307.68	54.98	21.38	0.00	0.10	0.03	
60	Mean	12.64	8.24	15.22	44.08	51.80	16.76	33.00	1380.40	1.08	1613.40	0.00	11639.40	405.40	83.60	0.00	7.16	3.42	
	S.D.	0.72	0.16	0.32	0.54	0.68	0.29	0.45	33.80	0.07	64.33	0.00	635.06	41.93	20.10	0.00	0.11	0.06	
90	Mean	12.09	8.38	15.41	45.56	53.71	16.97	33.49	1382.00	1.01	1577.71	0.00	10971.71	412.86	77.43	0.00	7.09	3.53	
	S.D.	0.38	0.10	0.13	0.38	0.35	0.08	0.15	34.07	0.04	53.09	0.00	398.18	25.51	13.47	0.00	0.06	0.04	

Sprague-Dawley rats were individually treated with 0% HCA-SX for 30, 60 and 90 consecutive days. Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean \pm S.D. of 5-7 animals.

Table 3B. Hematology and clinical chemistry results of 0% HCA-SX treated male Sprague-Dawley rats

		Male 0% HCA-SX																	
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity	
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dL	mEq/L	mEq/L	mg/dL	mg/dL	NA	
30	Mean	3.82	100.20	16.40	0.64	96.80	51.40	97.20	0.14	115.20	10.98	98.20	8.00	146.20	5.66	147.80	602.40	25.60	
	S.D.	0.09	2.69	0.33	0.01	4.09	4.34	2.46	0.01	0.95	0.14	0.26	0.07	0.17	0.09	4.79	14.50	0.91	
60	Mean	3.80	105.00	16.20	0.66	104.60	58.00	94.00	0.12	119.00	11.04	97.00	7.90	146.00	5.86	149.00	613.60	25.40	
	S.D.	0.10	3.50	0.30	0.01	6.03	4.29	3.45	0.01	5.97	0.11	0.14	0.11	0.14	0.10	4.84	10.69	0.67	
90	Mean	3.93	100.03	16.57	0.64	95.71	49.00	90.57	0.14	110.86	10.99	98.57	8.04	145.71	5.49	159.00	610.00	25.71	
	S.D.	0.09	3.12	0.26	0.01	7.19	2.74	4.31	0.01	1.39	0.05	0.18	0.05	0.14	0.07	2.02	7.06	0.59	

Sprague-Dawley rats were individually treated with 0% HCA-SX for 30, 60 and 90 consecutive days. Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean \pm S.D. of 5-7 animals.

Table 3C. Hematology and clinical chemistry results of 0.2% HCA-SX treated male Sprague-Dawley rats

		Male 0.2% HCA-SX																	
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticulocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin	
	Units	$\times 10^3/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fL	pg	%	$\times 10^3/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL	
30	Mean	12.74	8.45	15.28	45.96	52.00	17.38	32.70	1369.00	1.16	1464.20	0.00	10887.40	467.80	76.80	0.00	7.30	3.36	
	S.D.	0.72	0.10	0.17	0.38	0.32	0.22	0.38	60.82	0.07	53.32	0.00	782.19	45.93	14.05	0.00	0.12	0.03	
60	Mean	12.06	8.66	15.14	43.82	51.40	17.32	32.76	1385.00	1.04	1548.20	0.00	11802.40	406.60	78.40	0.00	7.38	3.30	
	S.D.	0.43	0.12	0.17	0.45	0.64	0.16	0.37	55.92	0.05	58.83	0.00	889.00	53.44	15.94	0.00	0.19	0.09	
90	Mean	13.04	8.38	14.84	45.00	53.57	17.67	33.74	1341.00	1.09	1509.57	0.00	10660.57	407.29	77.86	0.00	7.01	3.26	
	S.D.	0.27	0.13	0.17	0.38	0.55	0.18	0.13	22.99	0.03	67.25	0.00	455.99	23.33	15.55	0.00	0.12	0.05	

Sprague-Dawley rats were individually treated with an oral, chronic dose of 0.2% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 3D. Hematology and clinical chemistry results of 0.2% HCA-SX treated male Sprague-Dawley rats

