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Antiobesity activity of aqueous and ethanol extracts of *Enicostemma littorale* in high fat diet induced obese rats

Ankur Garg[1], Randhir Singh[2]

***Corresponding author:**

Ankur Garg

[1]Department of Pharmacology, Translam Institute of Pharmaceutical Education and Research, Meerut (U.P.), INDIA
*[2]Department of Pharmacology, M.M. College of Pharmacy, M.M. University, Mullana, Ambala, (Haryana), INDIA

Abstract

Obesity is a chronic disorder of global prevalence and associated with morbidity and mortality. Therefore, attention is being focused on the investigation of plant-based drugs used in the traditional medicine for the treatment of obesity. The presents work evaluates the anti-lipase and antiobesity activity of ethanol and aqueous extracts of *Enicostemma littorale* (ElEe & ElAe) (Genitanaceae) (Synonym – *Enicostemma axillare*) in HFD induced obesity in rats. Male Wistar rats weighing 150-200 g were divided into different group's i.e. Normal control, HFD control, Orlistat (lipase inhibitor), ElEe & ElAe at doses of 200, 400, 250 and 500 mg/kg p.o. Treatment was started after 6 weeks upto 12 weeks alongwith HFD (except normal control). Oxidative stress was measured by measuring MDA, SOD and GSH level. Obesity was assessed by measuring morphological parameters, serum glucose, serum cholesterol, triglycerides and HDL level. ElEe & ElAe at 200, 400, 250 and 500 mg/kg/orally significantly attenuated morphological parameters i.e. weight gain, BMI, WHR, obesity index and adiposity index respectively as compare to HFD control group. Similarly, serum glucose, triglyceride, total cholesterol and oxidative stress were found to be attenuated as compare to HFD control group. The aqueous and ethanol extracts of *Enicostemma littorale* exhibit significant anti-lipase and antiobesity activity in high fat diet induced obesity in rats.

Keywords: Herbal treatment, lipase inhibitor, Obesity, Oxidative Stress, Orlistat.

Introduction

Obesity is a common chronic disorder of carbohydrate and fat metabolism which is characterized by excessive fat deposition in adipose tissue and associated with chronic diseases, including type 2 diabetes mellitus, hypertension and dyslipidemia [1, 2]. Many factors affect the onset of obesity including satiety control, reduced levels of physical exercise, hormonal and genetic parameters which influence the metabolic pathways leading to increase in stored fat [3]. Obesity remains a major global public health issue because of its increasing prevalence, cutting across all sex age-groups, ethnicity or race [4]. Hence, there is need to search for novel anti-obesity drug to tackle the obesity problem. Some recent studies have focused on the search for herbal extracts that can suppress weight gain and body fat accumulation induced by a high-fat diet with less significant side effects [5]. Natural products/ dietary photochemicals have aroused considerable interest in recent years as potential therapeutic agents to counteract obesity.

Enicostemma littorale Blume (Genitanaceae) (Synonym – *Enicostemma axillare*) is an herbaceous plant that seems to be rich in medicinal compounds: major chemical constitutes of the plant are swertiamarin and gentianine [6, 7]. Apigenin, genkwanin, isovitexin, swertisin, saponarin, and gentiocrucine [8] are also reported to be present in minor amounts [9] and have anti–inflammatory [10], antimalarial [11], antimicrobial [12], antipyretic [13], antirheumatic [14], antipsychotic, antihelmintic [15], hypoglycemic, antioxidant [16], hepatoprotective and hepatomodulatory activities [17].

However, *Enicostemma littorale* (Genitanaceae) has not been investigated so far for its anti-obesity potential. Hence, present study was designed to evaluate anti-lipase and anti-obesity effect of *Enicostemma littorale* aqueous and ethanol extract in high fat diet (HFD) induced obesity in rats.

Methods

Plant material

Enicostemma littorale were purchased from CH. Devi Lal Herbal Nature Park - Chuharpur, Yamunanagar, Haryana, India, and authenticated by Dr. Shiddamallayyan N from the National Ayurveda Dietetics Research Institute, Bangalore, where a voucher specimen is preserved for further reference (Ref No. Drug authentication/SMPU/NADRI/BNG/2012-13/743)

Preparation of *Enicostemma littorale* ethanol and aqueous extracts

Powdered *Enicostemma littorale* (100 g) was placed in thimble of Soxhlet apparatus and extraction was carried out by using ethanol as solvent for 72 h. The extracts were filtered; ethanol was distilled off using rotary evaporator to remove excess solvent. The 25 g of

air dried ethanol *Enicostemma littorale* filtrate was soaked in 100 ml distilled water for 24 h. The extract was filtered by using muslin cloth. The aqueous & ethanolic extracts was then transferred separately into the empty beakers and evaporated to a thick paste on the water bath, maintained at 500C to get ethanol and aqueous extracts. Finally extracts was air dried thoroughly to remove all traces of the solvent. The dried extracts was then stored in an air tight container for anti obesity activity.

