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Review Article

DUBIOUS ANTI-OBESITY AGENT HCA FROM *GARCINIA*: A SYSTEMATIC REVIEW

ANKUR GOGOI[1*], NABAJYOTI GOGOI[1], BIJOY NEOG[2]

[1]Centre for Studies in Biotechnology, Dibrugarh University, Assam, India, [2]Department of Life Sciences, Dibrugarh University, Assam, India

Email: agogoibiotech@dibru.ac.in

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ABSTRACT

Obesity is a complex disorder of appetite regulation and energy metabolism controlled by specific biological factors. Whenever prevention fails, medicinal treatment of obesity may become an obligation and it is more fruitful when we can acquire the medicinal treatment directly from nature, which is more preferred and healthier rather than going for chemical and surgical treatment. Alternatively, inhibition of carbohydrate to fatty acid conversion reaction can lead to obesity control. This can be done by assay of Hydroxycitric acid [(-)-HCA], which inhibits the formation of ATP-citrate lyase, responsible for lipogenesis. HCA is a derivative of citric acid and found in *Garcinia* fruit as the principal acid. Many *in vitro* and *in vivo* studies have demonstrated that (-)-HCA suppresses the *de novo* fatty acid synthesis and lipogenesis. However, results from clinical studies showed both negative and positive anti obesity effects of (-)-HCA. In this review paper an attempt has been made to explore and give an insight of (-)-HCA taking account of the literature coverage on speckled topics: Its discovery, properties, extraction and estimation and its significance of role in anti-obesity activity.

Keywords: Hydroxycitric acid, HCA, *Garcinia*, Anti-obesity, ATP-citrate lyase, Lipogenesis.

INTRODUCTION

Obesity is becoming a catastrophic physiological challenge for the present generation human race and in the near future, it will overcome the consequences of under nutrition, which is a significant adjuvant to the poor and ill health conditions [1]. In fact, obesity is a complex disorder of appetite regulation and energy metabolism controlled by specific biological factors. Hypertension, metabolic syndrome, diabetes, dyslipidaemia, myocardial infarction, stroke and its related complications are normally associated with obesity. Whenever prevention fails, medicinal treatment of obesity may become an obligation and it is more fruitful when we can acquire the medicinal treatment directly from nature, which is more preferred and healthy rather than going for chemical and surgical treatment. Weight loss can be achieved with treatment. But after weight reduction, its long term maintenance is very difficult. Thus, the treatment often remains unsuccessful [2-4]. Inhibition of carbohydrate to fatty acid conversion reaction can lead to obesity control. This can be done by assay of Hydroxycitric acid [(-)-HCA], a derivative of citric acid found in *Garcinia* and in some varieties of tropical plants and has been established to be a potential metabolic regulator of anti-obesity activity and inhibitor of lipogenesis. The presence of (-)-HCA as the principal acid has added importance to the genus *Garcinia*. The genus *Garcinia* Linn., belonging to the Kingdom *Plantae*, Order *Malpighiales*, family *Clusiaceae* includes about 200 species found worldwide. *Garcinia* a commonly used flavoring agent is a large genus of evergreen trees or shrubs, distributed in tropical Asia, Africa, and Polynesia of which about 30 species are found in India. *Garcinia* species are found in forest lands, riversides and wastelands. These plants prefer evergreen forest, but sometimes they also thrive in areas with relatively low rainfall. It is also cultivated on a small scale. It does not require irrigation, spraying of pesticides or fertilizers [5-7].

HCA Unearthing

The fruits of *Garcinia* species are too acidic and sour in taste. The dried rinds of the fruit of *Garcinia* species are extensively used for cooking purposes. The organic acids present in the fruit are responsible for the bacteriostatic effect of the pickling medium. The fruits are also anthelmintic and useful in piles, dysentery, bilious affections, tumor, pains and heart complaints. Sun dried rinds are used as a garnish to give an acid flavor to curries and also for preparing syrup during hot summer days. In earlier studies principle acid present in *Garcinia cambogia* have been erroneously identified as tartaric and citric acid [8, 9]. Preliminary investigation for identification and separation of (-)-HCA was done with paper chromatography using *n*-butanol/acetic acid/water at a ratio of 4:1:5 and *n*-propanol/formic acid/water at a ratio of 4:1:5. The coloured spot development was achieved by spraying with 5% metavanadate. Isolation of the principal acid from the fruit rinds of *G. cambogia* and its identification as (-)-HCA on the basis of chemical and spectroscopic studies was done by Lewis and Neelakantan [10]. The absolute configuration was determined from Hudson's lactone rule, optical rotatory dispersion curves, circular dichrosim curves, and calculation of partial molar rotations [11].

The chemistry and properties of (-)-HCA and its lactone

HCA [$C_6H_8O_8$ (1, 2-dihydroxypropane-1, 2, 3-tricarboxylic acid)] has two asymmetric centers, thereby two pairs of diastereoisomers or four different isomers (I, II, III and IV) are possible [Figure: 1] [12]. Being a γ-hydroxy acid, it cyclizes readily to the corresponding lactone ($C_6H_6O_7$). The molar mass of HCA and HCA lactone are 208.12 and 190.106 g mol^{-1}respectively.

The absolute configurations of the HCA lactones of *garcinia* and *hibiscus* were determined to be (2*S*, 3*R*)-and (2*S*, 3*S*)-2-hydroxycitric acid-2, 5-lactone, respectively [fig. 2]. The structure and absolute configuration of the calcium hydroxycitrate and (-)-HCA lactone was done by X-ray crystallography [13, 14]. Stallings *et al.* have studied the crystal structures of the ethylenediamine salts of diastereoisomeric hydroxycitrates [15].

Table 1: Physical properties of HCA and lactone from *Garcinia* [10]

Properties	free acid	lactone
mp (˚c)		178
$[\alpha]^{20}{}_D$(deg)	-20	100
Crysal shape		needles
Hygroscopicity		slight
Solubility		high in alcohol and water; fair in ether
Paper chromatography (R_f)		
Butanol/formic acid/H_2O	0.24	0.42
Propanol/acetic acid/H_2O	0.26	0.36
Metavandate spray (5%)	Yellow	Raddish Orange

