Effect of Garcinia Cambogia as Weight Reducing Agents on the Morphology of Liver of Albino Mice in Pakistan

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Abstract

Background: Obesity is growing continuously, and people opt for pharmacological therapies, which might have negative side effects.

Objective: To study the effect of Garcinia Cambogia on body weight and safety profile regarding the morphology of the liver in albino mice.

Methodology: This animal experimental study was conducted on, Ninety male albino mice were used by diving into three groups containing thirty mice each. Group I, was Control group, group II and III were experimental groups, given drugs A (Slim Smart) and B (Ultra Slim Plus) respectively via oral gavage. Each group was further subdivided into subgroups "a", drug was given for 4 weeks, and "b", the drug was given for 8 weeks. Control group animals were given plain distilled water via oral gavage tube. Body weight and weight of the liver was measured and its gross features were observed.

Results: Mean body **w**eight of animals significantly increased in intervention groups, at 4 weeks, p=0.000 and 8 weeks p=0.000. Mean body weights of animals in experimental group II and III in 4 weeks categories were 33.92±3.23 grams in group II and 28.46±3.53 grams in group III. The mean relative tissue weight index at 4 weeks for group II was 6.66±0.67 and for group III was 5.71±0.41, p=0.004, while at 8 weeks were 5.88±0.64 and 5.91±0.69, p=0.713, respectively. When observed at 8 weeks time, the animals of the group I had the normal color of the liver while the two experimental groups had all the animals with the abnormally pale color of liver, p=0.001.

Conclusion: In this study, the herbal slimming agents, claiming to contain Garcinia Cambogia, did not reduce weight among albino mice, and are not safe for reducing weight.

Key words: Obesity, Pharmacological, Weight, Herbal, Garcinia Cambogia.

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Introduction

Obesity is growing continuously worldwide as a pandemic among all age groups.[1] Its prevalence has been on the increase for decades leading to deterioration in the quality of life for both adults and children.[1,2] Overall health consequences of obesity are serious and it is a prodromal factor for morbidity and mortality because of its association with various non-communicable chronic illnesses such as ischemic heart disease, osteoarthritis, type II diabetes mellitus, arterial hypertension, dyslipidemia, and cancer.[2,3,4] These diseases reduce the quality of life of the suffering human being, decrease his life expectancy, and represent a major socio-economic burden.[4,5] Obesity has also been found in a profound relationship with psychiatric conditions because of negative body image associated with increased weight and a lot of attention of society towards physical appearance.[6,7]

Overweight and obesity are important public health issues and burden which is estimated to increase steadily till 2030.[8] First-line treatment for the management of obesity is changing lifestyle, which involves inculcating healthy eating habits and exercise in daily routine.[8,9] Unfortunately, success rates are not high with this method because it relies on patients' behavior and will power.[10,11] People then resort to pharmacological therapies, which are sometimes expensive, inefficient and might have some negative side effects.[12] Surgical interventions are recommended only in cases of morbid obesity.[12,13] As a quick fix and to rapidly achieve weight loss goals, people turn towards readily available and relatively cheaper options, which can be harmful.[14] To fulfill this demand, an increasingly growing

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market of over the counter nutritional supplements seems a practical alternative, which is easily available to the general population.[15] Among these supplements, herbal products claiming to have natural ingredients in their composition, are being used generally as self-medication. There is an associated misconception among people that they uarantee safety because of their natural origin.[16,17] Among the multiple ingredients mentioned on various herbal slimming agents, Garcinia Cambogia is often claimed to be the most effective and important one.[18,19,20] Garcinia Cambogia and its fruit rind, are used extensively in culinary in South India, Northern Australia, and South Africa.[21,22,23] It is also used for medicinal purposes in various diseases such as rheumatic pain, hemorrhoids, ulcers, and bowel complaints.[24] Garcinia fruits have a rich source of hydroxy citric acid, which has gained tremendous attention in advertisements and commercial products as a promising agent for weight reduction.[25] It has been reported to reduce apatite, decrease lipogenesis, and fatty acid synthesis, thus decreasing body weight.[25,26,27] Due to its hype, many cheap, untested, over the counter medicinal products are available in the market, which claims to contain Garcinia Cambogia in them. They also rigorously advertise to have no side effects upon use and reduce weight like a magic potion over a period of a few weeks. Among the various weight loss and slimming products available in the local market of Pakistan, Slim Smart and Ultra Slim Plus are two extensively used formulations. They claim to reduce weight with no adverse side effects in spite of the fact that these herbal agents have never been tested in the laboratory. Their pharmaco-kinetics and pharmaco-dynamics are not known and they also mention other ingredients in their formulation whose drug interaction propensity with Garcinia Cambogia has not been analyzed. In this study, the aim was to provide evidence regarding the safety of the use of these products claiming to have Garcinia Cambogia. So this study was conducted to determine the effect of Garcinia Cambogia on body weight and safety profile regarding the morphology of liver in albino mice.

