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***RAPID PAPER***

# Safety of *Garcinia cambogia* Extract in Healthy Men: High-Doses Administration Study I

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**Abstract**: (−)-Hydroxycitric acid (HCA), a competitive inhibitor of ATP-citrate lyase, is frequently used in dietary supplements for weight loss. Recently, it was reported in a 52w toxicity study that Garcinia extract powder induced testicular atrophy in rats. In order to study the safety, Garcinia extract was administered to 10 healthy adult men at dose of 3,000 mg/day as HCA for 30 days. No toxicological changes were observed on anthropometric indices or clinical analysis. In addition, serum testosterone showed no change throughout the test period. In regard to subjective symptoms, very slight anorexia (two cases) and headache (one case) occurred. Therefore, we consider that Garcinia extract is safe as administered under the condition of this study.

**Key words**: Garcinia, hydroxycitric acid, testosterone

## 1 Introduction

(−)-Hydroxycitric acid (HCA), an active compound that is extracted from the rind of the fruit *Garcinia cambogia*, a native species of southern India, is an inhibitor of ATP-citrate lyase, a cytosolic enzyme that plays a crucial role in energy storage (1-5). The role of HCA is thus inhibitor in the production of acetyl CoA, and thereby might induce "body fat loss" *via* two mechanisms: 1) inhibition or limitation of the capacity for *de novo* lipogenesis (6), and 2) inhibition of malonyl CoA formation, which, in turn, would activate carnitine palmitoyl transferase I activity and increase $\beta$-oxidation (7,8).

Recently, Onomura *et al*. (9) reported that Garcinia extract decreased body weight, body mass index (BMI), and fat in the abdominal area under the condition of dietary intervention. In addition, we previously reported that HCA's efficacy depended on initial visceral fat area (VFA) values and was obvious in subjects whose initial VFA was greater than 90 $cm^2$ (10). From these reports, the anti-obese effective dose of Garcinia is considered

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to be from 750 mg/day to 1,000 mg/day as HCA.

On the other hand, we reported the influence of administration of a surplus dose of Garcinia for reviewing its safety (11). As a result, the no observed adverse effect level (NOAEL) of Garcinia as HCA was more than 4,000 mg. In addition, we examined the influence of 3,000 mg HCA daily, and recognized no adverse event on blood examination or in terms of subjective symptoms. Because the report was a pilot examination of the surplus quantity, the duration (10 days) of administration was short.

In March of 2002, however, National Institute of Health Sciences (NIHS) reported that Garcinia extract powder had been found to induce testicular atrophy in F344 male rats by 52w toxicity study (NIHS internal report). This was the first report of reproductive toxicity of Garcinia.

Therefore, the purpose of this investigation was twofold: first, to evaluate the influence of surplus doses on blood examination findings and on subjective symptoms for 30 days (i.e., three times the previous administration period), and, second, to evaluate the influence of surplus doses on serum testosterone level in healthy men.

## 2 Experimental

### 2·1 Subjects

The subjects were 10 Japanese men whose ages ranged from 26 to 56 years. All subjects were generally healthy and had no chronic constipation, diarrhea, or gastroenterological disorders. Other exclusion criteria are given in **Table 1**.

Because this study used human volunteers, it was carried out with due respect for the spirit of the Helsinki Declaration and was approved by the Institutional Review Board of the FANCL Corporation. The procedures were fully explained to all the volunteers in advance, and all subjects gave their informed consent before participating.

### 2·2 Test Sample

The Garcinia extract used was the "soluble type", and it was provided by Nippon Shinyaku (Kyoto, Japan). The HCA concentration was 60% as determined by HPLC. The test material was provided as a tablet containing 185.2 mg Garcinia extract. Subjects were instructed to take nine tablets 30 min before each meal (27 tablets per day). The total daily dose was 5,000 mg Garcinia extract, containing 3,000 mg HCA.

### 2·3 Analysis Method for Determination of HCA Content in Garcinia Extract

Sample of soluble Garcinia extract was weighed approximately 100 mg accurately and put into 100 mL volume of flask and added 5 mL of 1N NaOH. After further addition of 5 mL of 1N NaOH, the solution was left for 5 minutes at room temperature. 10 mL of 1N HCl, 5 mL of $HClO_4$ (10% w/w) and 10 mL of internal standard were added and the total volume was adjusted to 100 mL with distilled water. The solution was filtered through 0.2 $\mu$m membrane filter. 20 $\mu$l of the aliquot was withdrawn for analysis.