High fat diet-induced obesity

The male Wistar rats (150-200 g) were procured from animal house facility of Translam Institute of Pharmaceutical Education and Research, Meerut (U.P.), India and then housed in standard polypropylene cages and maintained under controlled room temperature (22 ± 2 C) and humidity (55 ± 5%) with 12 h light and 12 h dark cycle. All the rats were provided with commercially available rodent chow diet (Amrut rat feed, Nav Maharastra Chakan Oil Mills Ltd., Delhi, India) and tap water *ad libitum*. After 1 week of acclimatization with free access to rodent chow diet and water, animals were used in the study. The guidelines CPCSEA, Government of India were followed and protocol was approved by the Institutional Animal Ethics Committee. Rats were fed with prepared HFD and water *ad libitum* for the period of 12 weeks. Composition of the experimental diet (g/kg diet) was according to the formula of Srinivasan et al. [18] with some modifications shown in Table 1.

Composition Of Hfd

Ingredients	Diet (g/kg)
Powdered NPD	375
Lard	290
Casein	265
Corn oil	10
Cholesterol	10
Vitamin and mineral mix	45
Dl-Methionine	03
Yeast powder	01
Sodium chloride	01

Experimental design

In this study, a total of 42 rats were used and divided into seven groups of 06 rats each.

Group I: Normal Control rats were maintained on standard chow diet and water *ad libitum* for twelve weeks. No treatment was given to these rats.

Group II: High Fat Diet Control rats were maintained on high fat diet for twelve weeks to induce obesity.

Group III: Orlistat (Standard) (30 mg/kg/day p.o., 6 weeks) was administered to rats along with high fat diet at the end of sixth week and continued up to the end of the twelve weeks.

Group IV-V: Ethanol extract of *Enicostemma littorale* (200 & 400 mg/kg/day p.o., 6 weeks) was administered to rats alongwith high fat diet at the end of sixth week and continued up to the end of the twelve weeks.

Group VI-VII: Aqueous extract of *Enicostemma littorale* (250 & 500 mg/kg/day p.o., 6 weeks) was administered to rats alongwith high fat diet at the end of sixth week and continued up to the end of the twelve weeks.

All the drugs were administered by oral gavage once a day. Food and water intake was measured daily for the period of 12 weeks at the same time on per cage basis and the average food and water consumed were calculated. At the end of the experimental period (on 85th day), the animals were anesthetized with Diethyl ether, following overnight fasting. Blood was drawn by retro-orbital method into a tube and the serum was obtained by centrifugation. After collection of blood, rats were sacrificed; Retroperitoneal (RET), epididymal (EPI), mesenteric (MES) adipose tissue and liver were excised immediately, rinsed with phosphate buffer saline and weighed. The serum, liver and adipose tissue samples were stored at –70 C until analysis.

Morphological Parameters to measure obesity

The body weights were determined once a week. Body mass index (BMI), WHR, Adiposity index, Obesity index was calculated from formula:

BMI = body weight (g)/ length2 (cm2) [19].

Waist-hip ratio [20].

Adiposity index = (sum of the weights of perirenal white adipose tissue (WAT), retroperitoneal WAT, and epididymal WAT divided by body weight 100) [20].

Obesity index = (body weight of rat/nasoanal length (mm) 104) [20].

Sample collection

At the end of the experimental period, all rats were sacrificed and blood samples were collected. Sera were separated and stored in aliquots at -20 C till used for estimation of lipid profile including; total cholesterol, triglycerides, LDL-cholesterol, and HDL-cholesterol by enzymatic colorimetric methods using commercial kits. Then the abdomen were opened, liver and adipose tissues (Retroperitoneal, epididymal and mesenteric) were removed, washed three times in ice cold saline and blotted individually on ash-free filter paper, used for preparation of tissue homogenates for estimation of tissue Malondialdehyde (MDA), superoxide dismutase (SOD), reduced glutathione (GSH) levels and for histological sections.

Biochemical estimation

Estimation of total cholesterol

Total serum cholesterol was estimated by using Bayer Diagnostic kit (Bayer Diagnostic India Ltd).

Estimation of high density lipoprotein cholesterol

High-density lipoprotein cholesterol was estimated by using Bayer diagnostic kit (Bayer Diagnostic India Ltd.).

Estimation of triglycerides

Triglycerides level was estimated by using Erba Diagnostics Manheim, Germany kit.

Estimation of serum glucose

Total serum glucose was estimated by glucose-peroxides method.

Method for assessment of oxidative stress

The tissues were minced and washed repeatedly with the sucrose buffer pH 7.4 to remove adhering blood and 10% (w/v) homogenates were prepared using a Potter-Elvehjem type glass-Teflon homogenizer. Then the mitochondrial and postmitochondrial fractions were prepared [21].