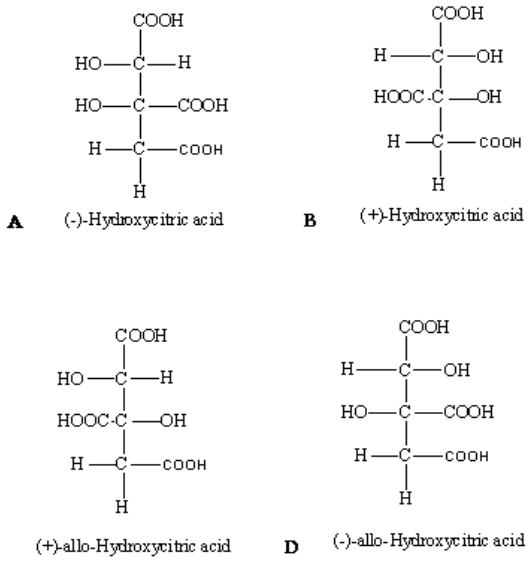

Fig. 1: Structural representation of isomers of HCA

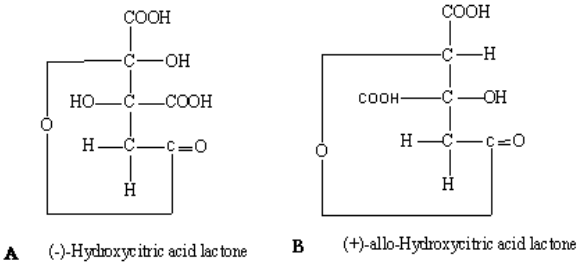

Fig. 2: Structural representation of HCA Lactones

A comparative physical properties of (-)-HCA and lactones from *Garcinia* are presented in table 1. The IR spectra of the ethyl ester showed ester and hydroxyl groups at 5.41-5.76 and 2.74-2.79 μ, respectively [10]. The structure of the (-)-HCA lactone was further established by IR and 1H NMR spectroscopy. The (-)-HCA lactone displayed strong IR bands at 3200, 1760, and 1680 cm^{-1}. ^1H NMR spectra of the (-)-HCA lactone showed two protons at the γ-carbon, which give an AB quartet at δ 2.53 and δ 2.74 with J = 17.1 Hz, and one proton at the α-carbon showing a singlet at δ 5.15 [16].

Importance

HCA is purported to be one of the active components in various over-the-counter weight-loss formulations and appetite-suppressor products. (-)-HCA being a potent inhibitor of ATP: Citrate lyase, which catalyzes the extramitochondrial cleavage of citrate to oxaloacetate and acetyl-CoA, limits the availability of acetyl-CoA units required for fatty acid synthesis and lipogenesis [17-23]. Many studies demonstrated both *in vitro* and *in vivo* that (-)-HCA suppresses the de novo fatty acid synthesis and lipogenesis. Extensive works have been carried out on plant systematics, distribution as well as on traditional medicinal uses and chemical constituent of *Garcinia* species in the last few decades. Some of these works are reviewed as below:

Extraction methods and estimation of HCA

Lewis *et al.* isolated the principal acid in the fruit rinds of *G. cambogia* and identified it as (-)-HCA on the basis of chemical and spectroscopic studies [10]. Boll *et al.* have reported that, the absolute configuration is determined from Hudson's lactone rule, optical rotatory dispersion curves, circular dichrosim curves, and calculation of partial molar rotations [11]. Glusker *et al.* have reported the structure and absolute configuration of the calcium hydroxycitrate and (-)-HCA lactone by X-ray crystallography [13, 14]. Stallings *et al.* Have reported the crystal structures of ethylenediamine salts of diastereoisomeric hydroxycitrates [15].

Upon concentration and evaporation free (-)-HCA gives (-)-HCA lactone. Acid-Base titration method gives total acidity of the extract, but concentrations of (-)-HCA and (-)-HCA lactone cannot be estimated separately. Gas chromatography (GC) estimation of (-)-HCA content of *G. cambogia* fruit was developed by Lowenstein *et al.*, which involves conversion of acid to volatile silyl derivative. Silylation requires completely dried samples, but free (-)-HCA readily cyclizes to gives its corresponding lactone upon drying. Thus the free (-)-HCA cannot be estimated [Figure: 3] [24]. Moffett *et al.* have developed a process for the aqueous extraction of (-)-HCA from *Garcinia* rinds. The extract was loaded onto an anion exchange column for adsorption of (-)-HCA, and it was eluted with sodium/potassium hydroxide for the release of (-)-HCA. The extract was passed through a cation exchange column to yield a free acid and have reported the preparation of (-)-HCA concentrate from *Garcinia* rinds with 23-54% (-)-HCA and 6-20% lactone [25, 26]. Jayaprakasha *et al.* have reported (-)-HCA has been found to be the major organic acid in *G. cambogia,* present in concentrations of 16–18%, using high-performance liquid chromatography (HPLC) method with 10 mM sulfuric acid as eluent. Citric and malic acids are present in Malabar tamarind in minor quantities. Again in 2000, they have reported on the determination of (-)-HCA in commercial samples of *G. cambogia* extracts by liquid chromatography using ultraviolet detection and in a subsequent study in 2002, on the determination of organic acids in the leaves and rinds of *G. indica* by HPLC, they have reported that (-)-HCA and its lactone can be quantified separately from the dilute extracts, without concentration and drying [27-29]. Jena *et al.* have reported on determination of organic acids in fresh leaves, fruits, and dried rinds of *Garcinia cowa* by high-performance liquid chromatography. The major organic acid was found to be (-)-HCA present in leaves, fruits, and rinds to the

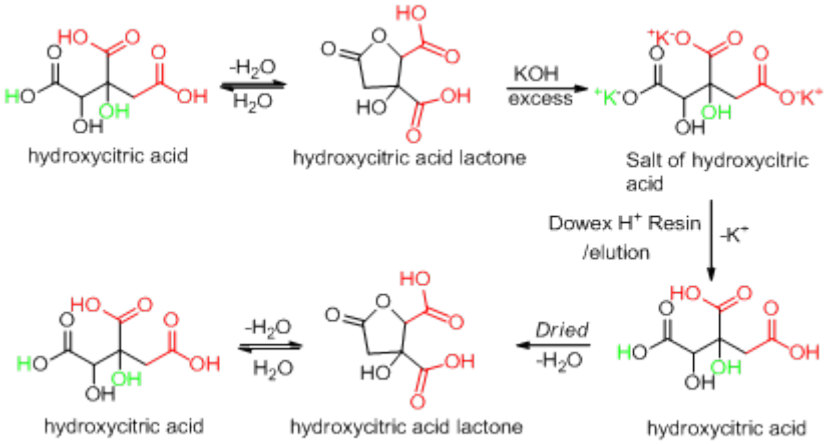

Fig. 3: Structural representation of HCA isolation and its subsequent conversion to corresponding lactone [40]

extent of 1.7, 2.3, and 12.7%, respectively. (-)-HCA lactone, and oxalic and citric acids are present in minor quantities [30]. In 2003, an improved liquid chromatography (LC) method for determination of organic acids in leaves, pulp, fruits, and rinds of *Garcinia* was developed. The commonly used LC method for analysis of organic acids in *Garcinia* extracts uses the direct application of the extracts in the column, which gradually reduces efficiency of the column and shortens its life. In the improved method, the interfering substances such as pigments and xanthones were effectively removed by passing the aqueous extract through an ODS cartridge [31].

