Comparative Study of the Effects of Orlistate and Green Coffee Bean Extract on Tongue Mucosa in Obese Rat Model

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Abstract: Background: Obesity is a condition of abnormal accumulation of body fat which is directly associated with increased risk of many chronic diseases. Orlistatis a lipase inhibitor commonly used as anti-obesity agent. Green coffee bean extract (GCBE) is one of the newest weight loss supplements. **Aim of study:** To evaluate and compare the effects of orlistat and GCBE on tongue mucosa in rats fed high fat diet (HFD). **Materials and Methods:** Twenty-four, adult male rats weighting 150 ± 7 g were allocated into four equal groups; control, HFD (20 g fat/100 g diet), HFD- orlistat treated (200 mg /kg diet) and HFD- GCBE treated (400 mg /kg bw) groups. After four weeks, the animals were weighted then euthanized and the tongue samples were processed for histological and ultrastructural study. ANOVA test was used to compare initial and final body weights between groups. **Results:** The tongue mucosa of HFD and HFD- orlistat treated groups showed atypical architecture in comparison with control however HFD- GCBE treated group revealed almost normal tongue mucosa. Statistical analysis of HFD- orlistat treated and HFD- GCBE treated groups showed non-significant difference between their initial and final body weights and their final body weights simulated each other although there were a significant decrease in their final body weightsbelow those of control and HFD groups. **Conclusion**: Obesity caused deleterious effects on rats' tongue mucosa. However, both orlistat and GCBE exerted potent weight loss effects. Unlike orlistat, GCBE had great ability to restore normal architecture of tongue mucosa.

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Key words: Obesity, Orlistat, Green coffee bean extract, Tongue mucosa
Abbreviations: GCBE: Green coffee bean extract; HFD: high fat diet.

1. Introduction

Obesity is a global problem associated with a number of chronic disorders including osteoarthritis, obstructive sleep apnea, gallstones, fatty liver disease, dyslipidemia, reproductive and gastrointestinal cancers, hypertension, coronary artery disease, heart failure and stroke [1,2]. Therefore, many medications have been approved to manage obesity and a significant increase in the prescriptions for those anti-obesity drugs is noticed over the years [3].

Orlistat (tetrahydrolipstatin) is the first agent of novel non- centrally acting anti-obesity agent which represents a hydrogenated derivative of lipstatin, produced by Streptomyces Toxytricini [4]. Orlistatacts locally in the gastrointestinal tract by inhibiting the absorption of dietary fats in the lumen of the stomach and small intestine [5]. It exerts its therapeutic activity by forming a covalent bond with the active serine residue site of gastric and pancreatic lipases. Thus, the inactivated enzymes are unavailable to hydrolyze dietary fat in the form of triglycerides into absorbable free fatty acids and monoglycerides. As undigested dietary triglycerides are not absorbed, the resulting caloric shortage has a positive influence on weight regulation [6,7]. Beside its anti- obesity effect, orlistat appears to have anti-diabetic and anti-atherogenic properties and may help prevent metabolic syndrome in the overweight people [8].

Green coffee is raw coffee beans that have not been roasted. Evidence is accumulating from numerous researches concerning the use of green coffee beans extract (GCBE) as one of the newest weight loss supplements [9-11]. GCBE enhances energy metabolism and reduces lipogenesis by down regulating SREBP-1c (Sterol regulatory element-binding protein) and related molecules, which leads to the suppression of body abdominal and liver fat accumulation by inhibition of macrophages infiltration into adipose tissues [12]. Moreover, GCBE possess effective inhibitory effect against pancreatic lipase [13]. In conjunction with its anti- obesity effect, researches demonstrated that GCBE ameliorates disorders such as atherosclerosis, type 2 diabetes and insulin-resistance [14-16] and regulates hypertension, vasoreactivity and glucose metabolism [17-19]. Most of biological effects and weight losing properties of GCBE has proposed to be related to its plentiful cholorogenic acid content, a natural phenolic compound formed by the esterification of cinnamic acids and exerts series of health benefits [20-23].

The oral cavity is the target organ for a number of various abnormalities that develop from side effects

of medications including anti-obesity drugs [24]. Thus, the aim of this study was to evaluate and compare the effect of two imperative anti-obesity supplements (orlistat and green coffee bean extract) on the tongue mucosa in obesity- induced rat model.