		Male 0.2% HCA-SX																	
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity	
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dL	mEq/L	mEq/L	mg/dL	mg/dL	NA	
30	Mean	3.80	180.40	17.20	0.76	91.00	56.80	83.00	0.14	131.80	10.92	99.00	8.00	146.00	6.26	149.40	603.80	25.00	
	S.D.	0.12	3.91	0.17	0.02	3.99	3.80	5.15	0.01	6.88	0.11	0.24	0.07	0.24	0.02	3.60	12.68	0.84	
60	Mean	3.64	99.20	17.60	0.74	95.80	59.00	83.00	0.14	143.20	11.26	98.20	8.22	146.60	6.16	150.60	609.40	25.20	
	S.D.	0.16	5.46	0.18	0.03	5.43	3.72	4.12	0.01	5.63	0.03	0.36	0.05	0.23	0.06	5.30	9.74	1.04	
90	Mean	3.76	103.14	17.14	0.70	100.86	56.14	90.43	0.13	144.43	11.03	97.86	7.99	146.57	5.84	150.14	609.00	24.86	
	S.D.	0.06	1.92	0.13	0.01	5.41	1.65	2.24	0.01	3.82	0.06	0.17	0.07	0.23	0.07	2.80	7.57	0.65	

Sprague-Dawley rats were individually treated with an oral, chronic dose of 0.2% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 3E. Hematology and clinical chemistry results of 2.0% HCA-SX treated male Sprague-Dawley rats

		Male 2.0% HCA-SX																
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticuloocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin
	Units	$\times 10^3/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fl	pg	%	$\times 10^9/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL
30	Mean	13.40	8.76	15.46	45.12	51.40	17.58	33.38	1231.60	1.12	1560.60	0.00	11707.80	386.60	73.80	0.00	7.04	3.32
	S.D.	0.49	0.19	0.20	0.53	0.77	0.10	0.32	51.94	0.07	90.46	0.00	1020.28	40.43	15.64	0.00	0.18	0.06
60	Mean	13.22	8.26	15.18	44.68	52.80	17.30	32.58	1199.80	1.08	1588.60	0.00	11128.00	407.20	75.80	0.00	7.10	3.34
	S.D.	0.51	0.10	0.21	0.44	0.52	0.10	0.29	41.49	0.07	67.32	0.00	911.42	48.73	25.24	0.00	0.11	0.08
90	Mean	12.30	8.69	15.43	45.65	53.22	17.03	34.23	1203.93	1.14	1538.33	0.00	12088.92	416.25	76.18	0.00	7.03	3.23
	S.D.	0.65	0.46	0.78	2.31	2.70	0.87	1.71	61.71	0.06	87.70	0.00	926.66	31.63	15.42	0.00	0.36	0.17

Sprague-Dawley rats were individually treated with an oral, chronic dose of 2.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 3F. Hematology and clinical chemistry results of 2.0% HCA-SX treated male Sprague-Dawley rats

		Male 2.0% HCA-SX																
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dl	mEq/L	mEq/L	mg/dL	mg/dL	NA
30	Mean	3.84	98.20	16.40	0.64	91.80	55.40	83.60	0.12	128.40	10.94	98.80	8.05	146.00	5.46	148.80	614.40	25.00
	S.D.	0.05	2.70	0.11	0.01	4.01	4.11	3.00	0.01	4.78	0.09	0.17	0.05	0.14	0.12	5.51	14.81	0.94
60	Mean	3.76	102.60	17.20	0.70	101.60	61.00	87.20	0.14	127.00	11.30	97.60	7.76	146.00	5.98	156.40	612.60	25.20
	S.D.	0.10	4.53	0.09	0.00	5.97	3.54	4.70	0.01	2.39	0.06	0.36	0.10	0.20	0.11	5.50	11.63	0.78
90	Mean	3.86	98.22	16.73	0.66	96.71	58.22	91.39	0.14	126.48	11.18	98.05	7.90	144.46	5.79	149.79	614.09	24.97
	S.D.	0.21	5.42	0.84	0.03	5.97	3.22	5.64	0.01	7.20	0.56	4.94	0.41	7.29	0.30	8.00	31.19	1.32

Sprague-Dawley rats were individually treated with an oral, chronic dose of 2.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 3G. Hematology and clinical chemistry results of 5.0% HCA-SX treated male Sprague-Dawley rats