Methodology

This animal experimental study was conducted at the University of Veterinary and Animal Sciences, Lahore (UVAS), on Ninety male albino mice were used by diving into three groups containing thirty mice each. Group I, was Control group, group II and III were experimental groups, given drugs A (Slim Smart) and B (Ultra Slim Plus) respectively via oral gavage. Each group was further subdivided into subgroups "a", drug was given for 4 weeks, and "b", drug was given for 8 weeks. Control group animals were given plain distilled water via oral gavage tube. Body weight and weight of the liver was measured and its gross features were observed. In this study, we selected male albino mice. Two over the counter, commercially available weight-reducing drugs, claiming a high quantity of Garcinia Cambogia, were selected and this included, “Slim Smart” and “Ultra Slim Plus”. These agents were in tablet form. Each tablet weighing 500 mg was crushed and mixed in 250 ml distilled water to form a suspension. Then 1 ml suspension containing roughly 2 mg of the drug, was given to the experimental animals via oral gavage. As these rodents make a very good model for studying various pathologies in humans because its pathophysiology resembles them. Ninety adult male albino mice weighing 20 to 25 g, were selected by consecutive non-probability sampling technique. Animals were kept in the research and experimental laboratory of the University of Veterinary and Animal Sciences, Lahore (UVAS). Animals were weighed before and after the experiment. Mice were caged in standard aluminum cages of size 42 x 21 x 20 cubic cm. Each cage contained fifteen mice. Cages were placed six inches apart to allow cross ventilation. Experimental animals were allowed to acclimatize for one week before the start of the animal. Environmental conditions were maintained constant at 22± 2°C, humidity 45-60%, and twelve hours light to dark cycle. They were fed with standard rodent diet and water ad libitum. Total of ninety animals were equally divided into three groups of thirty mice each. Group I was control group, Group II and III were experimental groups given drug A (Slim Smart) and drug B (Ultra Slim Plus) respectively. Each group was further subdivided into sub groups “a” and “b”. Sub group “a” animals were given the drug for four weeks duration and sub group "b” animals were given drugs for eight weeks duration. All the experimental animals were given drugs via gavage once daily for six days a week. Control group animals were given plain distilled water via oral gavage tube for the

purpose of exposing them to the stress of drug administration via this route and maintaining uniformity of the procedure. At the end of the experiment, animals were weighed by a digital weighing machine. They were then euthanized by intraperitoneal injection of 45 mg per kg bodyweight of pentobarbital and 0.325 mg per kg bodyweight of morphine. Afterward mice were dissected and the liver was removed from the body. Weight of liver was measured and its gross features were observed and noted in a predesigned proforma. Ethical approval was sought from the Institutional Ethics Committee.

Data were analyzed by SPSS 20.0. Values were presented as mean ± SD for each group. Comparison among groups was carried out by One Way ANOVA, followed by Tukey´s post hoc test. The statistical significance level was taken as P-value ≤ 0.5.

Results

1. Initial and final weights of animals.

No statistical difference in initial weights was observed in all the three groups either in 4 weeks or 8 weeks categories. (Table I) The mean final weight for the control group I was 24.40±2.0 grams for 4 weeks and 24.47±2.10 for 8 weeks category. The final weights of animals in experimental groups II and III increased in 4 weeks categories and their means were recorded as 33.92±3.23 grams in group II and 28.46±3.53 grams in group III.