Estimation of Malondialdehyde (MDA)

This method based on the formation of MDA as an end product of lipid per oxidation which reacts with thiobarbituric acid producing thiobarbituric acid reactive substance (TBARS), a pink chromogen, which can be measured spectrophotometrically at 532 nm and MDA standard was used to construct a standard curve against which readings of the samples were plotted [22].

Estimation of Superoxide dismutase (SOD)

The SOD activity was spectrophotometrically measured using a modified version of the method developed by Marklund and Marklund [23]. Briefly, SOD activity was detected based of its ability to inhibit superoxide-mediated reduction. One unit of SOD activity was defined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50% and was expressed as unit/g Hb and that from the tissue as unit/mg protein

Estimation of Reduced glutathione (GSH)

The method is based on the reduction of 5, 5 dithiobis (2-nitrobenzoic acid) (DTNB) with reduced glutathione (GSH) to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration and its absorbance can be measured at 405 nm by using a commercial kit was used (Biodiagnostic, Egypt) [24].

Histopathological analysis

For histological examination adipose tissue was collected and fixed in 10% neutral buffered formalin, embedded in paraffin. Standard sections of 5 mm thickness were cut, which were then stained with haematoxylin and eosin, and examined by light microscopy.

Drugs and chemicals

Orlistat was obtained from Ranbaxy Research Labs, Gurgaon, India; all other reagents used in this study were of analytical grade.

Statistical analysis

Statistical evaluation of analytical data was done by Student's t-test using the statistical software-GraphPad Prism 3.0. Data are expressed as the mean ± standard error (SE). The biochemical data for random glucose, lipid profile and fat pad weights were statistically analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni multiple comparison test $P < 0.05$, The effect of *Enicostemma littorale* aqueous and ethanol extract on feed intake, body weight, BMI, and Obesity index at different time points were statistically analyzed using repeated measure two way ANOVA followed by Bonferroni multiple comparison test $P < 0.05$ was set to be statistically significant.

Results

Morphological Parameter

Effect of Orlistat and *Enicostemma littorale* ethanol and aqueous Extracts on Body Weight, Body Mass Index (BMI), Waist Hip Ratio and Feed Intake of Rats

Obesity was induced in normal rats by feeding a high-fat diet for 12 weeks. The mean body weights of the seven experimental groups were similar at the start of the experiment.

A significant increase in body weight, body mass index (BMI) and waist hip ratio along with feed intake was observed in rats of HFD control group after 12 weeks as compared to normal control group. On the other hand, treatment with standard drug Orlistat (30 mg/kg, p.o.) once daily for six weeks, significantly ($P < 0.05$) decreased the body weight, BMI, waist hip ratio and feed intake as compared to HFD control group. Whereas, once daily treatment for six weeks with EIEe & EIAe (200, 400, 250 & 500 mg/kg; p.o.), resulted in significant attenuation of body weight, BMI, waist hip ratio and feed intake as compared to HFD control group (Figure 1 & Table 2).

Table:1- Effect of various doses of *Enicostemma littorale* aqueous and ethanol extracts on % change in body weight on Day 84.

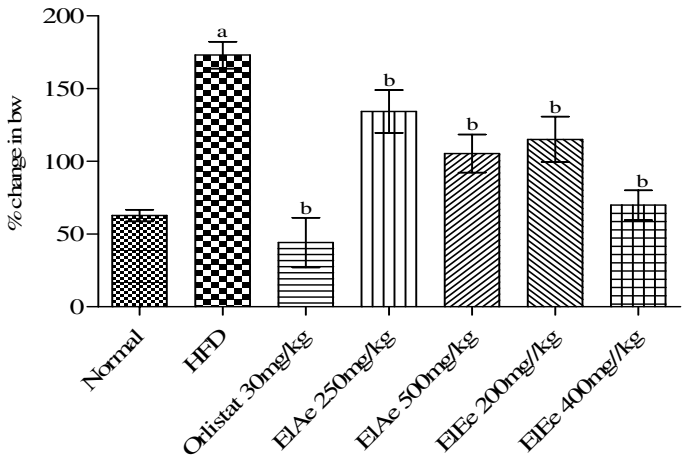


Table 2. Effect of various doses of *Enicostemma littorale* aqueous and ethanolic extracts on HFD-induced changes on BMI, feed intake in kilocalories (Kcal) and in gram, WHRatio, obesity index, adiposity index (%) on Day 84.