		Male 5.0% HCA-SX																	
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticulocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin	
	Units	$\times 10^3/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fl	pg	%	$\times 10^3/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL	
30	Mean	12.16	8.76	15.16	45.12	52.40	17.48	32.82	1251.00	1.04	1957.80	0.00	10715.60	351.20	73.00	0.00	7.06	3.20	
	S.D.	0.76	0.10	0.20	0.43	0.58	0.14	0.35	46.91	0.03	124.35	0.00	804.81	29.34	24.29	0.00	0.11	0.05	
60	Mean	13.76	8.24	16.14	45.62	53.40	17.52	33.68	1234.80	1.14	1992.20	0.00	11111.80	354.40	76.80	0.00	7.26	3.42	
	S.D.	0.33	0.13	0.17	0.26	0.67	0.12	0.25	23.97	0.07	101.19	0.00	946.92	22.79	15.93	0.00	0.15	0.04	
90	Mean	12.57	8.39	14.96	43.54	52.16	17.06	33.67	1233.86	1.00	1766.43	0.00	11142.57	376.00	57.29	0.00	7.13	3.41	
	S.D.	0.30	0.11	0.23	0.39	0.43	0.24	0.07	12.34	0.03	109.60	0.00	311.73	20.36	8.33	0.00	0.11	0.05	

Sprague-Dawley rats were individually treated with an oral, chronic dose of 5.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 3H. Hematology and clinical chemistry results of 5.0% HCA-SX treated male Sprague-Dawley rats

		Male 5.0% HCA-SX																	
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity	
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dL	mEq/L	mEq/L	mg/dL	mg/dL	NA	
30	Mean	5.62	104.40	17.00	0.64	94.20	56.40	95.80	0.14	119.20	11.08	98.60	8.14	146.20	6.08	149.80	612.00	24.80	
	S.D.	0.13	2.95	0.00	0.01	5.60	1.63	1.84	0.01	4.43	0.08	0.23	0.12	0.17	0.08	2.79	10.13	0.85	
60	Mean	3.98	101.00	16.60	0.68	90.20	58.40	89.80	0.12	133.00	10.84	98.80	7.66	145.60	5.52	151.80	600.60	25.80	
	S.D.	0.12	2.93	0.11	0.01	4.97	3.76	2.10	0.01	5.59	0.08	0.26	0.09	0.18	0.14	4.31	10.11	0.38	
90	Mean	3.50	106.29	16.43	0.63	91.57	56.00	92.00	0.13	117.86	10.94	99.29	8.10	145.71	5.56	149.86	612.29	25.00	
	S.D.	0.05	3.18	0.08	0.01	3.13	2.05	1.83	0.01	1.49	0.06	0.11	0.12	0.11	0.08	3.86	8.47	0.61	

Sprague-Dawley rats were individually treated with an oral, chronic dose of 5.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 4A. Hematology and clinical chemistry results of 0% HCA-SX treated female Sprague-Dawley rats

		Female 0% HCA-SX																
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticulocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin
	Units	$\times 10^3/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fL	pg	%	$\times 10^9/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL
30	Mean	6.46	7.49	14.60	39.80	53.80	19.02	33.64	1312.00	0.94	1529.20	0.00	10358.80	175.80	20.00	0.00	7.62	3.92
	S.D.	0.12	3.05	0.11	0.02	2.63	2.82	1.53	0.01	3.78	0.07	0.30	0.12	0.36	0.11	16.19	26.77	2.02
60	Mean	6.74	7.43	14.44	40.06	56.20	18.78	34.04	1392.60	0.96	1472.80	0.00	10679.40	170.80	20.40	0.00	7.72	3.90
	S.D.	0.10	2.97	0.18	0.02	2.79	2.67	3.72	0.00	3.18	0.09	0.73	0.25	0.58	0.10	18.51	23.13	2.73
90	Mean	6.61	7.38	14.19	40.03	55.86	18.96	33.80	1388.29	1.01	1501.00	0.00	10731.57	178.14	20.00	0.00	7.57	3.86
	S.D.	0.13	0.05	0.08	0.28	0.34	0.08	0.25	33.66	0.04	64.60	0.00	690.86	12.50	3.34	0.00	0.11	0.05

Sprague-Dawley rats were individually treated with 0% HCA-SX for 30, 60 and 90 consecutive days. Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean \pm S.D. of 5-7 animals.