HCA content was determined by HPLC. Standard HCA was purchased from Wako Pure Chemical Industries, Ltd. Shimazu Liquid chromatography System C-R7A with 210 nm UV detector was used for analysis. ULTRON PS-80H column (Shinwa Chemical Industries, Ltd., 8 mm$\phi$ × 30 cm) and ULTRON PS-80H guard column (Shinwa Chemical Industries, Ltd., 8 mm$\phi$ × 5 cm)

**Table 1** Inclusion and Exclusion Criteria.

| |
|---|
| Inclusion: |
| · Healthy men aged 20 to 65 years-old |
| · Provision of written informed consent |
| Exclusion: |
| · Chronic constipation, diarrhea and gastroenterological disorder |
| · Use other than the Garcinia product of the sample of this study |
| · Dysfunction of liver, kidney, heart and hematology disease |
| · History of drug hypersensitivity or allergic condition that might interfere with the study |
| · Any abnormality of potential clinical significance |

were selected for the analysis. Mobile phase was $H_2O$ (pH 2.2 by $HClO_4$). And the flow rate was 0.81 mL/min.

### 2·4 Study Design

The study had an open design, and was carried out at the Tsukasa Clinic (Higashi-matsuyama, Saitama). The test period was 30 days.

### 2·5 Anthropometric Measurements

All measurements were performed by a trained investigator. The parameters of the anthropometric values, which were recorded every 10 days, were height, weight, body fat ratio (bioimpedance analysis), waist circumference, and hip circumference.

### 2·6 Blood Sampling and Clinical Analysis

Blood samples were collected from the subjects between 9:30 and 11:30 h after overnight fasting, which began at 22:00 h on the previous night.

Laboratory parameters were RBC, WBC, hemoglobin, hematocrit, MCV, MCH, MCHC, platelet, triacylglycerol, total cholesterol, AST (GOT), ALT (GPT), blood urea nitrogen, creatinine, glucose, Na, K, Cl, insulin and testosterone. Clinical laboratory data were measured at 0, 10, 20 and 30 days.

### 2·7 Subjective Symptoms

The parameters of the subjective symptoms, which were recorded every 10 days, were anorexia, headache, constipation, diarrhea, and so on. Subjective symptoms were diagnosed by the attendant doctor.

### 2·8 Statistical Analysis

Values were expressed by mean ± SEM. The changes from the initial values were examined by Wilcoxon signed-rank test. A P value of less than 0.05 was considered to indicate statistical significance. Statistical calculations were performed with StatView for Windows Ver.5.0 (SAS Institute, Cary, NC).

## 3 Results

All subjects completed the 30-days protocol.

### 3·1 Anthropometric Indices

Anthropometric indices are shown in **Table 2**. No significant changes were observed in any indices in comparison with initial value.

### 3·2 Blood Analysis

The results of clinical laboratory tests are shown in **Table 3**. In hemoglobin, MCH, MCHC, creatinine, and K, significant changes were observed in comparison with initial values, but these changes were transient. Therefore, it was judged that Garcinia was not the causative agent of these changes. In terms of serum testosterone level, the values at days 0, 10, 20, and 30 were 408.5 ± 39.7 , 473.4 ± 53.0, 430.5 ± 40.4, and 435.8 ± 38.8 (ng/dL), respectively, and no significant change was observed in comparison with the initial value (**Fig. 1**).

### 3·3 Subjective Symptoms

Subjective symptoms, judged subsequent to administration, were observed five cases with three subjects. These indices were anorexia (two cases), headache (one case), and constipation (two cases). The causal relationship of Garcinia to these subjective symptoms was unclear; although the symptoms appeared under administration period, it was judged by the attendant doctor that they did not constitute a clinical problem because