In a study on the presence of (-)-HCA and its estimation using HPLC and acid-base titration method in two months old fruits and the leaves of four species of *Garcinia* namely G. *gummi-gutta, G. indica, G. tinctoria* and *G. cowa*. Among the four species studied G. *tinctoria* had very low (-)-HCA in leaves and fruits. It was revealed that fruit rinds contained more amount of (-)-HCA in comparison with leaves. The magnitude of (-)-HCA in leaves and fruit rinds followed in the order of G. *gummi-gutta>G. indica>G. cowa*>G. *tinctoria* irrespective of the methods studied. The titration method gave more amount of (-)-HCA than HPLC. This is due to the estimation of total acidity (all organic acids) in titration method, whereas in HPLC only (-)-HCA was determined [32]. Muensritharam *et al.* have developed Capillary zone electrophoresis (CZE) method for quantitative determination of (-)-HCA and (-)-HCA lactone in herbal products of *Garcinia atroviridis* Griff. Advantages of the developed CZE method over previous HPLC methods include simple sample preparation, faster analysis time within 5 minutes and better resolution of analytes [33]. The microwave power has been used successfully to extract (-)-HCA from the dried rinds of *G. oblongifolia.* The method gave effective result with reduced time for extraction at 25 min and higher extraction yields of total amount of organic acids as compared to Soxhlet extraction and pressure cooker distillation [34].

Naveen *et al.* reported on chemical constituents of the fruit rind and seed of *G. gummi-gutta*, analyzed by Atomic Absorption Spectroscopy and did proximate analyses and physicochemical analyses of the extracts of *G. gummi-gutta* [35-37]. Kumar *et al.* developed a rapid, sensitive and simple reverse-phase HPLC-PDA method with solvent system consisting of a mixture of acetonitril-water (90:10,v/v) and methanol-acetic acid (99.5:0.5,v/v) as a mobile phase followed by photo diode array (PDA) detection of peaks at 220 and 276 nm with run time less than 25 minute for the simultaneous identification and quantification of HCA lactone (HCAL), isoxanthochymol (Isoxan) and xanthochymol (Xan) in different extracts of *G. indica* [38]. Upadhyay *et al.* developed a simple HPLC sensitive isocratic method for the detection and quantification of HCA in *G. combogia.* Content of HCA present in plant extract has been found to be in the range of 50% using reversed-phase HPLC with ultraviolet detection. The methanolic extract of *G. cambogia* analyzed by this method found to contain 50-55% HCA [39]. Recently Gogoi *et al.* have reported on the development of a cost effective and improved HPLC analysis method using water as mobile phase for estimation of HCA in the fruits of G. *lanceaefolia* [40].

HCA stability and preparation of derivatives

Genus *Garcinia* contains 20-30% of (-)-HCA as its principal acid. But, (-)-HCA is susceptible to lactonization upon evaporation and concentration. Therefore the preparation of stable derivative was a necessity. Martius *et al.* have succeeded in synthesizing the four possible stereoisomers of hydroxycitrate. Among these four possible stereoisomers, (-)-HCA is found in *Garcinia* and (+)-allo-HCA in *Hibiscus* species [41,10,12]. Salt of potassium hydroxycitrate was prepared by extracting (-)-HCA from the fruits of *Garcinia* and the extract was treated with potassium hydroxide to yield potassium hydroxycitrate precipitate [42]. The soluble metal double salt of (-)-HCA was prepared by treating aqueous extract of (-)-HCA with different metal hydroxides and metal chlorides [43]. Sodium hydroxycitrate was prepared by treating the aqueous extract of (-)-HCA with aqueous sodium hydroxide at 80 °C [44].

Scrutinizing the Role of (-)-HCA in anti-obesity activities

Flourished nutraceutical industries and increasing interest in scientific credibility gave a thrust to many companies and scientist. The evaluation of the potential toxicity of weight control supplement is of the utmost importance as it requires long term continuous consumption in order to maintain its effects. With the dilemmatic reports demonstrating the efficacy of *Garcinia* extract or HCA without any toxicity and as an active ingredient in some products, showing potential toxicity; for that, to draw a fine line was of a necessity as of the topic concern. Weight gain occurs when the limited capacity for storing glycogen in the liver and muscles is attained, and beyond this point excess glucose is converted into fat and stored in fat cells throughout the body. Watson *et al.* reported inhibition of ATP: citrate Oxaloacetate lyase by (-)-HCA with purified enzyme from rat liver. ATP: citrate lyase (ATP: citrate Oxaloacetate lyase, EC 4.1.3.8) a citrate cleavage enzyme found abundantly in animal tissues and has been mentioned to play a physiological role in lipogenesis from carbohydrate and gluconeogenesis [45]. The synthesis of fatty acid takes place in the cytosol and acetyl-CoA in mitochondria.

The oxidation of pyruvate to acetyl-CoA in mitochondria leads to conversion of carbohydrate into fat. The primary source of carbon atoms for fatty acid synthesis is the acetyl-CoA. From the mitochondria acetyl group of acetyl-CoA are transferred to the cytosol for fatty acid biosynthesis. To transfer acetyl-CoA from mitochondria to cytosol requires conversion of acetyl-CoA to citrate. In the mitochondrial matrix condensation of acetyl-CoA with oxaloacetate produces citrate, which can bypass the obstacle. After transport of citrate to cytosol by the tricarboxylate transport system, acetyl-CoA is regenerated from citrate by ATP: citrate lyase in the following catalytic reaction:

$$\text{Citrate} + \text{CoA} + \text{ATP} \rightarrow \text{Oxaloacetate} + \text{Acetyl} - \text{CoA} + \text{ADP} + \text{Phosphate}$$ [46-53].

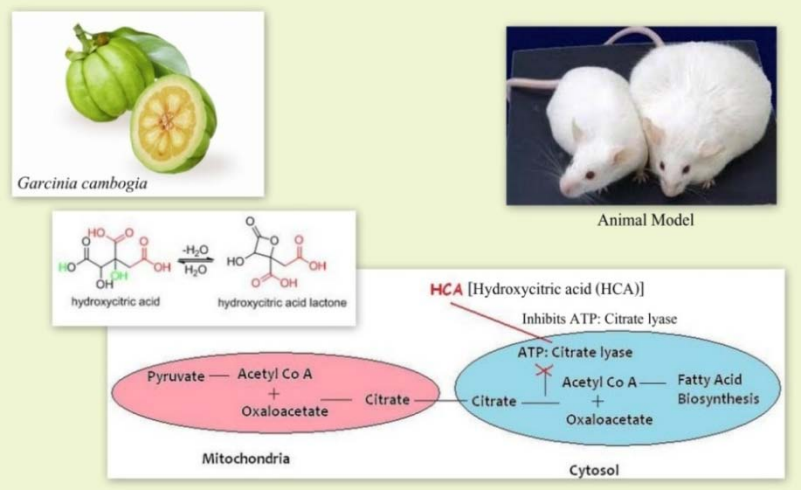

Fig. 4: Table of Contents Graphic (Graphical Abstract)