When final weights were compared group-wise in 4 weeks category, experimental group II had significantly higher weights as compared to the control group I (p=0.000) and experimental group III (p=0.000). Experimental group III also had significantly higher final weights as compared to the control group I with p-value 0.002. In 8 weeks category experimental group II had significantly higher weight as compared to control group I and experimental group III with p-values 0.000 and 0.000, respectively. The p-value for difference between the control group I and experimental group III was 0.010. It was observed that 100 gm food used to get finished by the evening in cages of experimental group animals. So 150 gm diet was added in their cages. Thus experimental group animals were consuming an additional 50 gm food.

Table-I: Weights of animals at the start of the experiment and at the end at 4 and 8 weeks time.

Time	Group	Initial weight(g)		Final weight		P-value
		Mean	Standard Deviation	Mean	Standard Deviation	
4 Weeks	Group I	24.39	1.93	24.40	2.00	0.000
	Group II	24.39	1.93	33.92	3.23	
	Group III	24.39	1.93	28.46	3.53	
8 Weeks	Group I	24.53	1.76	24.47	2.10	0.000
	Group II	24.53	1.76	33.16	4.89	
	Group III	24.53	1.76	28.42	3.00	

1. Weight of Liver and Relative Tissue Weight Index.

The liver weights though were different for three groups in both 4 weeks and 8 weeks categories but due to changes in body weight, the comparison was made for relative tissue weight index (RTWI) of the liver among three groups. The mean relative tissue weight index at 4 weeks for group II was 6.66±0.67 and for group III was 5.71±0.41, while at 8 weeks time these were 5.88±0.64 and 5.91±0.69 respectively. (Table II)

Table-II: Liver weights and their relative tissue weight index for animals at the end of the experiment at 4 and 8 weeks time.

Time	Group	Liver weight (gm)		Relative tissue weight index		P-value
		Mean	SD	Mean	SD	
4 Weeks	Group I	1.41	0.26	5.80	1.18	0.004
	Group II	2.26	0.27	6.66	0.67	
	Group III	1.62	0.18	5.71	0.41	
8 Weeks	Group I	1.49	0.32	6.14	1.31	0.713
	Group II	1.94	0.26	5.88	0.64	
	Group III	1.68	0.29	5.91	0.69	

The relative tissue weight index was found significantly different at 4 weeks with p-value 0.004 while insignificant at 8 weeks with p-value 0.713. Group-wise comparison revealed that group II had significantly higher RTWI as compared to group I and group III with p-values 0.017 and 0.007 respectively, while the difference between group I and III was insignificant with p-value 0.944. The difference among the group I and II was insignificant (p=0.732), the group I and III (p=0.785) and group II and III (p=0.995), at 8 weeks.

3. Colour of Liver

All the animals sacrificed at 4 weeks had the normal color of the liver for all the three groups. When observed at 8 weeks, the animals of the group I had the normal color of the liver while the two experimental groups had all the animals with the abnormally pale color of the liver. (Table III) The difference was found significant with p-value 0.001. When compared group-wise, group II and group III had a significant difference from the group I with p-values =0.001, while group II and III had no difference at all.

4. Texture of Liver

Based on the visual impression, the texture of the liver in control, as well as experimental groups, was normal. Therefore in statistical analysis, this parameter appeared as a constant.

Discussion

Garcinia Cambogia, extensively used as a flavoring agent, has currently gained popularity as a weight reducing supplement.[22] The mechanism of its anti-obesity activity is by decreasing food intake and fat deposition in the body by inducing satiety, increasing oxidation of fatty acids, and reducing de-novo lipogenesis.[26] A detailed study was carried out to see the response of the liver to the intake of weight-reducing agents, which contained Garcinia Cambogia. Animals remained healthy and active throughout the duration of the experiment.

The mean initial weights of the animals of subgroup Ia, IIa, and IIIa were the same as 24.39±1.93 grams. The mean initial weights of subgroup Ib, IIb and IIIb were also the same 24.53±1.76 grams. The final weights of both experimental groups increased instead of decreasing. The mean final weights of subgroup Ia, IIa, and IIIa were 24.40±2.0 grams, 33.92±3.23 grams, and 28.46±3.53 grams respectively. The mean final weights of subgroups Ib, IIb and IIIb were 24.47±2.10 grams, 33.16±4.89 grams, and 28.42±3.00. A study carried out by Semwal RB et al in April 2015 showed that Garcinia Cambogia exerts its anti-obesity effect by increasing satiety and fat oxidation by regulating serotonin levels and decreasing denovo-lipogenesis. They experimented with both in vivo and in vitro models.[22] In another study designed by Heo J et al in the year 2016, it was suggested that Garcinia Cambogia alleviates adiposity and weight gain in the high-fat diet-fed mice by altering the composition of the microbiota of the gut.[23] In the year 2016, Sripradha R, et al analyzed the effects of Garcinia Cambogia extract on male Wistar rats and concluded that it ameliorates hyperlipidemia induced by high-fat diet thus reducing fat deposition and obesity.[24] The results in our study are in contrast to the previous studies, which strongly advocate the weight-reducing effect of Garcinia Cambogia containing dietary supplements. Contrary to expectation, Garcinia Cambogia did not show an increase in satiety. The most probable reason behind this weight gain was the non-restricted food intake of mice.