2. Materials and methods

Animals

Twenty-four, adult male Sprague–Dawley rats weighting 150 ± 7 g each were housed at Faculty of Medicine, Tanta University in individual cages and received a standard diet for rodents and tap water ad libitum. Room temperature and humidity were maintained at 23 C and 60%, respectively. The light cycle was fixed at 12 h. All animal experiments were carried out in accordance with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals (NIH Publication, Number 85-23, Revised 1985). After one week acclimatization period, the rats randomly divided into four equal groups (each compromised of six rats); **Group I** (control group): the rats of this group were received standard diet for rodents and kept without any treatment throughout the whole period of the experiment. **Group II** (HFD group): this group acted as control positive wherein the rats of this group were fed on high fat diet (HFD) contained 20 g fat/100 g diet by weight (fat contained19 g of butter oil and1g of soybean oil to provide essential fatty acids) for four weeks to induce obesity in rats [25] and kept without any treatment throughout the whole period of the experiment. **Group III** (HFD- orlistat treated): rats of this group were kept on HFD supplemented with orlistat (Sigma Pharmaceutical Industries, Egypt) at a dose of 200 mg/kg diet for four weeks [26]. **Group IV** (HFD- GCBE treated): rats of this group were kept on HFD and were given GCBE (Puritan's Pride, Egypt) orally by a gastric tube at a daily dose of 400 mg/kg of body weight for four weeks [27].

Body weight measurement

Individual body weight was recorded at day 0 (initial body weight) and weekly for all rats. At the end of the experimental period (day 30), treatment and food were stopped for 12 hours then animals were weighed (final body weight) before sacrifice.

Animal euthanasia and samples collections

After measuring the final body weight, rats were anesthetized with ketamine at a dose of 50 mg/kg body weight and the whole tongue was removed from all rats and dissected from the midline into two halves. The right halves were prepared for light microscopic (LM) examination while the left ones were prepared for scanning electron microscopic (SEM) examination. Subsequently, the animals were scarified with overdose of anesthetic, according to the ethical guidelines, confirmed with cervical dislocation.

Light microscope (LM):

Immediately after collection of right halves of tongues, specimens were fixed with 4% buffered formalin solution then dehydrated by a graded ethanol series and embedded in paraffin. Serial sections (5µm) were cut and stained with hematoxylin and eosin (H & E). Subsequently, the histological sections were examined by LM (Leica ICC50 HD) at Faculty of Medicine, Tanta University.

Scanning electron microscope (SEM):

The left halves of the tongues were instantly fixed (2.5% glutaraldehyde for 72 hrs and post-fixation in 1% OsO4 for 24 hrs) then dehydrated in graded ethanol, dried, mounted on stubs and coated with gold using a sputter coater that converted electrically non-conductive samples into conductive ones hence enabled a tightly focused electron beam to be scanned across the sample surface by SEM (JEOL JSM-636 OLA at an accelerating voltage of 15kv) at Faculty of Medicine, Tanta University.

Statistical analysis

All measurements data of initial and final body weights for each group were presented as mean ± standard deviation and statistical analyses were done using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test to reveal statistical significance of difference among groups. Values of $P < 0.05$ indicated a statistically significant difference. All statistics were performed with SPSS (statistical package for social sciences) version 11.0 (SPSS Inc, Chicago, IL, USA).

3. Results

Body weight measurement

Statistical analysis of initial and final body weights demonstrated significant increase in final body weights of control and HFD groups in comparison with their initial body weights. However, non-significant difference was found between the initial and final body weights of both HFD- orlistat treated and HFD- GCBE treated groups (Table 1). Also, there was a significant increase in the final body weights of HFD group in comparison with those of control, HFD- orlistat treated and HFD- GCBE treated groups. On the other hand, non- significant difference was found between the final body weights of HFD- orlistat treated and HFD- GCBE treated groups although both groups exhibited significant decrease in their final body weightsbelow that of control group (Table 2).