Cheema-Dhadhi *et al.* reported inhibition of citrate cleavage enzyme by both free (-)-HCA and (-)-HCA lactone [54]. Szutowicz *et al.* reported (-)-HCA to be a potent inhibitor of citrate cleavage enzyme in rat brain synaptosomes [55]. In similar studies, it was found that out of the four isomers of HCA only (-)-HCA was the potential inhibitor of ATP [15,21]. Yamada *et al.* have reported that, (2S, 3R)-HCA, inhibits pancreatic α-amylase and intestinal α-glucosidase, leading to a reduction in carbohydrate metabolism *in vitro* [56]. Lowenstein *et al.* incorporated ^{3}H from $3H_2O$, to measure the rate of fatty acid synthesis in rat liver and found that (-)-HCA strongly inhibited fatty acid synthesis by Rat liver *in vivo* [17]. Sullivan *et al.* have reported that, the *in vivo* rate of lipogenesis was markedly decreased from [^{14}C] alanine following the administration of (-)-HCA either intraperitoneally or intravenously. Fatty acid and cholesterol syntheses were significantly inhibited by the oral administration of (-)-HCA only when the compound was given before the feeding period. In 1974, they have reported that oral administration of (-)-HCA to rats significantly depressed the *in vivo* lipogenic rates in a dose dependent manner in the liver, adipose tissue, and small intestine and caused significant reductions in body-weight gain, food consumption, and total body lipid [18-20, 23].

Pharmacological approaches for treating the obese patient, (-)-HCA has been used as a potentially safe and effective pharmacological intervention for obesity for at least 30 years [57]. In isolated hepatocytes Beynen *et al.* observed inhibition of fatty acid synthesis by (-)-HCA, when fatty acid synthesis takes place from glucose [58]. Indicating that (-)-HCA acts as an inhibitor of lipogenesis when cytoplasmic acetyl-CoA is produced by citrate cleavage enzyme reaction. But (-)-HCA activated fatty acid synthesis when the source of acetyl-CoA is acetate [59-62]. Hood *et al.* have shown that (-)-HCA reduced the synthesis of fatty acids from lactate and glucose in bovine adipose tissue and rat adipose tissue, respectively, and suggested that the conversion of lactate to fatty acids probably occurs by way of citrate. In the (-)-HCA treated rat liver cells, there was a decrease in the formation of phospholipids and triglycerides from lactate [63].

A significant reduction in food intake, decrease in the feed efficiency ratio, body-weight gain, epididymal fat, and serum triglyceride in the albino rats was observed by feeding lipogenic diets along with (-)-HCA. The reduction in appetite in (-)-HCA-fed rats were due to the specific effect of (-)-HCA ingestion. It was not due to alterations of taste of the fed diet as the control group fed with citrate taking consideration of the close structural relationship of (-)-HCA to citrate and it is reasonable that (-)-HCA does not affect the taste of the food [64]. Vicario *et al.* have also reported the inhibition of lipogenesis from lactate in rat brain by (-)-HCA, and the transfer of lactate carbons through the mitochondrial membrane is accompanied by the translocation of citrate [65]. Clouatre *et al.* reported (-)-HCA exerts its anti-obesity effect by inhibiting ATP citrate lyase, consequently inhibiting the cleavage of citrate to oxaloacetate and acetyl-CoA, a key molecule, which plays a critical role in energy storage as fat. Here, instead of wasting energy to synthesize fat, the energy is diverted to the production of glycogen in the liver and muscles. This slows the production of fatty acids, cholesterol, and triglycerides with the net effect of reduced fat production and storage [66].

Several studies have found a positive effect of (-)-HCA administration alone or in combination with other ingredients on appetite, energy intake, body weight loss, fat oxidation or energy expenditure, but some studies did not. Heymsfield *et al.* performed a randomized, double blind, placebo controlled trial to prove the efficacy of (-)-HCA as a potential anti obesity agent. But their findings failed to detect either weight loss or fat mobilizing effects of (-)-HCA beyond those of placebo [67]. Kriketos *et al.* hypothesis study failed to demonstrate an effect of (-)-HCA on Respiratory Quotient (RQ) or Energy Expenditure (EE) in the fasted state either during rest or during moderately intense exercise on a cycle ergometer under laboratory conditions in male adults consuming a typical mixed diet. They did not detect an effect of (-)-HCA on circulating concentrations of blood substrates associated with fat oxidation and regulation of glucose metabolism. It is possible, however, that their (-)-HCA treatment was not sufficiently long or that the dose used was insufficient to inhibit the enzyme citrate lyase under the influence of a typical mixed diet [68]. Mattes *et al.* study did not support a satiety effect of HCA on weight loss through suppression of hunger. No effects of the HCA were observed on appetitive variables. The active treatment group did not exhibit better dietary compliance or significant correlations between appetitive variables and energy intake or weight change. The study concluded that the compound may be more useful for weight maintenance after an initial loss [69]. But Mahendran *et al.* have reported the modulating effect of *G. gummi-gutta* extract on the ethanol induced peroxidation and on lipids and lipoprotein composition in dexamethasone administered rats [70].

As reported earlier, when dietary supplements contain high glucose, HCA significantly reduces body weight regain and was not diminished when the diet contained 24 % of the energy as fat. Leonhardt *et al.* studied whether dietary supplementation of HCA reduces food intake and body weight regain in rats after 10–15% weight loss. In their study HCA reduced body weight regain after substantial body weight loss, and the effects are presumably linked to its inhibiting effect on lipogenesis, suggesting a very strong effect of HCA on food intake and increased energy expenditure [71].

Kovacs *et al.* hypothesized that HCA supplementation might affect appetite and Body Weight (BW) regulation by increasing fat oxidation and metabolic rate, reflected by an increase in EE and combination of HCA and Medium-Chain Triglycerides (MCT) may have a stronger effect on fatty acid oxidation and consequently on satiety compared to HCA alone. But the results did not support the

hypothesis [72]. Westerterp-Plantenga *et al.* did assessment of the effects of daily administration of HCA on Energy Intake (EI) and satiety in overweight men and women for a period of 2 weeks and found that HCA might not primarily be a weight loss agent, as indicated by the minor changes observed in body weight, but might be effective in preventing weight regain in humans [73]. The dose and time dependent effect of HCA-SX was studied by Shara *et al.* in Sprague-Dawley rats over a period of 90 days. The study indicated that HCA-SX is safe and efficacious in weight management under the conditions employed in the study but did not cause any changes in hepatic and testicular lipid peroxidation, DNA fragmentation, or histopathological changes [74].

Hayamizu *et al.* found that extracts of *G. cambogia can* efficiently improve glucose metabolism and display leptin-like activity. The precise mechanism for suppression of leptin and insulin production was not clear. Their findings indicated that upregulation of leptin production is accompanied by enhanced insulin production. They concluded that further studies are required to clarify if the leptin-like activity and its mechanism are effectively due only to the HCA presence or the remaining 40% components in the extract have an important role [75]. In order to study the safety and the influence of *Garcinia* extract on androgen in humans, Hayamizu *et al.* administered *Garcinia* extract to human subjects at a dose of 3,000 mg/day for 30days to evaluate the influence of surplus doses on Blood and subjective symptoms and influence of surplus doses on serum testosterone level in healthy men. Their study confirmed the safety of *Garcinia* by high dose administration study in healthy man [76]. In their subsequent studies on the effects of 12 weeks of *G. cambogia* extract administration on visceral fat accumulation and on body indices, they reported that *G. cambogia* may be useful for the prevention and reduction of the accumulation of visceral fat and there was no rebound effect observed when treated with placebo at the end of the treatment period and did not affect the markers of reproductive toxicity such as serum testosterone, estrone and estradiol levels. The result of serum testosterone levels of the study was supported by their previous work. Additional human studies are required to confirm these findings [77, 78].