**Table 2** Effects of *Garcinia cambogia* on Body Weight, Body Mass Index, Body Fat Ratio and Waist/Hip ratio.

| | 0 day | 10 days | 20 days | 30 days |
|---|---|---|---|---|
| Body weight (kg) | 70.8 ± 2.5 | 70.8 ± 2.3 | 70.6 ± 2.4 | 70.8 ± 2.4 |
| Body mass index (kg/m$^2$) | 24.6 ± 0.7 | 24.7 ± 0.7 | 24.6 ± 0.7 | 24.7 ± 0.7 |
| Body fat ratio (%) | 24.5 ± 1.0 | 24.0 ± 0.1 | 23.5 ± 0.1 | 24.3 ± 0.1 |
| Waist circumference (cm) | 84.7 ± 2.7 | 84.8 ± 2.7 | 84.5 ± 2.7 | 83.7 ± 2.5 |
| Hip circumference (cm) | 93.2 ± 1.1 | 93.3 ± 1.6 | 92.9 ± 1.6 | 92.9 ± 1.2 |
| Waist / Hip ratio | 0.91 ± 0.02 | 0.91 ± 0.02 | 0.91 ± 0.02 | 0.90 ± 0.02 |

Values are Means ± SEM.

**Table 3** Effects of *Garcinia cambogia* on Hematology, Hemobiochemistry and Endocrinology.

| | 0 day | 10 days | 20 days | 30 days |
|---|---|---|---|---|
| Hematology | | | | |
| White blood cells (/$\mu$L) | 7560 ± 772 | 6531 ± 538 | 6522 ± 571 | 6447 ± 653 |
| Red blood cells ($\times 10^4/\mu$L) | 510.9 ± 8.8 | 512.7 ± 9.5 | 507.3 ± 8.4 | 505.3 ± 11.5 |
| Hemoglobin (g/dL) | 15.8 ± 0.2 | 15.6 ± 0.3 | 15.5 ± 0.3* | 15.5 ± 0.3 |
| Hematocrit (%) | 46.2 ± 0.8 | 45.8 ± 0.8 | 46.2 ± 0.8 | 46.3 ± 0.7 |
| MCV (fl) | 90.6 ± 1.6 | 89.5 ± 1.9 | 91.1 ± 1.6 | 91.8 ± 1.9 |
| MCH (pg) | 30.9 ± 0.6 | 30.5 ± 0.6* | 30.5 ± 0.5* | 30.7 ± 0.6 |
| MCHC (%) | 34.2 ± 0.2 | 34.1 ± 0.4 | 33.5 ± 0.3** | 33.5 ± 0.2 |
| Platelets ($\times 10^4/\mu$L) | 25.2 ± 1.6 | 25.2 ± 1.7 | 25.7 ± 1.6 | 26.0 ± 1.6 |
| Hemobiochemistry and endocrinology | | | | |
| Triacylglycerol (mg/dL) | 195.9 ± 54.8 | 167.6 ± 37.3 | 186.3 ± 65.8 | 220.9 ± 78.9 |
| Total cholesterol (mg/dL) | 203.2 ± 8.5 | 195.2 ± 9.4 | 202.7 ± 7.7 | 201.0 ± 10.3 |
| AST (IU/L) | 26.0 ± 2.3 | 30.0 ± 3.4 | 29.6 ± 3.7 | 27.5 ± 2.9 |
| ALT (IU/L) | 32.1 ± 5.0 | 34.9 ± 5.7 | 39.3 ± 7.6 | 38.2 ± 7.0 |
| Blood urea nitrogen (mg/dL) | 15.5 ± 1.4 | 14.9 ± 0.9 | 14.1 ± 0.8 | 14.4 ± 0.9 |
| Creatinine (mg/dL) | 1.05 ± 0.04 | 1.00 ± 0.04* | 1.02 ± 0.05 | 0.98 ± 0.05 |
| Glucose (mg/dL) | 89.1 ± 4.1 | 87.7 ± 1.8 | 94.2 ± 6.1 | 97.7 ± 8.8 |
| Insulin ($\mu$U/mL) | 12.8 ± 4.0 | 8.8 ± 2.3 | 12.9 ± 4.1 | 13.8 ± 3.6 |
| Na (mEq/L) | 143.1 ± 0.6 | 143.2 ± 0.5 | 142.8 ± 0.5 | 141.4 ± 0.5 |
| K (mEq/L) | 3.9 ± 0.1 | 4.2 ± 0.1* | 4.0 ± 0.1 | 4.2 ± 0.2* |
| Cl (mEq/L) | 101.0 ± 0.7 | 101.4 ± 1.1 | 101.7 ± 0.9 | 101.1 ± 0.8 |

Values are Means ± SEM.

Wilcoxon signed-rank test *$p<0.05$, **$p<0.01$.

