ORIGINAL ARTICLE

Effect of Garcinia Cambogia Containing Commercially Available Weight Reducing Agents on Morphology of Hepatocytes – An Experimental Study

Ponum Mirani[1], Gulafshan[2], Agha Nadeem Ahmad[3], Jazib Andleeb[4]
Faiza Mehboob[5] and Naveed Akhter[6]

ABSTRACT

Background and Objective: Obesity is the adverse outcome of modern living which has affected both the physical and mental health. Slim Smart and Ultra Slim Plus are the most frequently purchased over the counter products by desperate obese persons in Pakistan having Garcinia cambogia (GC) as the main active ingredient. This study is conducted to determine the effect of GC containing Slim Smart and Ultra Slim Plus drugs on the morphology of hepatocytes in male albino mice.

Methods: Ninety albino mice were divided into control group A, experimental groups B and C receiving Slim Smart and Ultra Slim Smart respectively. Each group was further divided into subgroup I and II and the drug was administered to experimental groups for 4 and 8 weeks respectively via oral gavage. After the completion of experiment, histological examination of liver was conducted.

Results: Marked enlargement of hepatocytes was observed in experimental groups B and C (both I & II) along with ballooning degeneration and fatty change in the cytoplasm.

Conclusion: Both Slim Smart and Ultra Slim Plus has hepatotoxic effects resulting in increase in hepatocyte size, ballooning degeneration and fatty change in liver cells.

KEYWORDS: Fatty change, Garcinia cambogia, Hydroxycitric acid, Hepatocytes, Vacuolar degeneration, Weight reducing agents.

How to Cite This:

Mirani P, Gulafshan, Ahmad AN, Andleeb J, Mehboob F, Akhter N. Effect of Garcinia *cambogia* containing commercially available weight reducing agents on morphology of hepatocytes – An experimental study. *Biomedica. 2020; 36 (1): 38-42*

1. Ponum Mirani
   Multan Medical and Dental College, Multan
2. Gulafshan
   King Edward Medical University, Lahore
3. Agha Nadeem Ahmad
   Nishtar Medical University
4. Jazib Andleeb
   CMH Institute of Medical Sciences, Bahawalpur
5. Faiza Mehboob
   Multan Medical and Dental College, Multan
6. Naveed Akhter
   Rai Medical College. Sargodha

Correspondence to:
Dr. Ponum Mirani. Assistant Professor
Multan Medical and Dental College, Multan
E-mail:drkamranuhs@gmail.com

- Received for Publication: 19-11-19
- Revision Received: 06-02-2020
- Accepted for Publication: 16-03-2020

INTRODUCTION

Presently obesity is highly prevalent both in childhood and adolescence due to sedentary life style such as intake of processed food, long hours of sitting over social media and reduced physical activity.[1] Excessive fat deposition in body is associated with multiple morbidities such as diabetes, hypertension, atherosclerosis, metabolic syndrome, fatty liver disease, depression, anxiety and respiratory disorders.[2] All over the world, obesity related health issues are mounting and greatly affecting the national health budget.[3]

In the modern era, frequent avoidance of healthy food (with appropriate proportion of omega-3 and omega-6 fatty acids) that results in alteration of microbial composition, ultimately

disturbs the dynamic bacterial growth in gut leading to weight gain.[4] Adipokines and cytokines produced by unnecessary adipose tissue triggers metabolic imbalance which perpetuates diabetes, hypertension and even carcinomas such as prostatic, colon, breast, ovarian, endometrial and pancreatic cancer.[5,6] Prevention and management of obesity will not only combat the above-mentioned health issues but will also improve the mental and psychological well-being of individuals suffering from social disgrace.[7]

Due to lack of awareness and resources, people of developing countries are fascinated towards over the counter available herbal and allopathic drugs claiming rapid weight loss but lacking any authenticity or legal approval. In Pakistan, *Garcinia cambogia* (GC) containing, Slim Smart and Ultra Slim Plus (brand names) are being sold by the chemists on a larger scale. GC has long been used for the treatment of intestinal parasites, bowel deregulation and rheumatism, however, recently it has gained popularity owing to its weight reducing effect.[8] The active agent in GC organic extract is hydroxyl citric acid which has hypo-lipidemic, anti-diabetic, anti-inflammatory, anti-cancer, anti-helminthic and anti-cholinesterase activity both in vivo and in vitro models.[9] It has been investigated that hydroxycitric acid reduces the body fat by promoting lipolysis, proteinogenesis at hepatic level and ATP consumption by raising the metabolic rate.[10,11]

The current study is designed to determine the effects of Slim Smart and Ultra Slim Plus on morphology of hepatocytes on microscopic examination and micrometry.