Para meter	Normal Chow Diet Control	High Fat Diet Control	Orlistat 30 mg/kg	*Enicostemma littorale*			
				Aqueous extract		Ethanol extract	
				250 mg/kg	500 mg/kg	200 mg/kg	400 mg/kg
BMI	1.13 ± 0.087	1.57 ± 0.10a	0.98 ± 0.08b	1.50 ± 0.12b	1.15 ± 0.06b	1.34 ± 0.10b	1.10 ± 0.19b
Feed Intake(gm)	21.66 ± 6.31	17.18 ± 2.88a	5.17 ± 1.47b	12.18± 1.14b	8.70 ± 0.58b	14.47 ± 1.17b	7.65 ± 0.81b
Feed intake (Kcal)	78 ± 22.74	61.86 ± 4.83a	18.61 ± 5.29b	43.86± 4.08b	31.32± 4.27b	52.08± 4.20b	27.54± 2.92b
WHRatio	0.84 ± 0.023	1.09 ± 0.016a	0.92 ± 0.43b	0.98± 0.08b	0.97± 0.013b	1.08± 0.018b	0.93± 0.011b
Obesity Index	345.9 ± 8.07	368.5 ± 6.06a	333.7± 3.17b	364.6 ± 9.37b	337.6 ± 7.75b	336.4 ± 8.34b	320.8 ± 11.23b
Adiposity Index (%)	2.68 ± 0.08	5.00 ± 0.20a	2.89 ± 0.23b	4.57 ± 0.22b	3.80 ± 0.09b	5.19 ± 0.32b	3.2 ± 0.09b

All values are represented as mean ± S.E; a = $P < 0.05$ *vs* Normal Chow Diet control, b = $P < 0.05$ *vs* HFD control.

Effect of Orlistat and *Enicostemma littorale* aqueous and ethanol Extracts on Fat Pad Weights, Total Fat, Obesity Index and Adiposity Index of Rats

The fat pad weights (Epididymal, Mesenteric, Retroperitoneal and Total fat) significantly increased in HFD control rats as compared to those of normal control rats. The once daily oral treatment of animals with standard drug (Orlistat), EIEe & EIAe (200, 400, 250 & 500 mg/kg; *p.o.*), for six weeks significantly ($P < 0.01$) attenuated the fat pad weights, total fat, obesity index and adiposity index as compared to HFD control group (Table 3 & 4)

Table 3. Effect of various doses of *Enicostemma littorale* aqueous and ethanolic extracts on HFD-induced changes on antioxidant enzyme activities on Day 84.

Para meter	Normal Chow Diet Control	High Fat Diet Control	Orlistat 30 mg/kg	*Enicostemma littorale*			
				Aqueous extract		Ethanol extract	
				250 mg/kg	500 mg/kg	200 mg/kg	400 mg/kg
MDA (nmol/mg protein)	23.02 ± 0.88	32.68 ± 2.11a	21.43 ± 0.65b	30.21 ± 1.42b	27.65 ± 1.07b	29.45 ± 1.21b	27.75 ± 1.64b
GSH (µg/mg protein)	30.07 ± 3.86	12.26 ± 1.28a	28.67 ± 2.14b	20.43 ± 1.33b	26.84 ± 1.26b	23.36 ± 1.61b	27.04 ± 1.52b
SOD (unit/mg protein)	7.54 ± 0.17	5.48 ± 0.04a	6.86 ± 0.12b	5.38 ± 0.17b	6.65 ± 0.19b	5.92 ± 0.49b	6.79 ± 0.34b

Table 4. Effect of various doses of *Enicostemma littorale* aqueous and ethanolic extracts on HFD-induced changes on various fat pads on Day 84.

Para meter	Normal Chow Diet Control	High Fat Diet Control	Orlistat 30 mg/kg	*Enicostemma littorale*			
				Aqueous extract		Ethanol extract	
				250 mg/kg	500 mg/kg	200 mg/kg	400 mg/kg
Epididymal Fat (gm)	2.83 ± 0.49	7.4 ± 0.62a	2.75 ± 0.69b	5.38 ± 0.93b	3.58 ± 0.73b	5.98 ± 0.98b	3.08 ± 0.60b
Retroperitoneal Fat (gm)	1.26 ± 0.54	4.26 ± 0.65a	1.22 ± 0.65 b	3.6 ± 0.55b	2.45 ± 0.489b	3.38 ± 0.55b	2.05 ± 0.24b
MesentericFat (gm)	2.86 ± 0.25	7.06 ± 0.55a	2.67± 0.52 b	6.06 ± 0.81b	4.73 ± 0.63 b	5.63 ± 0.63b	3.08 ± 0.65b
Total Fat (gm)	6.97 ± 0.49	18.75 ± 1.34a	6.63 ± 1.44 b	15.05 ± 1.83b	10.87 ± 0.56b	15.0 ± 1.63b	8.21 ± 0.89b

All values are represented as mean ± S.E; a = $P < 0.01$ *vs* Normal Chow Diet control, b = $P < 0.01$ *vs* HFD

Biochemical Parameters

Effect of Orlistat and *Enicostemma littorale* aqueous and ethanol Extracts on High Fat Diet Induced Changes in Blood Glucose Level of Rats

Random blood glucose levels were measured at the end of study. Feeding with high fat diet for 12 weeks significantly increased the blood glucose level in HFD control group as compared to normal control group. Further, the once daily per oral treatment with Orlistat (standard drug) 30 mg/kg for six weeks significantly decreased blood glucose level as compared to HFD control group. Also, the treatment of animals with EIEe & EIAe (200, 400, 250 & 500 mg/kg, *p.o.*) for six weeks show significant ($P < 0.01$) difference in blood glucose level, as compared to HFD control rats (Figure 2).

