Effect of Over the Counter Untested Weight Reducing Agents on the Histology of Liver of Albino Mice

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ABSTRACT

Background and Objective: *Obesity is a rapidly growing morbidity, putting lives of the affected individuals at risk and badly affecting the quality of their life. Garcinia Cambogia is claimed to be the most effective natural ingredient present in these slimming agents. This study was designed to investigate the effects of the Garcinia Cambogia on liver histology.*

Methods: *Ninety male albino mice were randomly divided into three groups containing thirty mice each. Group I served as Control group and group II and III were experimental groups administered with drugs A (Slim Smart) and B (Ultra Slim Plus) respectively via oral gavage, whereas control group was only given equivalent volume of distilled water. Animals were sacrificed and liver were inspected for congestion and lymphocytic infiltration on histological examination.*

Results: *Histological examination revealed that group II and III had significantly larger number of inflammatory cells/HPF as compared to group I (P-value = 0.001). Group II and III were having no significant differences when compared for inflammatory cells (P-value = 0.172).Features of congestion were present in 60 (100%) animals of group II and III when compared to group I (P-value = 0.001).*

Conclusion: *Garcinia Cambogia containing weight reducing drugs tested in this experiment adversely affected the histology of liver. Both Slim Smart and Ultra Slim Plus have hepatotoxic effects resulting in marked cellular infiltration and congestion.*

KEYWORDS: *Obesity, Pharmacological, Weight, Herbal, Garcinia Cambogia.*

INTRODUCTION

Global rise in the incidence of obesity is leading to rise in several health problems.[1,2] Obesity can cause dyslipidemia, hypertension, ischemic heart diseases, arthritis and cancer.[3,4] Obese patients develop insulin resistance leading to type II diabetes mellitus which is a prodromal factor increasing the risk of morbidity and mortality in obese population.[5-7] Fighting obesity is difficult as well as challenging because of the recently developing culture of zero physical activity, excessive intake of junk foods and overall rich economic status.[8,9] At the same time society demands up to the mark physical appearance and good health. Maintaining the optimum health with sedentary life style and excessive food intake is a difficult task. As a quick solution and short cut, people are turning towards the use of weight reducing drugs and supplements available in the market.[10-12] Since the market is deficient in providing authentic pharmacologically tested and approved drugs to overweight people, many untested non-pharmacological, dietary supplements claiming to contain medicinal plants are in widespread use as slimming agents.[13,14] Most of the slimming aids have Garcinia Cambogia in its ingredients, which is a tropical fruit whose extract contains hydroxy citric acid which is the main active ingredient inhibiting fatty acid synthesis and storage of glycogen.[15,16] It has been used in the past as a traditional medicine for the cure of ulcers, hemorrhoids, tumors, fever, dysentery and diarrhea.[17,18] In present era, globalization has led to excessive consumption of these medicinal products because of increase in the demand of these herbal commodities.[19-21] The problem arises with these untested supplements exerts their harmful effects on individuals who are unaware of the safety index of these supplements. It is because the these dietary supplements or slimming agents are being sold into the marker without clinical trial phase for the evaluation of their safety and efficacy.[22-24] General population remains at peace because of the inherent misconception regarding these products being composed of organic and natural ingredients thus totally safe for consumption.[25,26] These products are rampantly consumed as self-medication without physician's prescription by purchasing from local market, over the counter medicine or ordering online.[27-29]

This study was conducted to reveal the potential hazards of these slimming products when used by

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common people. In this study two most commonly available formulations of weight reducing agents containing Garcinia Cambogia were tested for their hepatotoxic effects

METHODS

This was an experimental study approved by ethical committee and institutional review board of King Edward Medical University Lahore in 2017 vide letter No. 205/RC/KEMU. Duration of the study was one year. The research was conducted on albino mice. Research animals were housed at the animal house of University of Veterinary and Animal Sciences Lahore (UVAS). All research animals were kept in ventilated aluminum cages having standard chow and com cob bedding with free access to food and water. Mice cages were placed in a room with 12 hours light/dark cycle at 25-30° temperature, 40-70% humidity and luminosity by natural lighting. Mice were kept in the above mentioned pre-designed climatic condition for one week before starting the experiment for acclimatization. All research mice were obtained from the breeding unit of department of theriogenology. All procedures carried out on animals followed the directions and guidelines of Institutional Animal Use and Care Committee of UVAS.