METHODS

This research proposal was approved by the Institutional Review Board of King Edward Medical University Lahore Vide Letter No. 207/RC/KEMU and was conducted over a period of one year, from December 2017 to December 2018. The randomized control trial consisted of ninety adult male albino mice (Mus Musculus) weighing 30-40 gm, which were held captive in standard aluminum cages under appropriate environment at animal house of University of Veterinary and Animal Sciences, Lahore. The animals were fed commercially prepared feed and water ad libitum. By using consecutive, non-probability sampling technique, the animals were divided into three groups, A, B and C each consisting of thirty animals. Group A was control group given plain distilled water, Groups B and C were experimental groups given drugs Slim Smart and Ultra Slim plus respectively. All the groups were further divided into subgroups I and II. Animals of subgroup I was given drug for 4 weeks duration and those of subgroup II for 8 weeks duration, once daily.

Human equivalent dose for albino mice is 80mg per kg body weight. Each tablet weighing 500mg was crushed and mixed in 250 ml distilled water to obtain a concentration of 2mg per ml. So 2mg drug mixed in 1ml distilled water was given to mice according to its weight, once daily by oral gavage. After the intervention period, each mouse was euthanized and dissected to explore the abdominal cavity and the internal organs were revealed gently. The liver specimen was put in 10% formalin for preservation, fixation and later processed for the histological examination. Slides were assessed and observed via Nikon light microscope.

For measuring the size of hepatocytes, micrometry was performed on three slides from each animal's liver and twenty hepatocytes in which nucleus was located in the center, were randomly selected from each slide. The stage micrometer of 1mm length and 100 divisions (each measuring 10μm) was mounted on the stage of microscope. Under 4x magnification the ocular micrometer (placed in right eye piece of the microscope) was coincided with stage micrometer scale. Transverse, oblique and antero-posterior dimensions were measured and average of the three values was taken. Then, mean of the average values of all the sixty hepatocytes was calculated.

Fatty change on light microscopic examination is defined as pale colorless vacuole in the cytoplasm of hepatocytes i.e. nucleus displaced to one side and cytoplasm occupied by single large or multiple small vacuoles. Ballooning degeneration refers to appearance of enlarged hepatocytes with small central nucleus and wispy/cobweb-like cytoplasm having variously sized cytoplasmic vacuoles, outlined by a single membrane when viewed by light microscope.

STATISTICAL ANALYSIS

Statistical Package for Social Sciences (SPSS) version 24.0 was used to analyze the data. Mean and standard deviation were given for quantitative variables and group mean differences among the groups was made by applying One Way ANOVA test followed by post-hoc Tukey's test. Frequencies and percentages were given for qualitative variables. Chi-square test was applied to observe associations between qualitative variables. A p-value ≤ 0.05 was considered statistically significant.

RESULTS

The mean hepatocyte size of the experimental animals reveals a significantly higher value (P = 0.001) in the experimental groups as compared to the respective control groups (Fig. 1).

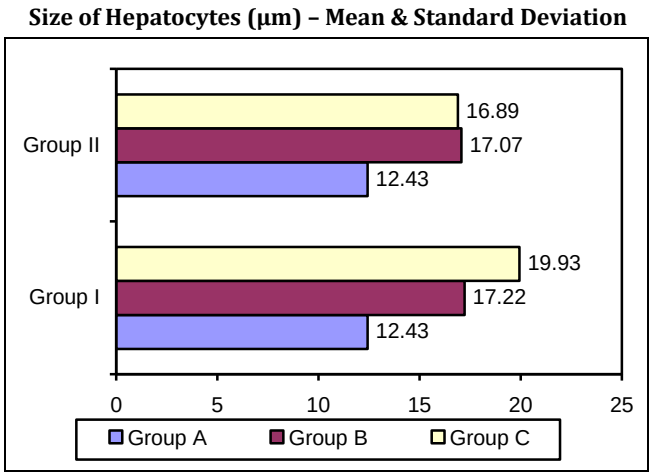

Fig.1: Bar chart showing size of hepatocytes on micrometry (Mean and Standard deviation).

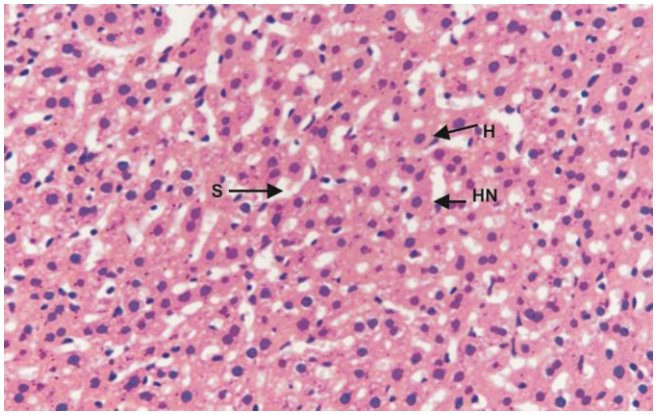

Fig.2: Photomicrograph of cross section of liver of albino mice of control group A-I showing hepatocytes (H), hepatocyte nucleus (HN), hepatic sinusoids (S). (H&E, 400X).