Preuss *et al.* have reported that optimal doses (4667 mg) of (-)-HCA-SX alone and in combination with niacin-bound chromium (NBC) and a standardized *Gymnema sylvestre* extract (GSE) given 30–60 min. before meals, is highly bioavailable, efficacious and safe as weight-management supplements and 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 [79, 80]. Soni *et al.* Systematically reviewed on the available safety and toxicity literature on HCA to determine its safety in-use. On the basis of scientific procedures, which included human, animal, analytical, and other scientific studies, and history of exposure and use; they concluded that, the consumption of HCA or HCA-SX at a dose level of 2800 or 4667 mg/day, respectively, is considered safe [81]. In a study by Saito *et al.* in Zucker rats, being genetically predisposed to obesity was treated with (-)-HCA showed that high doses (778 mg HCA/kg BW/d and higher) led to significant suppression of epididymal fat accumulation, but also had high testicular toxicity. But it was not observed at 389 mg HCA/kg BW/d or 51 mmol HCA/kg diet and thus this level was deemed to be the No Observed Adverse Effect Level (NOAEL) [82]. This study has been criticized by Burdock *et al.* (2005) for possible contamination of the (-)-HCA used and various design flaws [83].

Downs *et al.* have reported that, the structural characteristics of a novel $Ca^{2+}$and K^{+}bound (-)-HCA salt (HCA-SX or Super CitriMax) make it completely water soluble as well as bioavailable. Both Ca and K act as buffers in pH homeostasis. HCA-SX has been shown to increase serotonin availability, reduce appetite, increase fat oxidation, improve blood lipid levels, reduce body weight, and modulates a number of obesity regulatory genes without affecting the mitochondrial and nuclear proteins required for normal biochemical and physiological functions [84]. Deshmukh *et al.* have reported on the safety of HCA-SX for long-term human consumption, which may eradicate obesity and related disorders including cardiovascular dysfunctions, diabetes and arthritis. In their developmental two generation reproductive toxicity study, HCA-SX was not found to be teratogenic in the Sprague-Dawley rat and did not cause maternal toxicity, adverse effects on the gravid uteri, soft tissue abnormalities, skeletal abnormalities or external abnormalities in the fetuses of the treated animals. Based on the results of this study, the NOAEL of HCA-SX is determined as 1240 mg/kg/day [85].

According to Brandt *et al.*, the effects of HCA on body weight gain were accounted for entirely by the reduction in feed intake. It has no lasting beneficial effects on hypertriglyceridemia and hyperinsulinemia and leads to the accumulation of liver lipids [86]. When rats fed with high-lipid diet, liver fattening partly occurs. But the lipid content did not lead to severe cellular liver degeneration and damage. Marked fat infiltration was observed in hepatocytes of animals fed high-lipid diet. Livers of animals fed *G. cambogia* added diet showed moderate fat infiltrations of the hepatocytes [87]. In a subsequent study on the preventive effects of dietary *G. cambogia* extract on lipid metabolism and serum activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma-glutamyle transferase (GGT) in rats fed high-lipid diet caused an increase in serum lipid indices, but the study dose failed to decrease the rise in serum lipid indices [88].

In a review by Onakpoya *et al.* examined the efficacy of HCA as a weight reduction agent, using data from randomised clinical trials (RCTs) with suitable data for statistical pooling. A small, statistically significant difference in weight loss favouring HCA over placebo (MD: −0.88 kg; 95% CI: −1.75, −0.00) was revealed in the meta-analysis. The evidence from RCTs suggested that HCA generates a short term weight loss. However, the magnitude of this effect is small, is no longer statistically significant when only rigorous RCTs are considered, and its clinical relevance seems questionable and concluded that future trials should be more rigorous, longer in duration, and better reported [89].

Natural food supplements with high flavonoid content are often claimed to promote weight-loss and lower plasma cholesterol in animal studies, but human studies have been more equivocal. Kim *et al.* studied to determine on the effectiveness of natural food supplements containing *G. cambogia* extract (GCE) to promote weight-loss and lower plasma cholesterol and to examine whether these supplements have any beneficial effect on lipid, adipocytokine or antioxidant profiles. GCE supplementation failed to promote weight-loss or any clinically significant change in % body fat [90]. But, in their subsequent study on investigation of the anti-obesity activities of Rapha diet®; a preparation containing *G. cambogia* extract in mice with dietary obesity demonstrated to attenuate body weight gain of high-fat diet (HFD) fed animals, not only by decreasing the accumulation of body fats and the size of adipocytes, but also by modulating lipid profiles without side effects such as energy deficiency-related fatigue [91]. When *G. cambogia* extract was supplemented with atherogenic diet, it improves the effect on performance metrics, and serum non-esterified fatty acids (NEFA) and C-reactive protein (CRP) levels in 1 year old, female Sprague-Dawley rats. Large doses of *G. cambogia* can lead to a substantial increase in serum NEFA concentrations which may be due to the increased fat degradation [92]. When Altiner *et al.* studied the effect on serum lipoprotein (a), apolipoproteins A1 (apo A1) and B (apo B), and total cholesterol levels, they concluded that, diet containing 65% HCA was insufficient to lower atherosclerotic lipoprotein levels [93].

Chuah *et al.* in their two reviews, established a comprehensive safety profile of HCA as a dietary supplement for treating obesity taking account of cytotoxicity, genotoxicity, acute toxicity, subchronic safety, two-generation reproductive and teratogenicity studies. The clinical studies verified the safety of HCA and HCA-SX for Human consumption and reported that those products which possess adverse effects are either polyherbal or multi-component in nature and concluded that the comprehensive scientific evidence had shown NOAEL at levels up to 2800 mg/day, suggesting its safety for use [94, 95].

Supplementation of HCA can significantly lower visceral fat accumulation and adipocyte size *via* inhibition of fatty acid synthase

activity and its mRNA expression in visceral adipose tissue, along with enhanced enzyme activity and gene expression involved in adipose fatty acid β-oxidation. HCA supplementation can protect against HFD-induced obesity by modulating the adipose fatty acid synthesis and β-oxidation, but induces hepatic fibrosis, inflammation and oxidative stress [96]. HCA has been found to be protective against the liver toxicity associated with ethanol and dexamethasone administration, and to maintain serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase at near normal levels. In both animal and clinical literature, elevated intakes of HCA *per se* have not led to signs of inflammation or hepatotoxicity. The compound has been found to reduce markers of inflammation in the brain, intestines, kidney and serum [97].