A total sample size of 90 adult male albino mice of eight weeks age was selected having 20-30 grams weight. Animals were distributed into three groups by consecutive non-probability sampling method. Group I was labeled as control group, group II and III were labeled as experimental groups. Group II animals were given drug A (Slim Smart) and group III animals were given drug B (Ultra Slim Plus). Each group containing thirty mice was further sub-divided into subgroups "a" and "b" having 15 mice each. In subgroup "a" animals were sacrificed at the end of 4th week and in subgroup "b" animals were sacrificed at the end of 8th week. Control group animals were given plain distilled water and experimental groups were given drugs in suspension form. Suspension was formulated by crushing a tablet of 500 mg and stirring its powder in 250ml distilled water thus obtaining a concentration of 2mg per ml distilled water. Animals were then given 1 ml suspension via oral gavage to maintain uniformity in the experimental process. The adequate dose chosen was 80 mg of drug per kg body weight.[3]

After the completion of experiment in each subgroup, mice were anesthetized by intraperitoneal injection of sodium pentobarbital 45 mg/kg body weight. Morphine at a dose of 0.3-0.5 mg/kg body weight was also given intraperitoneally as analgesic. Sacrifice of animals was followed by dissection for rapid fixation of liver for further observations. Weight of liver was measured and its gross anatomical features were observed to note any abnormal finding. Liver specimen was then immersed in 10% formalin for a period of 24 hours. After fixation, the samples were assessed and 0.5 cm pieces of organs were cut from three large lobes especially where tissue appeared abnormal or different. Liver tissues were processed according to the standard methodology and tissue blocks were prepared. Blocks were sectioned and mounted on glass slides and stained with Hematoxylin and eosin (H&E). Slides were observed under light microscope. Adobe photoshop version 7.0 was used for adjusting the captured images.

Cellular infiltration was measured as inflammatory cells present per high power field (HPF). Among the inflammatory cells, only lymphocytes were counted as they are a characteristic feature of drug induced hepatitis. Lymphocytes were identified as cells having oval to bean shaped uniformly darkly stained nucleus with barely visible thin rim cytoplasm. These specific cells were counted at high power field of 400X. Tissue was labeled congested if endothelial cells of portal venules or blood vessels were found edematous.

STATISTICAL ANALYSIS

The data was collected on a predesigned proforma and was analyzed by SPSS version 20. Data for cellular infiltration was described as mean ± SD for all the groups. Mean differences between the groups were com-pared by One Way ANOVA. Tukey's test was applied for post hoc analysis. Features of congestion were described as frequency and percentages in all the groups. Among the groups, comparison was made by Chi-square test. P value of ≤ 0.05 was considered as statistically significant.

RESULTS

Cellular Infiltration (No. of Inflammatory Cells/HPF)

There was no cellular infiltration by inflammatory cells/HPF in group Ia and Ib. The mean number of inflammatory cells/HPF recorded in group IIa was 29.87 ± 9.91 and in group IIIa was 39.20 ± 22.08 and this difference was statistically significant (p = 0.001). The mean number of inflammatory cells/HPF in group IIb and IIIb were 33.67 ± 4.81 and 34.33 ± 8.63 respectively and this difference was also statistically significant *(P = 0.001)* (Table 1 & 2).

The difference between group IIa and IIIa was in-significant with P-value 0.172. Similarly, group IIb and IIIb had insignificant differences for the mean number of inflammatory cells with P-value *0.945* (Table 1 & 2).

Features of Congestion

Features of congestion were present in all animals of group IIa, IIIa, IIb and IIIb (Fig. 1 & 2). The overall difference was significant as well as the difference of group I from group II and III with *P-value of 0.001*, Table-3 & 4.

Table-1: Comparison of number of inflammatory cells/HPF for animals in all the three groups (One way ANOVA).

Variables	*No of inflammatory cells/HPF Mean ± SD*	*P-value One way Anova*
Group Ia	0.00	0.001*
Group IIa	29.87 ± 9.91	
Group IIIa	39.20 ± 22.08	
Group Ib	0.00	0.001*
Group IIb	33.67 ± 4.81	
Group IIIb	34.33 ± 8.63	

*statistically significant

Table-2: Group wise comparison of number for inflammatory cells/HPF for animals in all groups (Post Hoc Tukey's Test).

(I) Group	*(J) Group*	*Mean Difference (I-J)*	*Std. Error*	*P-value*
Group Ia	Group IIa	-29.87*	5.10	0.001*
	Group IIIa	-39.20*	5.10	0.001*
Group IIa	Group IIIa	-9.33	5.10	0.172
Group Ib	Group IIb	-33.67*	2.08	0.001*
	Group IIIb	-34.33*	2.08	0.001*
Group IIb	Group IIIb	-0.67	2.08	0.945

*statistically significant

Table-3: Comparison of features of congestion among animals of all groups.

Group	*Features of Congestion*						*P-value*
	Present		*Absent*		*Total*		
	n	*%*	*n*	*%*	*n*	*%*	
Group Ia	0	0.0	15	100.0	15	100.0	0.001*
Group IIa	15	100.0	0	0.0	15	100.0	
Group IIIa	15	100.0	0	0.0	15	100.0	
Group Ib	0	0.0	15	100.0	15	100.0	0.001*
Group IIb	15	100.0	0	0.0	15	100.0	
Group IIIb	15	100.0	0	0.0	15	100.0	

Table-4: Group wise comparison of features of congestion among all the three groups.