Table 4B. Hematology and clinical chemistry results of 0% HCA-SX treated female Sprague-Dawley rats

		Female 0% HCA-SX																
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dL	mEq/L	mEq/L	mg/dL	mg/dL	NA
30	Mean	3.78	48.20	15.60	0.72	74.80	38.20	91.60	0.12	151.60	10.90	98.40	6.46	144.80	5.66	330.40	590.60	56.00
	S.D.	0.12	3.05	0.11	0.02	2.63	2.82	1.53	0.01	3.78	0.07	0.30	0.12	0.36	0.11	16.19	26.77	2.02
60	Mean	3.82	48.80	18.40	0.68	75.20	37.80	89.00	0.10	133.00	10.98	98.80	6.62	145.00	5.52	330.20	590.60	56.20
	S.D.	0.10	2.97	0.18	0.02	2.79	2.67	3.72	0.00	3.18	0.09	0.73	0.25	0.58	0.10	18.51	23.13	2.73
90	Mean	3.84	47.86	17.43	0.66	74.43	38.71	89.57	0.13	133.14	11.06	99.00	6.51	145.14	5.40	330.14	590.43	56.43
	S.D.	0.03	1.43	0.11	0.01	1.82	1.85	2.38	0.01	4.35	0.04	0.64	0.19	0.35	0.06	19.04	17.62	2.11

Sprague-Dawley rats were individually treated with 0% HCA-SX for 30, 60 and 90 consecutive days. Animals were sacrificed on 30, 60 and 90 days of treatment. Each value represents the mean \pm S.D. of 5-7 animals.

Table 4C. Hematology and clinical chemistry results of 0.2% HCA-SX treated female Sprague-Dawley rats

		Female 0.2% HCA-SX																
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticulocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin
	Units	$\times 10^9/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fl	pg	%	$\times 10^9/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL
30	Mean	6.24	7.32	14.10	40.52	54.80	18.78	34.02	1313.60	0.94	1504.00	0.00	10237.00	164.80	13.20	0.00	7.54	3.90
	S.D.	0.32	0.04	0.15	0.39	0.30	0.17	0.12	96.49	0.05	67.77	0.00	196.61	9.18	5.90	0.00	0.21	0.09
60	Mean	6.16	7.53	13.92	40.32	55.00	18.42	33.26	1315.40	1.18	1512.00	0.00	10994.20	168.80	13.00	0.00	7.48	4.02
	S.D.	0.55	0.10	0.14	0.47	0.58	0.12	0.48	47.86	0.06	54.13	0.00	431.93	19.49	3.59	0.00	0.10	0.05
90	Mean	6.54	7.62	14.33	40.23	56.14	18.93	33.63	1354.29	0.87	1516.57	0.00	10796.71	156.43	15.43	0.00	7.71	3.87
	S.D.	0.39	0.06	0.06	0.20	0.25	0.11	0.12	32.28	0.03	83.14	0.00	391.66	5.55	4.23	0.00	0.14	0.03

Sprague-Dawley rats were individually treated with an oral, chronic dose of 0.2% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 4D. Hematology and clinical chemistry results of 0.2% HCA-SX treated female Sprague-Dawley rats

		Female 0.2% HCA-SX																
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dL	mEq/L	mEq/L	mg/dL	mg/dL	NA
30	Mean	3.90	48.00	15.80	0.68	85.00	39.00	89.60	0.12	122.00	11.32	99.20	6.40	144.80	5.48	331.80	590.20	55.80
	S.D.	0.08	2.48	0.09	0.02	4.20	1.64	2.95	0.01	3.11	0.11	0.36	0.30	0.17	0.07	17.20	19.82	4.17
60	Mean	3.78	48.40	16.20	0.76	81.80	41.00	92.00	0.14	124.20	10.98	99.00	6.60	144.80	5.58	332.20	591.00	56.40
	S.D.	0.13	1.87	0.09	0.01	3.08	1.76	3.78	0.01	3.64	0.06	0.51	0.23	0.61	0.19	17.19	14.33	3.72
90	Mean	3.81	48.00	15.86	0.67	84.00	40.86	90.43	0.11	124.57	11.01	99.57	6.64	146.43	5.46	332.14	589.00	55.71
	S.D.	0.05	1.53	0.13	0.01	1.94	1.27	1.43	0.01	2.75	0.09	0.42	0.15	0.25	0.08	15.35	16.02	2.01