Light Microscopic findings:

The dorsal tongue surface of the control group showed normal long finger like projections of filiform papillae consisted of a lamina propria core covered by a keratinized stratified squamous epithelium and had normally shaped epithelial rides (Figure 1, A). In HFD

group, the filiform papillae of almost normal pattern however some filliform papillae loss their characteristic conical appearance in some areas (Figure 1, B). On the other hand, HFD- orlistat treated group exhibited ill- defined filiform papillae with few, ill-defined and shallow epithelial ridges. Moreover, loss of classical epithelial cells stratification and defective keratinization were noticeable in some areas (Figure 1, C and C^-). Interestingly, in HFD- GCBE treated group, the filiform papillae exhibited almost normal architecture with almost restoration of normal epithelial stratification and keratinization and regular pattern of epithelial ridges comparable to that of control group (Figure 1, D and D^-).

Table 1: Diagram showing comparison between initial and final body weights among different group

Body weights		Range	Mean ± S. D	t. test	p. value
Group I	Initial	150 – 157	154 ± 2.65	19.674	0.001*
	Final	212 – 231	220.6 ± 7.09		
Group II	Initial	151 – 157	154 ± 2.55	31.360	0.001*
	Final	269 – 289	281 ± 8.69		
Group III	Initial	150 – 157	153.6 ± 3.05	1.385	0.204 NS
	Final	151 – 164	157.8 ± 6.06		
Group IV	Initial	150 – 157	154.4 ± 3.21	1.341	0.217 NS
	Final	153 – 160	157 ± 2.92		