(I) Group	*(J) Group*	*Chi-square*	*Df*	*P-value*
Group Ia	Group IIa	26.13	1	0.001
	Group IIIa	26.13	1	0.001
Group IIa	Group IIIa	Nd	Nd	---
Group Ib	Group IIb	26.13	1	0.001
	Group IIIb	26.13	1	0.001
Group IIb	Group IIIb	Nd	Nd	---

*Nd: not detected

DISCUSSION

This study was conducted to observe the response of liver to the intake of weight reducing agents having Garcinia Cambogia. The mean number of inflammatory cells/HPF representing cellular infiltration in experimental groups II and III were significantly higher as compared to control group *(P-value = 0.001)*. Cellular infiltration by lymphocytes is characteristic of drug induced hepatitis as published in a study carried out by Sintaychu Keb-ede et al in year 2016.[29] Keri E Luns-ford et al mentioned in their study on Garcinia Cambogia and its asso-ciation with hepatic failure in 2016 that cellular infiltration is a characteristic finding in the form of diffuse infiltration or focal aggregates which can be mild, moderate or severe depending upon the extent of liver damage.[19] Tanvir Haque et al in 2016 reported in their study that in the absence of any evidence of viral hepatitis, lymphocytic infiltrates represent drug induced hepatitis which in this study is associated with Garcinia Cambogia supplementation.[28] Thus, the results of current study correspond with the findings of previous studies.

Features of congestion were found in all the animals of experimental group II and III in contrast to control group I. The overall difference of group II and III from group I was significant with P-value of 0.001. In a study conducted by Young Je Kim in 2018 on high fat diet fed mice, it was proposed that congestion is re-presented by edematous endothelial cells of portal venules or blood vessels. This is a non-specific finding which can be associated with multiple causative factors.[25] In current study, the cause could be Garcinia Cambogia induced hepatitis and hepatocellular injury.

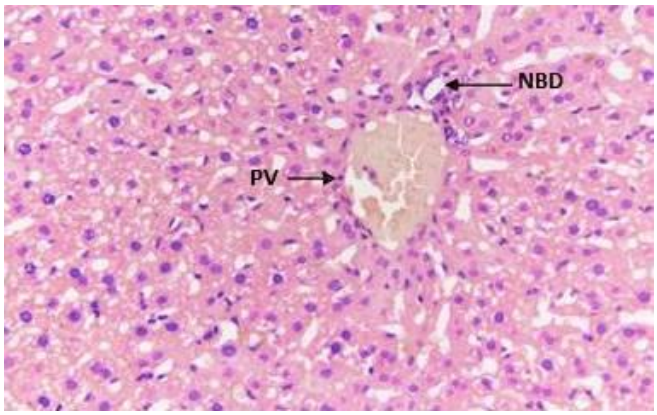

Fig:1. *Photomicrograph of cross section of liver of albino mice of control group Ia showing portal vein (PV), normal bile duct (NBD). (H&E, 400X).*

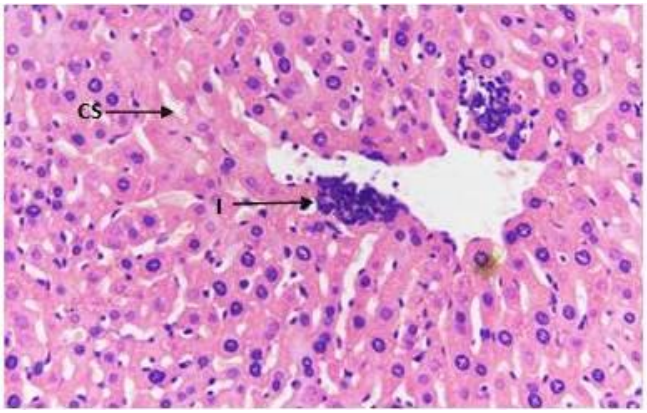

Fig:2. *Photomicrograph of cross section of liver of albino mice of experimental group IIa cellular infiltration by lymphocytes (I), congested sinusoids (CS). (H&E, 400X).*

CONCLUSION

Garcinia Cambogia containing weight reducing drugs tested in current experiment adversely affected the histology of the mice liver. Both Slim Smart and Ultra Slim Plus have hepatotoxic effects resulting in marked cellular infiltration and congestion.

LIMITATIONS OF STUDY

Small sample size and single center study were the limitations of the current study.

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AUTHOR'S CONTRIBUTION

PM, RTK, G, KA, FM and RR: Contributed substantially to the concept, designing, analysis and drafting of the research work. They revised it critically for proof reading and final approval for publishing of this article. They all agree to be accountable for ensuring the accuracy and integrity of work.

DISCLAIMER

None.

CONFLICT OF INTEREST

There is no conflict of interest of any of the authors.

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None to disclose.

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