Oral sustained release matrix tablet of anti-obesity drug from *G. cambogia* has been formulated in an attempt to design a dosage form that manifests desirable release profile. An antiobesity liquid formulation of *G. cambogia* extract, L Carnitine and Chromium Picolinate was developed by Patel *et al.*, which gives good microbial stability and pharmacological activity. The formulation was prepared by using coconut water as base and flavour in different combinations. In Ayurveda, it is said that sour flavours such as those from *Garcinia* activate digestion. *Garcinia* is considered to make foods more filling and satisfying and has been used routinely for many centuries with no toxicity [98, 99].

CONCLUSION

Present day generation is threatened with increased body mass index (BMI: defined as the mass in kilograms divided by the square of the height in meters) or we can say obesity. Though losing weight has become a modern day obsession, in the developed world, various factors do contribute to the increased number of overweight and obese individuals. The treatment of obesity should be done as a physiological disorder, not as a physical disorder [2]. Whenever prevention fails, medicinal treatment of obesity may become an obligation and it is more fruitful when we can acquire the medicinal treatment directly from nature, which is more preferred and healthy rather than going for chemical and surgical treatment. The preliminary research based on laboratory and animal experiments suggests that (-)-HCA may be a useful weight loss aid. (-)-HCA has been demonstrated in the laboratory to reduce the conversion of carbohydrates into stored fat by inhibiting certain enzyme processes. Animal research also indicated that (-)-HCA suppresses appetite and food intake to induce weight loss.

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CONFLICT OF INTERESTS

The authors hereby declares no competing financial conflict of interest.

REFERENCE

1. Kopelman PG. Obesity as a medical problem. Nature 2000;404(6778):635-43.
2. Bray GA, Tartaglia LA. Medicinal strategies in the treatment of obesity. Nature 2000;404:672-7.
3. Fogelholm M, Kukkonen-Harjula K, Oja P. Eating control and physical activity as determinants of short-term weight maintenance after a very-low-calorie diet among obese women. Int J Obes 1999;23:203-10.
4. Westerterp-Plantenga MS, Kempen KP, Saris WHM. Determinants of weight maintenance in women after diet-induced weight reduction. Int J Obes 1988;22:1-6.
5. CSIR: New Delhi, India. The Wealth of India (Raw Materials); 1956;4:99-108.
6. Hooker JD. The flora of British India; Vol. I. L. Reeve and Co. Ltd, Kent, England; 1874. p. 259-70.
7. Kanjilal UN, Kanjilal PC, Das A. Flora of Assam: Vol. I. Printed at Jay Offset Press: Delhi; 1940. p. 103-10.
8. Sreenivasan A, Venkataraman R. Chromatographic detection of the organic constituents of Gorikapuli (*Garcinia cambogia* Desr.) used in pickling fish. Curr Sci 1959;28:151-2.
9. Kuriyan KI, Pandya KC. A note on the main constituents of the dried rind of the fruit of *Garcinia cambogia.* J Indian Chem Soc 1931;8:469.
10. Lewis YS, Neelakantan S. (-)-Hydroxycitric acids-The principal acid in the fruits of *Garcinia cambogia.* Phytochemistry 1965;4:619-25.
11. Boll PM, Else S, Erik B. Naturally occurring lactones and lactames III. The absolute configuration of the hydroxycitric acid lactones, Hibiscus acid and Garcinia acid. Acta Chem Scand 1969;23:286-93.
12. Lewis YS. Isolation and properties of hydroxycitric acid. Methods Enzymol 1969;13:613-9.
13. Glusker JP, Minkin JA, Casciato CA. Absolute configuration of the naturally occurring hydroxycitric acids. Arch Biochem Biophys 1969;132:573-7.
14. Glusker JP, Minkin JA, Casciato CA. The structure and absolute configuration of the calcium salt of garcinia acid, the lactone of (-)-hydroxycitric acid. Acta Crystallogr 1971;B27:1284-93.
15. Stallings W, Blount TF, Srere PA. Structural studies of hydroxycitrate and their relevance to certain enzymatic mechanisms. Arch Biochem Biophys 1979;193:431-48.
16. Jayaprakasha GK, Sakariah KK. Determination of (-)-hydroxycitric acid in commercial samples of *Garcinia cambogia* extracts by liquid chromatography using ultraviolet detection. J Liq Chromatogr Relat Technol 2000;23:915-23.
17. Lowenstein JM. Effect of (-)-Hydroxycitrate on fatty acid synthesis by rat liver *in vivo*. J Biological Chem 1971;246(3):629-32.
18. Sullivan AC, Hamilton JG, Miller ON. Inhibition of lipogenesis in rat liver by (-)-hydroxycitrate. Arch Biochem Biophys 1972;150:183-90.
19. Sullivan AC, Triscari J, Hamilton JG. Effect of (-)-hydroxycitrate upon the accumulation of lipid in the rat. II. Appetite Lipids 1974;9:129-34.
20. Sullivan AC, Triscari J, Hamilton JG. Effect of (-)-hydroxycitrate upon the accumulation of lipid in rat I Lipogenesis. Lipids 1974;9:121-8.
21. Sullivan AC, Singh M, Sere PA. Reactivity and inhibitor potential of hydroxycitrate isomers with citrate synthase, citrate lyases and ATP citrate lyases. J Biol Chem 1977;252:7583-90.
22. Triscari J, Sullivan AC. Comparative effects of (-)-hydroxycitrate and (+)-allo-hydroxycitate on acetyl CoA carboxylase and fatty acid and cholesterol synthesis *in vivo*. Lipids 1977;12:357-63.
23. Sullivan AC. Effect of (-)-hydroxycitrate on lipid metabolism. In Modification of Lipid Metabolism; Academic Press: New York; 1984. p. 143-74.
24. Lowenstein JM, Bruneugraber H. Hydroxycitrate. Methods Enzymol 1981;72:486-97.
25. Moffett SA, Bhandari AK, Ravindranath B. Hydroxycitric acid concentrate and food products prepared therefrom US; 1996.
26. Moffett SA, Bhandari AK, Ravindranath B. Hydroxycitric acid concentrate and food products prepared therefrom US; 1997.
27. Jayaprakasha GK, Sakariah KK. Determination of organic acids in *Garcinia cambogia* (Desr.) by high-performance liquid chromatography. J Chromatogr A 1998;806:337-9.
28. Jayaprakasha GK, Sakariah KK. Determination of (-)-hydroxycitric acid in commercial samples of *Garcinia cambogia* extracts by liquid chromatography using ultraviolet detection. J Liq Chromatogr Relat Technol 2000;23:915-23.
29. Jayaprakasha GK, Sakariah KK. Determination of organic acids in leaves and rinds of *Garcinia indica* (Desr.) by LC. J Pharm Biomed Anal 2002;28:379-84.
30. Jena BS, Jayaprakasha GK, Sakariah KK. Organic acids from leaves, Fruits, and Rinds of *Garcinia cowa*. J Agric Food Chem 2002;50:3431-4.
31. Jayaprakasha GK, Jena BS, Sakariah KK. Improved liquid chromatographic method for determination of organic acids in Leaves, Pulp, Fruits, and Rinds of *Garcinia.* J AOAC Int 2003;86(5):1063-8.