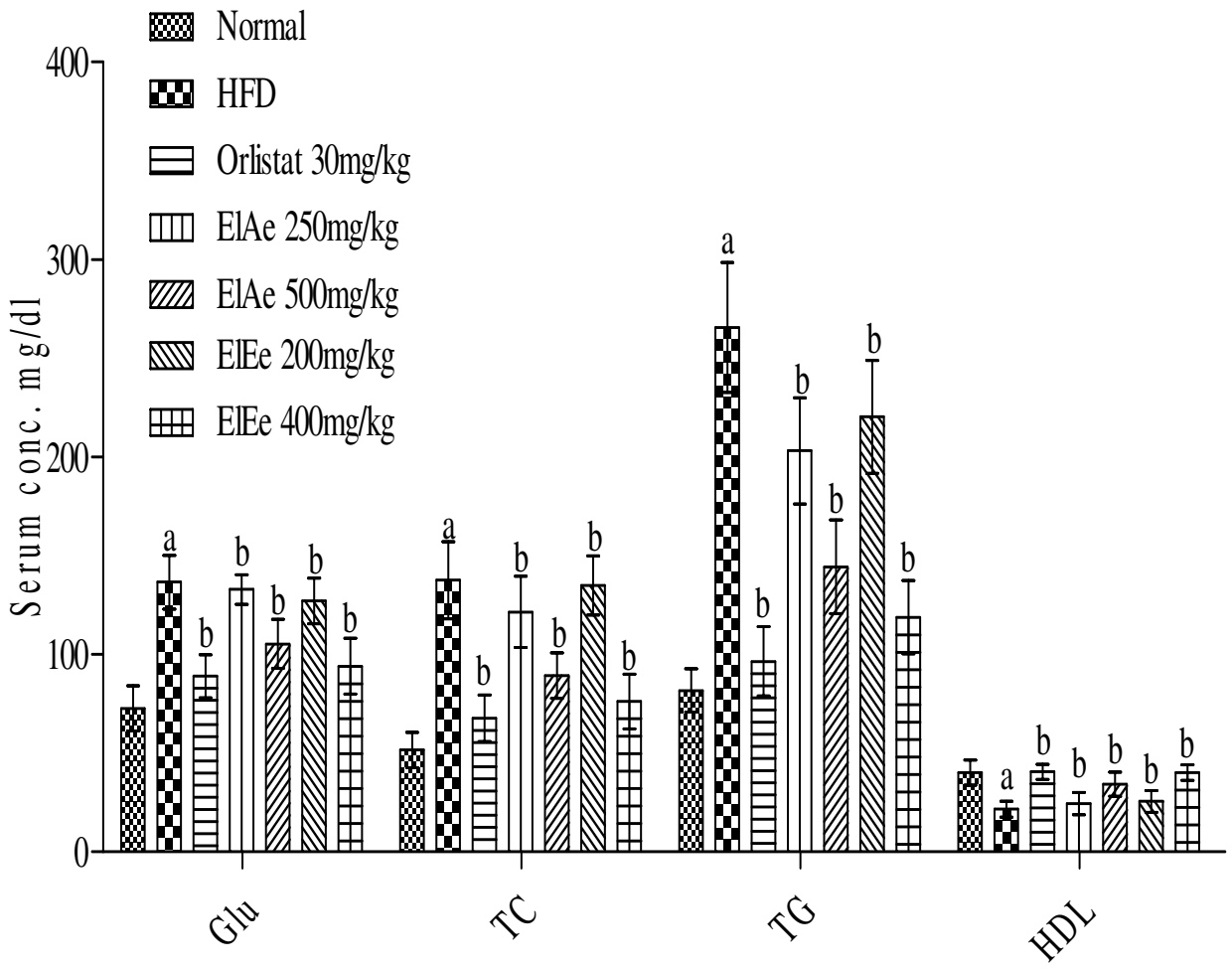
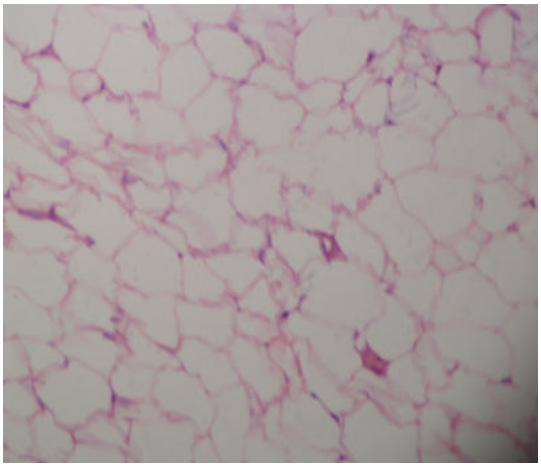