Sprague-Dawley rats were individually treated with an oral, chronic dose of 0.2% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 4E. Hematology and clinical chemistry results of 2.0% HCA-SX treated female Sprague-Dawley rats

		Female 2.0% HCA-SX																
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticulocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin
	Units	$\times 10^3/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fL	pg	%	$\times 10^3/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL
30	Mean	6.68	7.54	13.84	41.70	55.60	18.74	33.48	1359.20	0.98	1524.60	0.00	10823.60	156.00	12.80	0.00	7.64	3.92
	S.D.	0.16	0.05	0.11	0.52	0.18	0.14	0.41	73.57	0.05	84.72	0.00	308.74	9.35	5.72	0.00	0.18	0.11
60	Mean	6.96	7.35	13.74	41.14	54.80	18.62	33.72	1353.60	0.94	1503.40	0.00	10727.00	166.00	21.20	0.00	7.80	3.90
	S.D.	0.41	0.07	0.23	0.25	0.43	0.19	0.16	57.65	0.04	91.87	0.00	362.22	12.53	3.96	0.00	0.12	0.11
90	Mean	6.93	7.38	13.87	41.33	54.86	18.61	33.81	1349.29	0.97	1513.43	0.00	10657.86	177.43	16.86	0.00	7.81	3.76
	S.D.	0.23	0.07	0.10	0.33	0.15	0.07	0.15	33.16	0.04	74.85	0.00	376.59	7.86	3.33	0.00	0.10	0.10

Sprague-Dawley rats were individually treated with an oral, chronic dose of 2.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 4F. Hematology and clinical chemistry results of 2.0% HCA-SX treated female Sprague-Dawley rats

		Female 2.0% HCA-SX																
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dL	mEq/L	mEq/L	mg/dL	mg/dL	NA
30	Mean	3.80	46.80	16.20	0.70	78.80	42.60	94.00	0.12	123.20	11.26	99.20	6.40	143.60	5.56	331.80	587.60	54.60
	S.D.	0.10	1.54	0.17	0.01	2.17	1.63	2.52	0.01	4.17	0.10	0.09	0.09	0.77	0.12	18.33	15.47	3.65
60	Mean	3.88	50.60	16.60	0.70	77.20	42.20	92.60	0.12	124.40	11.00	99.80	6.48	144.80	5.40	331.00	592.00	58.00
	S.D.	0.09	2.38	0.11	0.02	1.56	1.20	2.70	0.01	2.41	0.08	0.55	0.15	0.52	0.12	14.56	16.37	2.70
90	Mean	3.91	51.86	16.71	0.70	77.57	41.14	92.57	0.13	123.00	11.11	99.14	6.31	144.29	5.50	332.14	590.14	54.57
	S.D.	0.03	1.55	0.07	0.03	1.42	0.67	2.53	0.01	3.70	0.09	0.45	0.16	0.49	0.06	15.77	9.90	1.69

Sprague-Dawley rats were individually treated with an oral, chronic dose of 2.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 4G. Hematology and clinical chemistry results of 5.0% HCA-SX treated female Sprague-Dawley rats