P value< 0.05 *: significant NS: not significant

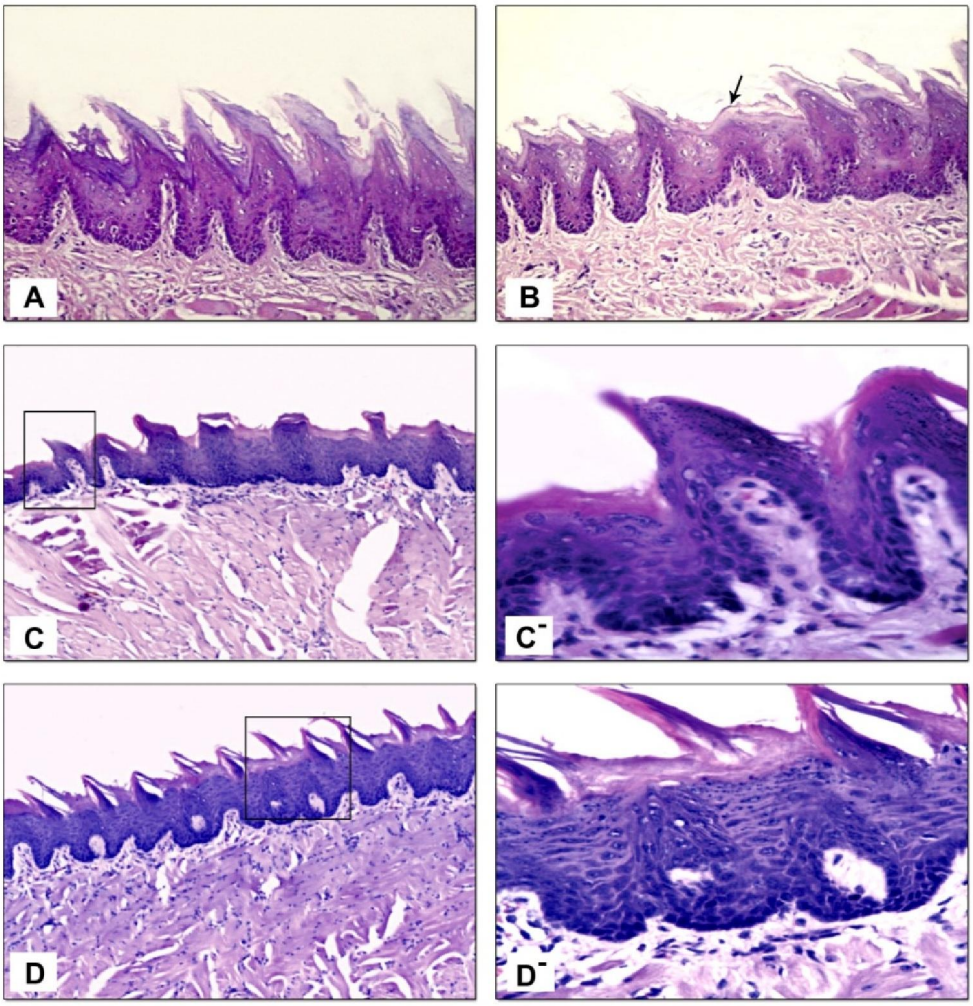

Figure 1: Photomicrographs of the tongue dorsal mucosa of different groups: (A) Control group shows normal architecture of filiform papillae with numerous, sharp and long finger- like projections (H & E X 200). (B) HFD group shows filiform papillae of almost normal pattern however ill-defined conical projections of filliform papillae could be seen in some areas (arrow) (H & E X 200). (C) HFD- orlistat treated group shows ill- defined filiform papillae with few and shallow epithelial ridges (H & E X 100). (C^-) Higher magnification of boxed area in (C) shows loss of normal epithelial cells pattern and keratinization (H & E X 400). (D) HFD- GCBE treated group shows regular filiform papillae with almost normal architecture (H & E X 100). (D^-) Higher magnification of boxed area in (D) showing almost normal epithelial stratification and keratinization (H & E X 400).

Table 2: Diagram showing comparison of the final body weights between different groups

Final body weights	Group I	Group II		Group III	Group IV
Range	212 – 231	269 – 289		151 – 164	153 – 160
Mean ± SD	220.6 ± 7.09	281 ± 8.69		157.8 ± 6.06	157 ± 2.92
F test	58.974				
P value	0.001*				
G I & G II	G I & G III	G I & G IV	G II & G III	G II & G IV	G III & G IV
0.001*	0.001*	0.001*	0.001*	0.001*	0.849 ^{NS}