Figure 2.Effect of various doses of *Enicostemma littorale* aqueous and ethanol extracts on HFD-induced changes on random serum glucose and lipid profile on Day 84.

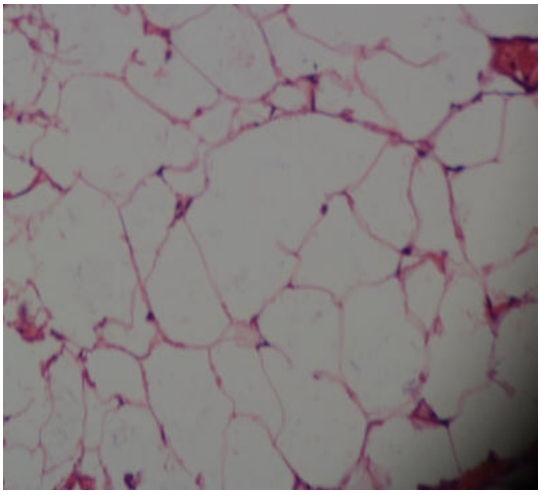
All values are represented as mean ± S.E; a = $P < 0.01$ *vs* Normal Chow Diet control, b = $P < 0.01$ *vs* HFD control. HFD- High Fat Diet, GLU-glucose, TC-total cholesterol, TG-triglyceride, HDL- High Density Lipoprotein

Effect of Orlistat and *Enicostemma littorale* aqueous and ethanol Extracts Treatment on High Fat Diet Induced Changes in Lipid Profile of Rats

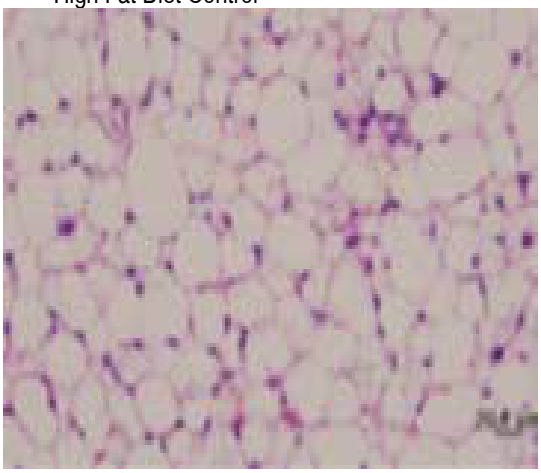
The evaluation of serum lipid profile of experimental animals was carried out for all groups. There was statistically significant ($P < 0.01$) increase in total cholesterol (TC) and triglycerides (TG) along with decreased high density lipoprotein (HDL) in HFD control group, as compared to normal control group. The once daily oral administration of Orlistat for six weeks along with HFD significantly decreased the levels of TC and TG with increase in HDL as compared to HFD control group. Also, the once daily treatment with ElEe & ElAe (200, 400, 250 & 500 mg/kg, *p.o.*), for six weeks significantly attenuated the levels of TC and TG with increase in HDL as compared to HFD control group and comparable to standard drug (Orlistat) treatment (Figure 3).