No fatty change was observed in groups A-I and B-I while in group C-I it was observed among 12 (80.0%) of the animals. Group A-II animals had no fatty change in hepatocytes while 10 (66.7%) animals of group B-II and 11 (73.3%) of group C-II had fatty change in hepatocytes (Fig. 2&3). The difference was statistically significant when compared among the groups *(P-value < 0.001)*.

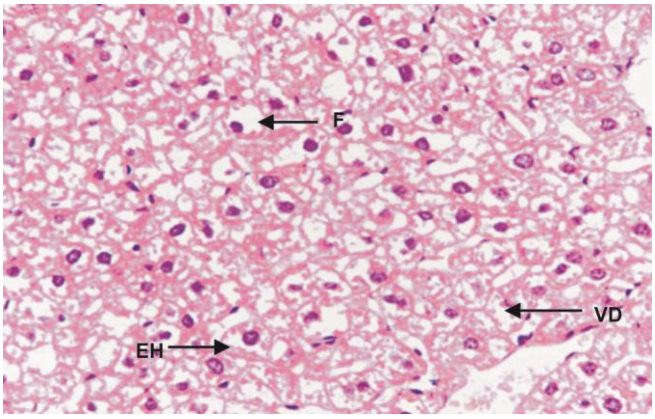

Fig.3: Photomicrograph of cross section of liver of albino mice of experimental group C-II showing enlarged hepatocytes (EH), vacuolar degeneration (VD), fatty change (F). (H&E, 400X).

There was no vacuolar degeneration observed in control groups A-I and A-II animals while all the animals (100%) of groups B-I, B-II, C-I and C-II showed vacuolar degeneration of hepatocytes (Fig. 2&3). The difference was statistically significant among the groups having p-value < 0.001.

DISCUSSION

In the present study, there was a significant and simultaneous increase in the size of hepatocytes and vacuolar degeneration among the Slim Smart and Ultra Slim Smart treated groups, which are indicators of cellular injury.[12] The major active ingredient present in these two drugs is Garcinia *cambogia*. Fazelipour[13] described hepatocyte size increase and cytoplasmic vacuolization as indicators of degeneration of hepatocytes in an animal study conducted to investigate the drug induced liver damage. These findings are also in agreement with the work of Crescioli et al. who investigated the case series and literature review regarding GC containing herbal products induced liver injury and reported the release of

inflammatory markers (Tumor necrosis factor-α and monocyte chemoattractant protein-1) leading to impaired liver functions (evidenced by raised serum ALT and AST).[2] They elaborated that underlying mechanism of GC induced hepatocellular injury is the excessive production of reaction oxygen species due to lipid peroxidation and raised mRNA levels of oxidative stress related genes.[2] The finding of current study are contrary to those of Pittler and his colleagues, who thoroughly investigated the herb, induced liver injury and concluded that the adverse effects of most of the weight reducing herbs exceed their benefits hence their usage must be discouraged with the exception of few including GC.[14]

After four weeks duration, all the animals of three groups didn't show any noticeable fatty changes, however, after eight weeks of drug exposure, significant fatty changes were observed. Fatty changes in liver are categorized as non-alcoholic fatty liver diseases present as steatosis (with or without hepatocellular injury) or even inflammation and fibrosis.[15] In contrast to the findings of present study, Shara et al. reported that hydroxycitric acid doesn't promote oxidative stress and has no adverse effect over any vital body organ.[11]Lunsford[16] reported a case study regarding fulminant hepatic failure in 34 years old male patient receiving 80mg capsule of GC, thrice daily for the last five months. Liver biopsy revealed ballooning of hepatocytes representing vacuolar degeneration along with apoptotic bodies. In this study, the histopathological findings like enlarged hepatocytes, vacuolar degeneration and fatty change, are suggestive of "Non-alcoholic Fatty Liver" according to the disease patterns designed by Haque and colleagues.[12]

The results of the study under discussion are in agreement with those of Kim et al. who demonstrated GC induced hepatic injury through histological findings suggestive of collagen deposition and inflammation. They investigated the weight reducing role of GC in high fat diet induced obese mice at cellular and genetic levels and the outcome was effective weight reduction along with hepatocellular injury owing to high oxidative stress.[17]

CONCLUSION

Garcinia *cambogia* containing weight reducing drugs tested in this study caused hepatic injury by disrupting the morphology of liver in experimental animals. The active compound in these drugs is thus potentially hepatotoxic, irrespective of duration of use.