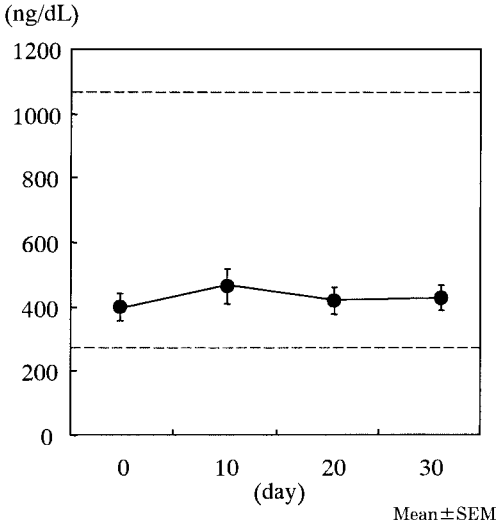


**Fig. 1** Effects of *Garcinia cambogia* on Serum Testosterone in Healthy Men (n=10). Broken line represents the reference interval of testosterone.

the symptom severities were slight.

## 4 Discussion

In our previous safety report, we found that Garcinia at 3,000 mg/day as HCA for 10 days exerted no influence on healthy volunteers (11). In this study, we examined the influence of Garcinia over a longer period (30 days) at the same dose. To our knowledge, this study is the first report on the influence of Garcinia on androgen in humans.

In terms of body weight, BMI, and other anthropometric indices, no significant change was observed in response to Garcinia. These results are similar to those in a previous report, which were considered to be due to an absence of overweight in these subjects. In clinical analysis, no changes definitively caused by Garcinia were observed.

In terms of subjective symptoms, anorexia, headache, and constipation were observed, but only to a slight degree; none of these symptoms was determined to be a clinical problem. No subject was removed from the

study protocol for treatment-related adverse events.

In our previous safety report (11), serum insulin was found to decrease significantly at 10 days, and same change tendency was observed after 10 days administration in this study too. But in this study, it was not statistically significant, and insulin level returned to an initial value at 20 and 30 days. From results of two studies, change of insulin was finally judged as transient drifting. Therefore, we have confirmed more safety on Garcinia use because of no influence to serum insulin.

NIHS reported pathological examination findings in testes including atrophy of seminiferous tubules, edema in seminiferous tubular interstitia, degeneration of germ cells, loss of germ cells, vacuolation of Sertoli cells, proliferation of Leydig cells, and calcification of seminiferous tubules (NIHS internal report). Thus, we investigated the influence of Garcinia on testosterone in order to evaluate its potential for reproductive toxicity in men. Testosterone, testicular main hormone, is synthesized from cholesterol in the Leydig cells. However, no significant change in testosterone was observed in comparison with the initial value; therefore, any influence of Garcinia on the Leydig cells might be unlikely. The difference of influence of Garcinia between NIHS study and our study may depend on administration dose. In this study, the administration dose was 3,000 mg/day as HCA, which was administered at 36.9 to 50.8 mg/kg/day to the enrolled subjects. NIHS reported that testicular toxicity dose was 1629.1 mg/kg/day as HCA. Consequently, the clinical dose used in this study was from 1/45 to 1/32. Therefore the dose of this study may be lower level for influencing of testosterone. However, the general dose used for anti-obesity is 1,000 mg/day as HCA; thus, a dose of 3,000 mg/day was sufficiently excessive.

There have been several reports of atrophy of the testes caused by general food items. Cocoa powder demonstrated an increasing incidence of bilateral testicular atrophy, and aspermatogenesis was present in Sprague-Dawley CD male rats receiving 5% as admixture at 78 weeks (12). Green tea catechin demonstrated a decrease in the mean absolute weight of the testes in F344 rats fed 5% green tea catechin powder at 8 weeks (13). However, no clinical report has confirmed these effects of catechin or cocoa powder.

The influence of Garcinia over a longer treatment period to healthy men and its effect on Sertoli cells remain to be elucidated. Our future work will include broader investigation of the safety of Garcinia, not only in terms of its effects on sex hormones but also in terms of general safety indices, in greater detail.

## 5 Conclusion

We examined the safety of Garcinia by high-dose administration study in healthy men. The treatment dose was 3,000 mg/day as HCA for 30 days, and we observed anthropometric indices, conducted clinical analysis (including testosterone), and monitored subjective symptoms. There were no toxicological changes in anthropometric indices and clinical analysis, and no serious subjective symptoms were observed. Therefore, we consider Garcinia administration to be generally safe under the conditions of this study.

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