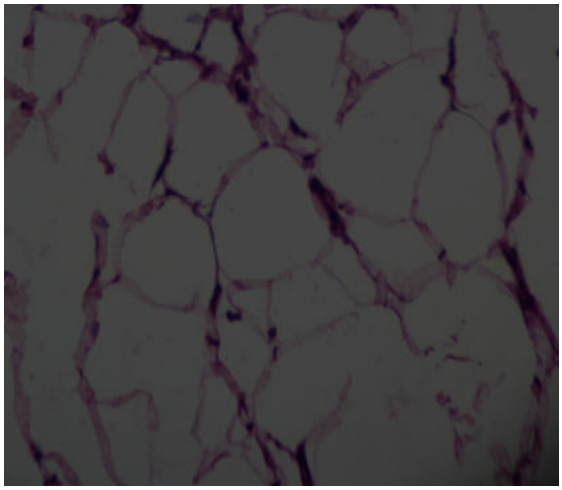
Normal Control



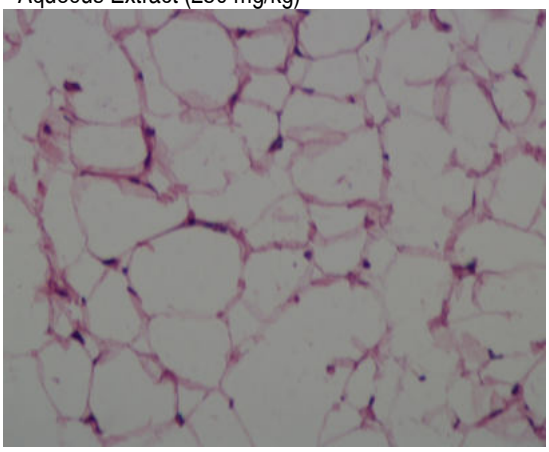
High Fat Diet Control



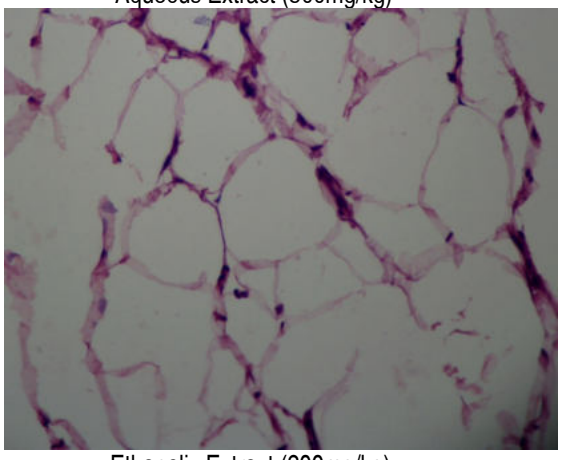
Orlistat (30mg/kg)



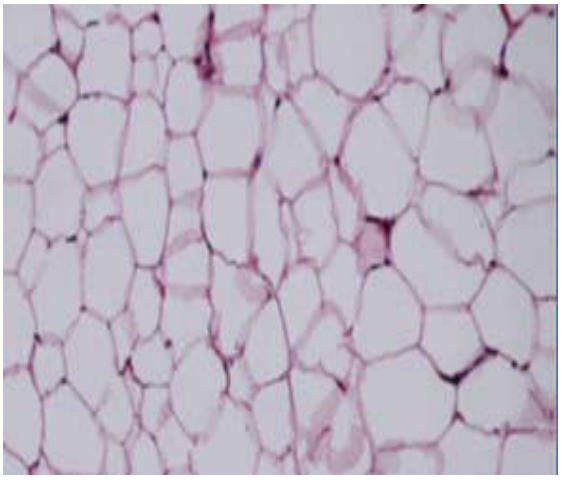
Aqueous Extract (250 mg/kg)



Aqueous Extract (500mg/kg)



Ethanolic Extract (200mg/kg)



Ethanolic Extract (400mg/kg)

Oxidative stress assessment

Effect of Orlistat and *Enicostemma littorale* aqueous and ethanol extracts on HFD Induced changes in MDA, SOD & GSH level of rats.

A significant (P < 0.05) decrease was observed in reduced glutathione (GSH), superoxide dismutase (SOD) whereas, Malondialdehyde (MDA) level was found to be increased in HFD control group as compared to normal control group (P< 0.005).The once daily oral administration of Orlistat for six weeks along with HFD significantly increased the levels of GSH and SOD with decrease in MDA when compared to HFD control group. Also, the once daily treatment with EIEe & EIAe (200, 400, 250 & 500 mg/kg, *p.o.*), for six weeks significantly attenuated the levels of GSH and SOD with decrease in (P < 0.005) as compared to HFD control group and comparable to standard drug (Orlistat) treatment (Table No.3)

Discussion

Fat enriched diet has been used to produce obesity, dyslipidemia and insulin intolerance in rodents [20, 25]. Thus in the present study high fat diet (HFD) is used for 12 weeks to produce obesity and dyslipidemia. It is documented that high fat diet has produced rapid weight gain in rats [20, 26]. The HFD rats weighed more than normal controls as consumption of high fat diet is thought to be one of the main factors [27]. Dietary fat is calorically dense, extremely palatable and easily over consumed [28]. The food intake (g and Kcal), body weight, body mass index, WHratio, obesity index were used as parameters in present study to assess obesity. The lipogenesis was upregulated by HFD in rats lead to elevation of plasma lipid levels [29] which is characterized by elevated TG levels [30] LDL-C levels [31] and decrease in serum HDL-C [32] in obese rats [25]. Further, feeding of high fat diet produced hyperglycemia in rats [33]. A high fat diet not only lowers glucose uptake but also inadequately suppresses hepatic glucose production stimulated by insulin leading to insulin resistance as well as hyperglycemia [34]. Nearly 50-80% of the dietary lipids are hydrolyzed by pancreatic lipases (PL) and released as their respective fatty acids (FAs) and monoglycerides (MGs). The released FAs and MGs form mixed micells with bile salts, cholesterol and lysophosphatidic acid and are absorbed into enterocytes where resynthesis of triglycerides (TGs) takes place. TGs are stored in adipocytes as their main energy source [35]. Thus, the observed high fat diet induced increased levels of serum glucose, triglycerides and total cholesterol may be due to increased pancreatic lipase activity. Therefore serum lipid levels (total cholesterol, LDL, HDL and triglycerides) and glucose levels were estimated in present study as the marker of hyperlipidemia and hyperglycemia. The weight and size of each of the three adipose tissues (epididymal, retroperitoneal, mesenteric fat depots) increased progressively due to the *ad libitum* HFD feeding as compared to normal control rats. Therefore, in the present study three adipose tissues were weighed as an index of adiposity and size of adipose tissue was examined histologically using light microscope.