32. Asish GR, Parthasarathy U, Zachariah TJ. A comparative estimation of (-) Hydroxycitric acid in different species of *Garcinia.* Hortic J 2008;21(1):26-9.
33. Muensritharam L, Tolieng V, Chaichantipyuth C. Capillary zone electrophoresis for separation and analysis of hydroxycitric acid and hydroxycitric acid lactone: Application to herbal products of *Garcinia atroviridis* Griff. J Pharm Biomed Anal 2008;46:577-82.
34. Vinh DO, Cuong DH, Thuong N. Extracting (-)-hydroxycitric acid from dried rinds of garcinia oblongifolia Champ. ex Benth by using microwave. J Korean Chem Soc 2011;55(6):983-7.
35. Naveen GPAN, Krishnakumar G. Atomic absorption spectroscopic analysis of fruit rind and seeds of *Garcinia* species. Indian J Sci 2012;1(1):61-3.
36. Naveen GPAN, Krishnakumar G. Biochemical analysis and seed oil Characterizations of *Garcinia indica, G. xanthochymus and G. gummi-gutta* for nutritional qualities. Indian J Sci 2012;1(1):71-3.
37. Naveen GPAN, Krishnakumar G. Traditional and Medicinal Uses of *Garcinia gummi-gutta* Fruit-A Review. Species 2013;4(10):4-5.
38. Kumar S, Sharma S, Chattopadhyay SK. Rapid and sensitive HPLC-PDA method for simultaneous identification and quantification of dietary weight reducing compound hydroxy citric acid lactone and chemo preventive compounds isoxanthochymol and xanthochymol in *Garcinia indica.* Int Food Res J 2013;20(1):397-402.
39. Upadhyay V, Tiwari A, Sharma N. Rapid determination & standardization of garcinia fruit extract of hydroxycitric acid (HCA) in *Garcinia cambogia* by HPLC. PHARMBIT 2013;27(1):25-31.
40. Gogoi A, Gogoi N, Neog B. Estimation of (-)-Hydroxycitric acid (HCA) in *Garcinia Lanceaefolia* Roxb. Using novel HPLC Methodology. Int J Pharm Sci Res 2014;5(11):4993-7.
41. Martius C, Maue R. Preparation, physiological behavior, and importance of hydroxycitric acid and its isomers. Z Physiol Chem 1941;269:33-9.
42. Majeed M, Badmaev V, Rajendran R. Potassium hydroxycitrate for the suppression of appetite and induction of weight loss US; 1998.
43. Balasubramanyam K, Chandrasekhar B, Ramadoss CS. Soluble double metal salt of group IA and IIA of (-)-hydroxycitric acid, process of preparing the same and its use in beverages and other food products without effecting their flavor and properties US; 2000.
44. Ibnusaud I, Puthiaparampil TT, Thomas B. Convenient method for the large-scale isolation of *Garcinia* acid US; 2000.
45. Watson JA, Fang M, Lowenstein JM. Tricarballylate and hydroxycitrate: Substrate and inhibition of ATP: citrate oxaloacetatae lyase. Arch Biochem Biophys 1969;135:209-17.
46. Watson JA, Lowenstein JM. Citrate and the conversion of carbohydrate into fat. J Biol Chem 1970;245:5993-6002.
47. Lowenstin JM. Citrate and the conversion of carbohydrate into fat. Biochem Soc Symp 1968;27:61-86.
48. Lehninger AL. Biochemistry. 2nd ed. Kalyani Publishers: New Delhi, India; 1983. p. 659-91.
49. Stryer L. Biochemistry. 3rd ed. Freeman: New York; 1988. p. 487-8.
50. Rawn JD. Biochemistry. Neil Patterson: Burlington, NC; 1997. p. 421-55.
51. Srere PA. The citrate cleavage enzyme. I. Distribution and purification. J Biol Chem 1959;234:2544-7.
52. Spencer A, Corman L, Lowenstein JM. Citrate and the conversion of carbohydrate into fat. Biochem J 1964;93:378-88.
53. D'Adamo AF Jr, Haft DE. An alternate of R-ketoglutarate catabolism in the isolated, perfused rat liver. J Biol Chem 1965;240:613-7.
54. Cheema-Dhadli S, Halperin M, Land Leznoff CC. Inhibition of enzymes which interact with citrate by (-)-hydroxycitrate and 1, 2, 3-tricarboxybenzene. Eur J Biochem 1973;38:98-102.
55. Szutowicz A, Stepien M, Lysiak W. Effect of (-)-hydroxycitrate on the activities of ATP citrate lyase and the enzymes of acetyl-CoA metabolism in rat brain. Acta Biochim Pol 1976;23:227-34.
56. Yamada T, Hida H, Yamada Y. Chemistry, physiological properties and microbial production of hydroxycitric acid. Appl Microbiol Biotechnol 2007;75(5):977-82.
57. Bray GA, Greenway FL. Pharmacological approaches to treating the obese patient. Clin Endocrinol Metab 1976;5:455-79.
58. Beynen AC, Geelen MJ. Effect of insulin and glucagon on fatty acid synthesis from acetate by hepatocytes incubated with (-)-hydroxycitrate. Endokrinologie 1982;79:308-10.
59. Barth C, Hackenschmidt J, Ullmann H. Inhibition of cholesterol synthesis by (-)-hydroxycitrate in perfused rat liver. Evidence for an extramitochondrial mevalonate synthesis from acetyl coenzyme A. FEBS Lett 1972;22:343-6.
60. Gregolin C, Ryder E, Warner RC. Liver acetyl coenzyme A carboxylase. II. Further molecular characterization. J Biol Chem 1968;243:4236-45.
61. Hashimoto T, Numa S. Kinetic studies on the reaction mechanism and the citrate activation of liver acetyl coenzyme A carboxylase. Eur J Biochem 1971;18:319-31.
62. Hackenschmidt J, Barth C, Decker K. Stimulation of acetyl-CoA carboxylase by (-)-hydroxycitrate. FEBS Lett 1972;27:131-3.
63. Hood RL, Beitz DC, Johnson DC. Inhibition by potential metabolic inhibitors of *in vitro* adipose tissue lipogenesis. Comp Biochem Physiol 1985;81(3):667-70.
64. Rao RN, Sakariah KK. Lipid-lowering and antiobesity effect of (-)-hydroxycitric acid. Nutr Res 1988;8:209-12.
65. Vicario C, Medina M. Metabolism of lactate in the rat brain during the early neonatal period. J Neurochem 1992;59:32-40.
66. Clouatre D, Rosenbaum M. The diet and health benefits of HCA (Hydroxycitric Acid). Keats Publishing: New Cannan CT; 1994.
67. Heymsfield SB, Allison DB, Vasselli JR. *Garcinia cambogia* (hydroxycitric acid) as a potential antiobesity agent: A randomized controlled trial. J Am Med Assoc 1998;280(18):1596-600.
68. Kriketos AD, Thompson HR, Greene H. (-)-hydroxycitric acid does not affect energy expenditure and substrate oxidation in adult males in a post-absorptive state. Int J Obes 1999;23:867-73.
69. Mattes RD, Bormann L. Effects of (-)-hydroxycitric acid on appetitive variables. Physiol Behav 2000;71:87-94.
70. Mahendran P, Devi CSS. The modulating effect of *Garcinia cambogia* extract on ethanol induced peroxidation. Indian J Pharmacol 2001;33(2):87-91.
71. Leonhardt M, Hrupka B, Langhans W. Effect of hydroxycitrate on food intake and body weight regain after a period of restrictive feeding in male rats. Physiol Behav 2001;74:191-6.
72. Kovacs EMR, Westerterp-Plantenga MS, Saris WHM. The effects of 2-week ingestion of (-)-hydroxycitrate and (-)-hydroxycitrate combined with medium-chain triglycerides on satiety, fat oxidation, energy expenditure and body weight. Int J Obes 2001;25:1087-94.
73. Westerterp-Plantenga MS, Kovacs EMR. The effect of (-)-hydroxycitrate on energy intake and satiety in overweight humans. Int J Obes 2002;26(6):870-2.
74. Shara M, Ohia SE, Yasmin T. 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. Mol Cell Biochem 2003;254:339-46.
75. Hayamizu K, Hirakawa H, Oikawa D. Effect of *Garcinia cambogia* extract on serum leptin and insulin in mice. Fitoterapia 2003;74:267-73.
76. Hayamizu K, Ishii Y, Shigematsu N. Safety of *Garcinia cambogia* extract in healthy men: high dose administration study I. J Oleo Sci 2003;52(9):499-504.
77. Hayamizu K, Ishii Y, Kaneko I. Effects of *Garcinia cambogia* (Hydroxycitric Acid) on visceral fat accumulation: A Double-Blind, Randomized, Placebo-Controlled Trial. Curr Ther Res 2003;64(8):551-67.
78. Hayamizu K, Tomi H, Kaneko I. Effects of *Garcinia cambogia* extract on serum sex hormones in overweight subjects. Fitoterapia 2008;79:255-61.
79. Preuss HG, Bagchi D, Bagchi M. Efficacy of a novel, natural extract of (-)-hydroxycitric acid (HCA-SX) and a combination of HCA-SX, niacinbound chromium and *Gymnema sylvestre* extract in weight management in human volunteers: a pilot study. Nutr Res 2004;24:45-58.
80. Preuss HG, Bagchi D, Bagchi M. 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. Diabetes Obes Metab 2004;6:171-80.