		Female 5.0% HCA-SX																
Days	Tests	WBC	RBC	Hemoglobin	Hematocrit	Mean corpuscular volume	Mean corpuscular hemoglobin	Mean corpuscular concentration	Platelet count	Reticulocyte count	Segmented neutrophils	Absolute banded neutrophils	Lymphocyte	Monocyte	Eosinophil	Basophil	Total serum protein	Total albumin
	Units	$\times 10^3/\text{mm}^3$	$\times 10^6/\text{mm}^3$	g/dL	%	fL	pg	%	$\times 10^9/\text{mm}^3$	% RBC	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	/mm ³	g/dL	g/dL
30	Mean	6.58	7.36	14.08	41.46	55.20	18.78	33.76	1253.40	1.14	1513.20	0.00	11956.40	165.60	22.00	0.00	7.60	3.96
	S.D.	0.32	0.08	0.12	0.53	0.52	0.18	0.09	42.66	0.15	61.18	0.00	1122.33	5.31	6.66	0.00	0.19	0.09
60	Mean	6.52	7.56	14.10	41.44	55.80	18.62	34.00	1214.80	0.92	1689.20	0.00	11764.60	165.80	21.60	0.00	7.74	3.66
	S.D.	0.47	0.10	0.20	0.66	0.30	0.11	0.14	43.63	0.06	149.34	0.00	473.18	7.22	4.78	0.00	0.08	0.06
90	Mean	6.66	7.37	14.09	40.90	56.00	19.01	33.86	1226.71	0.97	1596.00	0.00	11751.29	177.29	22.86	0.00	7.79	3.89
	S.D.	0.11	0.03	0.14	0.23	0.37	0.07	0.07	23.63	0.04	38.27	0.00	572.93	2.83	6.27	0.00	0.10	0.04

Sprague-Dawley rats were individually treated with an oral, chronic dose of 5.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

Table 4H. Hematology and clinical chemistry results of 5.0% HCA-SX treated female Sprague-Dawley rats

		Female 5.0% HCA-SX																
Days	Tests	Globulin	Alkaline phosphatase	Blood urea nitrogen	Creatinine	Aspartate amino-transferase	Alanine amino-transferase	Cholesterol	Total bilirubin	Glucose	Calcium	Chloride	Phosphorus	Sodium	Potassium	Iron	Total iron binding capacity	Iron/total iron binding capacity
	Units	g/dL	IU/L	mg/dL	mg/dL	IU/L	IU/L	mg/dL	mg/dL	mg/dL	mg/dL	mEq/L	mg/dL	mEq/L	mEq/L	mg/dL	mg/dL	NA
30	Mean	3.80	48.00	15.60	0.74	82.60	40.60	91.60	0.12	122.40	11.32	100.00	6.78	142.40	5.68	332.60	588.80	55.40
	S.D.	0.12	1.99	0.11	0.01	2.51	2.15	3.23	0.01	2.86	0.09	0.28	0.21	0.67	0.10	21.98	23.00	4.35
60	Mean	3.78	49.00	16.40	0.74	80.80	38.20	95.80	0.14	123.00	11.38	98.60	6.72	143.60	5.56	330.80	590.00	54.60
	S.D.	0.04	2.03	0.18	0.01	2.22	0.74	1.13	0.01	3.83	0.13	0.23	0.11	0.59	0.08	18.37	23.74	1.85
90	Mean	3.83	52.57	16.71	0.73	83.71	41.71	91.29	0.13	122.57	11.29	98.14	6.83	143.00	5.66	332.00	588.43	56.00
	S.D.	0.05	1.54	0.07	0.01	1.47	0.99	1.26	0.01	2.48	0.07	0.19	0.10	0.87	0.06	14.94	6.94	2.61

Sprague-Dawley rats were individually treated with an oral, chronic dose of 5.0% HCA-SX for 30, 60 and 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 \pm S.D. of 5-7 animals.

The minimal hepatocyte vacuolation seen in two animals may have indicated a metabolic problem associated with the experimental procedure, but one control animal had a similar lesion and the changes were limited and not considered significant.

Another change noted was within the glandular stomach of a number of animals in the 90 day group. There were scattered minimal or mild foci of gastric gland dilatation with accumulation of what appeared to be proteinaceous material in their lumens. No necrosis or inflammation was seen. These changes were noted in animals in the positive controls as well as all three experimental groups, but were minimal (only two animals) or not present in negative controls. There was no obvious indication of the change being either more severe or more numerous in any particular dose group. Since the positive control animals were also involved, the possibility of the change being associated with the gavage procedure or the diluent would certainly have to be considered. In any case, overall these appear morphologically to be minimal and not significant changes.

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.

Acknowledgements

The authors acknowledge IDEXX Veterinary Services, Sacramento, CA, USA, for performing histological studies. The authors thank Ms. Kristine Strong for technical assistance.

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