It was observed that those mice which were given Garcinia Cambogia had increased their food intake as compared to the mice of the control group who were given plain water only. Garcinia Cambogia might have increased basal metabolic rate leading to hunger thus leading to increased food intake and weight gain. From this we may interpret that diet restriction and fixing food portions might be necessary to get desired weight reduction from the intake of Garcinia Cambogia containing weight reducing drugs. As far as the effects on the liver are concerned, the weight of the liver was different in all three groups i.e control group I and experimental groups II and III, but due to changes in the body weight, relative tissue weight index of the liver was calculated. It was found out that the mean relative tissue weight index of subgroups IIa and IIb were 6.66±0.67 and 5.88±0.64 respectively. For IIIa and IIIb they were 5.71±0.41 and 5.91±0.69 respectively. Difference between control and experimental groups as regards relative tissue weight index was significant at 4 weeks time having p-value 0.004 while insignificant at 8 weeks time.

The difference between group II and control group I, was highly significant and also between groups II and III but insignificant between groups I and III. In a study done by Shara M et al in the year 2004, varying concentrations of hydroxycitric acid; an active ingredient of Garcinia Cambogia given to male and female Sprague Dawley rats have shown no significant effect on liver weight to body weight percentage.[27] The results of our study are in contrast to the previous study which showed no significant change in liver weight to body weight percentage. In our study increase in relative tissue weight index has been observed, but one cannot be sure as to whether it can be linked to hydroxycitric acid or not since the concentration of Garcinia Cambogia and its extract hydroxycitric acid were not precisely described because the brochures available with drug bottles do not mention any exact quantification of ingredients per tablet. That's why we don't know the exact concentration of hydroxycitric acid at 2mg per day dose of the drug given to the animal for 4 and 8 weeks duration. Similarly, this effect on weight might be due to some ingredient in drug A or B other than Garcinia Cambogia.

The color of the liver was normal reddish-brown in both experimental groups after 4 weeks of duration of drug intake. Colour changed to abnormally pale in all the animals of both experimental groups after 8 weeks of drug intake. The difference was found significant with a p-value <0.001. Change in the color of the liver after 8 weeks of study in both experimental groups can be attributed to an alteration in the histological architecture of hepatocytes due to Garcinia Cambogia induced toxicity.

The texture of the liver remained normal and smooth both after 4 and 8 weeks time of drug intake. Garcinia Cambogia intake seems to not affect the texture of the liver up till 8 weeks of intake. In a study carried out by Young-Je Kim et al in August 2018 on obesity-prone C57BL/6J mice. It was proposed that Garcinia Cambogia intake for 16 weeks in high-fat diet fed mice induced fibrosis due to exacerbation of collagen accumulation in liver and subsequent inflammatory response induced by Garcinia Cambogia.[25] Thus it might take a longer time, greater than 8 weeks to bring about change in the texture of the liver by altering the histological architecture due to fibrosis by active collagenosis. Limitations of the study include, only male albino mice were included, and that we did not have evaluated the presence of Garcinia Cambogia in the brand names used for weight reduction, hence the results may show the effects of Garcinia Cambogia or the brands claiming to have this as content.

Conclusion

In conclusion, these herbal slimming agents claiming to contain Garcinia Cambogia did not reduce body weight among albino mice and are not safe for reducing weight.

Author Contribution

PM: Conception of work , design of work and revising. **GA:** Design of work, Acquisition and analysis of data and drafting. **FM & MM:** Interpretation of data and revising. **MKA:** Interpretation of data, revising. **KM:** Conception of work, acquisition and revising.

All authors critically revised and approve its final version.

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