P value< 0.05 *: significant NS: not significant

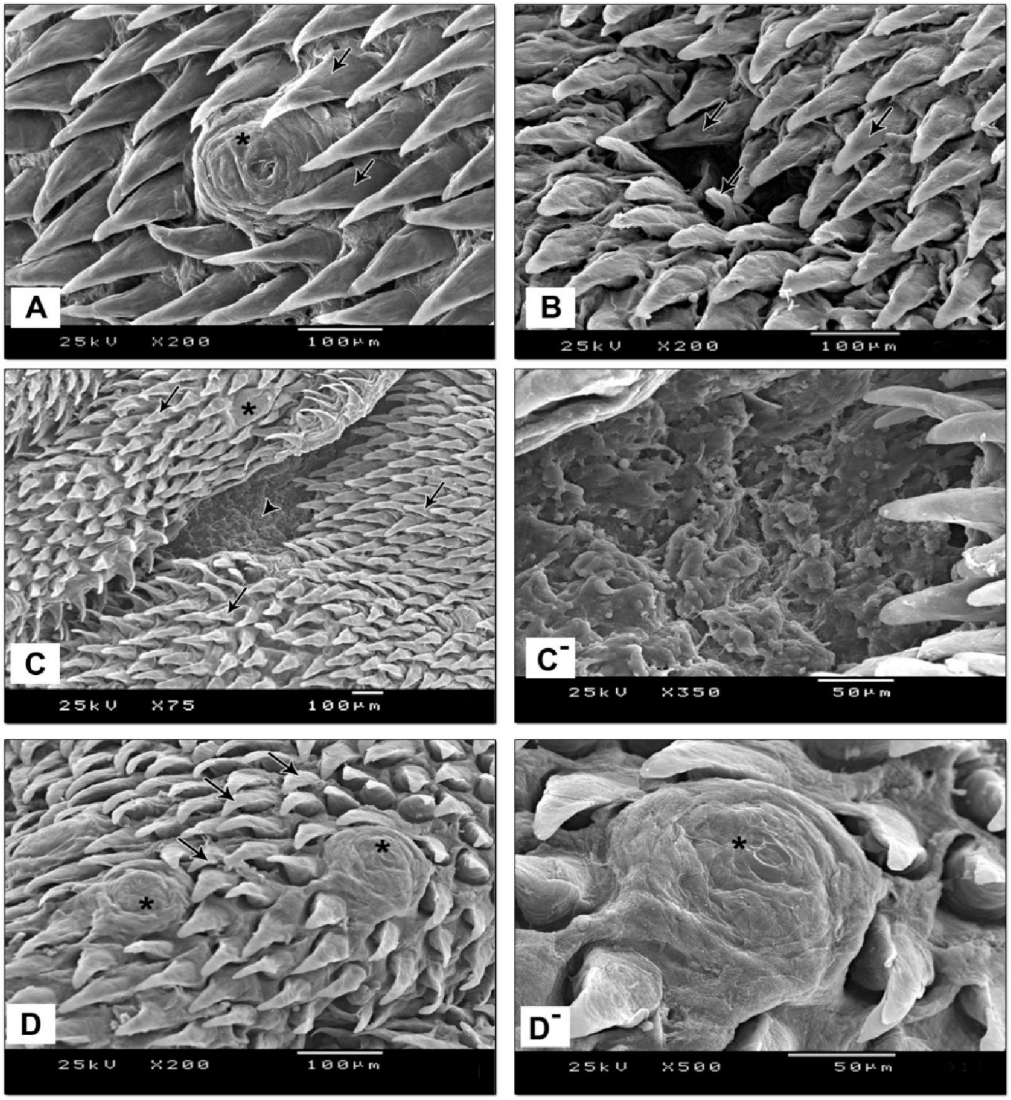

Figure 2: Scanning Electron micrographs of the tongue dorsal surfaces of different groups: (A) Control group shows typical conical shape filliform papillae with regular arrangement (arrows). Classical appearance of fungiform papilla is noticeable (*) (Mic. Mag. X 200). (B) Tongue mucosa of HFD group displays irregular arrangement of filliform papillae (arrows) and no apparent fungiform pappilae (Mic. Mag. X 200). (C) HFD- orlistat treated group shows irregularly arranged filliform papillae (arrows) with noticeable area devoid of papillae (arrow head). Fungiform papilla is visible (*) (Mic. Mag. X 75). (C^-) Higher magnification of (C) shows obvious area lacking tongue papillae (Mic. Mag. X 350). (D) HFD- GCBE treated group shows almost regular shape and arrangement of filiform (arrows) and fungiform (*) papillae (Mic. Mag. X 200). (D^-) Higher magnification of (D) shows almost typical fungiform papilla (*) (Mic. Mag. X 500)

Scanning electron microscopic findings:

Topographical examination of tongue dorsal surface of control group revealed regular arrangement of typical conical shape filliform papillae. Moreover, fungiform papillae with their classical appearance as dome shape eminences on the tongue mucosa were noticeable (Figure 2, A). In HFD group, unlike control group, the filiform papillae exhibited irregular arrangement and appeared to have wrinkled surface. Additionally, almost no detectable fungiform papillae on the tongue dorsal surface (Figure 2, B). HFD-orlistat treated group revealed irregular arrangement of conical shaped filliform papillae in almost all the surface besides scattered fungiform papillae that could be seen on the tongue mucosal surface of this group. However, noticeable area of tongue dorsal surface exhibited loss of tongue papillae (Figure 2, C and C^-). HFD- GCBE treated group appeared similar to control group and exhibited restoration of almost regular shape and arrangement of filiform papillae and regular distribution of almost normal fungiform papillae (Figure 2, D and D^-).

4. Discussion

The present study attempted to evaluate and compare the possible effects of two imperative and commonly used anti-obesity treatments, namely; orlistat and GCBE, on the tongue mucosa in HFD-induced obesity rat model.

In the current study, both HFD- orlistat treated and HFD- GCBE treated groups exhibited non-significant changes in their final body weights compared with their initial ones. Besides, non-significant difference was found between the final body weights of both groups in comparison with each other. These findings were coincided with several researches that documented the efficiency of orlistat and GCBE in weight loss and attenuation of diet-induced obesity [6-13,28].