LIMITATIONS OF STUDY

The impact of current study could be enhanced by investigating markers of cell death and oxidative stress at subcellular level as in our study only the morphological changes at cellular level have been discussed.

ACKNOWLEDGMENT

This work is attributed to all the supporting staff related to Anatomy Department, King Edward Medical University, Lahore.

CONFLICT OF INTEREST

None to declare.

FINANCIAL DISCLOSURE

None to disclose.

REFERENCES

1. Attia RT, Abdel-Mottaleb Y, Abdallah DM, Ei-Abhar HS, El-Maraghy. Raspberry ketone and Garcinia *cambogia* rebalanced disrupted insulin resistance and leptin signaling in rats fed high fat fructose diet. Biomed Pharmacother. 2019; 110: 500-9.
2. Crescioli G, Lombardi N, Bettiol A, Marconi E, Risalitit F, Bertoni M, et al. Acute liver injury following Garcinia *cambogia* weight-loss supplementation: case series and literature review. Intern Emerg Med. 2018; 13 (6): 857-72.
3. Antwi F, Fazylova N, Garcon MC, Lopez L, Rubiano R. The effectiveness of web-based programs on the reduction of childhood obesity in school-aged children: A systemic review. JBI Libr Syst Rev. 2012; 10 (42 Suppl.): 1-14.
4. Stenman LK, Burcelin R, Lahtinen S. Establishing a causal link between gut microbes, body weight gain and glucose metabolism in humans-towards treatment with probiotics. Benef Microbes. 2016; 7 (1): 11-22.

5. Ejarque M, Ceperuelo-Mallafre V, Serena C, Pachon G, Nunez-Alvarez Y, Terron-Puig M, et al. Survivin, a key player in cancer progression, increases in obesity and protects adipose tissue stem cells from apoptosis. Cell Death Dis. 2017; 8 (5): 2802.
6. Booth A, Magnuson A, Fouts J, Foster M. Adipose tissue, obesity and adipokines: role in cancer promotion. Horm Mol Biol Clin Investig. 2015; 21 (1): 57-74.
7. Williams LT, Germov J. The social determinants of obesity. In Germov J, editor, Second opinion: An introduction to health sociology. 6th ed. South Melbourne, Victoria: Oxford University Press, USA. 2019: p. 252-279.
8. Semwal RB, Semwal DK, Vermaak I, Viljoen A. A comprehensive scientific overview of Garcinia *cambogia*. Fitoterapia. 2015; 102: 134-48.
9. Fassina P, Adami FS, Zani VT, Machado IC, Garavaglia J, Grave MT, et al. The effect of Garcinia *cambogia* as coadjuvant in the weight loss process. Nutr Hosp. 2015; 32 (6): 2400-8.
10. Han N, Li L, Peng M, Ma H. (-)-Hydroxycitric acid nourishes protein synthesis via altering metabolic directions of amino acids in male rats. Phytother Res. 2016; 30 (8): 1316-29.
11. Shara M, Ohia SE, Schmidt RE, Yasmin T, Zardetto-Smith A, Kincaid A, et al. Physico-chemical properties of a novel (-)-hydroxycitric acid extract and its effect on body weight, selected organ weights, hepatic lipid peroxidation and DNA fragmentation, hematology and clinical chemistry, and histopathological changes over a period of 90 days. Mol Cell Biochem. 2004; 260 (1-2): 171-86.
12. Haque T, Sasaatomi E, Hayashi PH. Drug-induced liver injury: pattern recognition and future directions. Gut and Liver, 2016; 10: 27-36.
13. Fazelipour S, Kiaei SB, Tootian Z, Dashtnavard H. Histomorphometric study of hepatocytes of mice after using heroin. Int J Pharmacol. 2008; 4 (6): 496-9.
14. Pittler MH, Schmidt K, Ernst E. Adverse events of herbal food supplements for body weight reduction: Systemic review. Obes Rev. 2005; 6 (2): 93-111.
15. Brown GT, Kleiner DE. Histopathology of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis. Metabolism, 2016; 65 (8): 1080-6.
16. Lunsford KE, Bodzin AS, Reino DC, Wang HL, Busuttil RW. Dangerous dietary supplements: Garcinia *cambogia* associated hepatic failure requiring transplantation. World J Gastroenterol. 2016; 22 (45): 10071-6.
17. Kim YJ, Choi MS, Park YB, Kim SR, Lee MK, Jung UJ, et al. Garcinia *cambogia* attenuates diet-induced adiposity but exacerbates hepatic collagen accumulation and inflammation. World J Gastroenterol. 2013; 19 (29): 4689-701.

Author's Contribution

PM: Conception and design of study, Acquisition data.
GUL: Acquisition of data.
ANA: Analysis of data.
JA: Drafting of manuscript.
FM: Critical review and intellectual input.
NA: Data analysis and interpretation.