81. Soni MG, Burdock GA, Preuss HG. Safety assessment of (-)-hydroxycitric acid and Super CitriMax!, a novel calcium/potassium salt. Food Chem Toxicol 2004;42:1513–29.
82. Saito M, Ueno M, Ogino S. High dose of *Garcinia cambogia* is effective in suppressing fat accumulation in developing male Zucker obese rats, but highly toxic to the testis. Food Chem Toxicol 2005;43:411-9.
83. Burdock G, Soni M, Bagchi M. "*Garcinia cambogia* toxicity is misleading". Food Chem Toxicol 2005;43(11):1683-4.
84. Downs BW, Bagchi M, Subbaraju GV. Bioefficacy of a novel calcium-potassium salt of (-)-hydroxycitric acid. Mutat Res 2005;579:149-62.
85. Deshmukh NS, Bagchi M, Yasmin T. Safety of a novel calcium/potassium salt of (-)-Hydroxycitric acid (HCA-SX): II. Developmental toxicity study in rats. Toxicol Mech Methods 2008;18:443-51.
86. Brandt K, Langhans W, Geary N. Beneficial and deleterious effects of hydroxycitrate in rats fed a high-fructose diet. Nutrition 2006;22:905-12.
87. Ates A, Gursel FE, Altıner A. Effect of *Garcinia cambogia* extract on fatty liver in rats fed high lipid. Kafkas Universitesi Veteriner Fakultesi Dergisi 2011;17(6):1015-20.
88. Ates A, Gursel FE, Bilal T. Effect of dietary *Garcinia cambogia* extract on serum lipid profile and serum enzymes in rats fed high-lipid diet. Iranian J Vet Res 2012;13(1):1-7.
89. Onakpoya I, Hung SK, Perry R. The use of *garcinia* extract (Hydroxycitric Acid) as a weight loss supplement: a systematic review and meta-analysis of randomised clinical trials. J Obes 2011. doi: 10.1155/2011/509038 [Article in Press].
90. Kim J, Jeon S, Park KH. Does glycine max leaves or *Garcinia Cambogia* promote weight-loss or lower plasma cholesterol in overweight individuals: a randomized control Trial. Nutr J 2011;10:94.
91. Kim J, Kyung J, Kim D. Anti-obesity effects of Rapha diet® preparation in mice fed a high-fat diet. Lab Anim Res 2012;28(4):265-71.
92. Bilal T, Gursel FE, Ates A. Effect of *Garcinia cambogia* extract on body weight gain, feed intake and feed conversion ratio, and serum non-esterified fatty acids and C-reactive protein levels in rats fed with atherogenic diet. Iranian J Vet Res 2012;13(4):330-3.
93. Altiner A, Ates A, Gursel E. Effect of the antiobesity agent *Garcinia cambogia* extract on serum lipoprotein (a), Apolipoproteins A1 and B, and total Cholesterol levels in female rats fed atherogenic diet. J Anim Plant Sci 2012;22(4):872-7.
94. Chuah LO, Yeap SK, Ho WY. "*In vitro* and *in vivo* toxicity of *Garcinia* or hydroxycitric acid: a review,". Evidence-Based Complementary Altern Med 2012. doi: 10.1155/2012/197920. [Article in Press].
95. Chuah LO, Ho WY, Beh BK. Updates on antiobesity effect of *Garcinia* Origin (-)-HCA. Evidence-Based Complementary and Alternative Medicine 2013. doi: 10.1155/2013/751658 [Article in Press].
96. Kim YJ, Choi MS, Park YB. *Garcinia Cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation. World J Gastroenterol 2013;19(29):4689-701.
97. Clouatre DL, Preuss HG. Hydroxycitric acid does not promote inflammation or liver Toxicity. World J Gastroenterol 2013;19(44):8160-2.
98. Sethi A, Patidar D, Patidar N. Formulation of sustained release tablet of antiobesity drug *Garcinia cambogia*. Int J Pharm Res Dev 2012;3(12):56-63.
99. Patel R, Patel J, Kakkar S. Formulation and development of anti-obesity liquid formulation containing *garcinia cambogia* extract, l-carnitine & chromium picolinate. Indo Global J Pharm Sci 2013;3(1):40-51.