LMfindings of the present study revealed some changes in the characteristic appearance of some filliform papillae in HFD group. The negative impact of obesity on the oral cavity was conducted by several researches which concluded that obesity was inversely associated with increased risk of periodontal diseases and dental caries and associated with impaired immune function which in turn lead to increased susceptibility to infection [29-32]. Moreover, HFD could induce structural changes in different body organs. Hengar*et al.*2001 found that HFD caused histological changes in the rat`s kidney that could be the precursors of severe glomerular injury [33]. Likewise, Altunkaynak,2005 examined the liver in HFD-induced obesity rats and found severe changes in the histological structure of liver which considered as an indicator of liver damage [34]. To explain the destructive effects of obesity on body organs, Huang *et al.*2015 illustrated that oxidative stress is the main contributing factor in obesity-related diseases. This obesity-related oxidative stress can lead to sever cellular damage and dysfunction due to imbalance between pro-oxidants such as reactive oxygen specie (ROS) and reactive nitrogen species (RNS) antioxidants [35]. Moreover in the current LM results, HFD- orlistat treated rats revealed adversely affected histological structure of their tongue mucosa including ill- defined filiform papillae with loss of usual epithelial cells stratification and keratinization and presence of few and shallow epithelial ridges. These unpleasant effects of orlistat on tongue mucosa are compatible with the findings of Nairooz*et al.* 2010 who found that orlistat exerted bad effects on the structure of rat colonic mucosa [36]. Similarly, it was found that orlistate resulted in disturbance of normal architecture of the pancreatic acini and caused acute pancreatitis with elevated pancreatic enzymes [37, 38]. Also, Kaila *et al.* 2008 depicted that although researches have explored its effectiveness compared to other anti- obesity drugs, orlistat causes unpleasant adverse effects including gastrointestinal problems, fat-soluble vitamin deficiencies (vitamins A, D, E, and K) and serious liver injury which limit patient compliance for orlistat [39]. Interestingly in the present LM results, HFD- GCBE treated group revealed filliformpapillae of almost normal architecture similar to that of control group. This constructive effect of GCBE and its ability to alleviate the destructive effect of obesity on the tongue mucosa could be related to its antioxidant function that reduce oxidative stress since GCBE contains cholorogenic acid which is the main polyphenol in GCBE and is known to prevent health problems associated with oxidative stress including obesity [40].

Furthermore in this study, topographical examination of the dorsal surface of the tongue using SEM revealed results compatible with those of LM. HFD group showed irregular architecture of the filiform papillae and few numbers of fungiform papillae on the tongue dorsal surface comparable with that of control group. This matched the findings of Proserpio*et al.* 2016 who declared that obese individuals showing a significantly decreased number of fungiform papillae on their tongues and had lower taste sensitivity for all tastes (sweet, salty, sour and bitter) and for the fat sensation than normal weight individuals. This decreased fat sensitivity might be a contributing factor in the pathogenesis of obesity since this could lead to the consumption of excess dietary energy and weight gain [41]. The taste buds in the fungiform papillae contain fatty acid receptors and

mechanoreceptors thus a lower amount of fungiform papillae may decrease the perception of fat via declined tactile and chemosensory perception [42,43]. Moreover in the current SEM results, HFD- orlistat treated group revealed irregular arrangement of filliform papillae and some scattered fungiform papillae however an area deficient of tongue papillae was present on the tongue surface. These observations coordinated with that depicted by Taha*et al.* 2012 who verified the appearance of oral ulcers concomitant with the use of orlistat in human [44]. It is worth mentioning that the current study noxious atrophic influence of orlistat on tongue papillae might be secondary to the novel adverse effect reported by Martinez *et al.* 2013 who found that orlistat could induce macrocytic anemia and thrombocytopenia [45]. This consecutively could affect badly tongue health and architecture and resulted in atrophy of tongue papillae [46]. Interestingly, SEM results of the present HFD- GCBE treated group showed restoration of almost normal shape and distribution of filliform and fungiform papillae. These findings matched LM results of this group and matched the findings of Vinson *et al.* 2012 who confirmed the safety of GCBE and documented that no adverse effects associated with the use of GCBE [47].

In conclusion, Obesity has adverse effects that could harm tongue dorsal surface therefore anti-obestity medications are recommended to attenuate obesity and offset obesity- induced destructive effects. Although orlistat is a frequently used anti- obesity drug, this study proved its harmful effect on tongue mucosal architecture. On the other hand, GCBE intake is recommended as a novel and effective anti-obesity supplement able to alleviate the obesity harmfuleffectsand restore normal tongue mucosal architecture.

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