One of the key targets to treat obesity is the development of lipase inhibitors. Pancreatic lipase inhibitors which help to limit intestinal fat absorption at the intestinal stage have been proved as medications for the treatment of hyperlipidemic condition in animal models [36]. Orlistat, is one of the important drugs to reduce obesity by the potential action of pancreatic lipase inhibition. However, it showed some side effects like gastrointestinal effects, steatorrhea, oily stools, fecal spotting, diarrhoea, cholelithiasis, cholostatic and sub acute liver failure [37]. Therefore, research is focused on natural products, with special emphasis on pancreatic lipase inhibitors to combat obesity [36, 38-41]. The present study provides the first line of evidence of the antilipase and antiobesity effect of *Enicostemma littorale* aqueous (EIAe) and ethanol extracts (EIEe) in high-fat diet-fed rats, since this metabolic model of obesity reproduces human obesity better than the genetic obese models. Decrease body weight was due to lesser digestion and transport of dietary lipids in EIAe and EIEe treated high fat diet fed rats. *Enicostemma littorale* to rats with diet-induced obesity, significantly lowered plasma TC, TG levels, increased HDL-C levels and improved glucose tolerance. The hypertriglyceridemia observed in HFD fed rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of triglyceride enriched hepatic very low density lipoprotein (VLDL) and decreased triglyceride uptake in peripheral tissues [42]. Hypercholesterolemia may be attributed to increased dietary cholesterol absorption from the small intestine following the intake of HFD [43, 44]. Indeed, obesity is also associated with an unfavorable lipid profile or dyslipidemia [45, 46]. A decrease in the size of the adipocyte further support the argument that *Enicostemma littorale* suppresses high fat diet induced fat accumulation. The anti-obesity activity of EIAe and EIEe appears partly to be mediated by decreasing dietary fat absorption from the intestine via inhibition of pancreatic lipase activity. In other words, the effective inhibition of intestinal lipolysis

is possible if some of the molecules present in *Enicostemma littorale* could bind to rat lipase. The beneficial effect of high dose of *Enicostemma littorale* in preventing the high fat diet induced body weight gain has been observed to be almost similar to the effect produced by Orlistat. Moreover, our histological examinations revealed that the sizes of the adipocyte were significantly reduced in EIAe and EIEe treated rats (Figure3). However, EIAe and EIEe supplementation noticeably attenuated the extent of steatosis, suggesting that EIAe and EIEe may regulate lipid storage and mobilization in adipocyte. Obesity and hyperlipidemia synergistically promote systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS) and antioxidants [47]. ROS could react with polyunsaturated fatty acids, which lead to lipid peroxidation [48]. MDA is a by-product of lipid peroxidation and reflect the degree of oxidation in the body [49]. Possible mechanisms that generate oxidative stress in obesity include hyperglycemia, elevated lipid levels and inadequate antioxidant defenses [50].

In our study, the activities of GSH (a major endogenous antioxidant) and SOD decreased and MDA activity get increased in HFD-fed rats. Treatment of HFD-fed rats with EIAe and EIEe had reversed the activities of these enzymatic antioxidants. Therefore EIAe and EIEe treatment improves oxidative balance in HFD-fed obese rats.

Conclusively, observed reduction in body weight gain, feed intake, BMI, obesity index, serum lipids, glucose and decreased body fat pad weight suggests that EIAe and EIEe possesses significant anti-lipase and anti-obesity potential.

Abbreviations

EIEe	:	Enicostemma littorale ethanol extracts
EIAe	:	Enicostemma littorale aqueous extracts
HFD	:	High Fat Diet
ANOVA	:	Analysis of variance
aq.	:	Aqueous
b.w	:	Body weight
CPCSEA	:	Committee for the Purpose of Control and Supervision of Experiments on Animals.
MDA	:	Malondialdehyde
SOD	:	Superoxide dismutase
GSH	:	Reduced glutathione
BMI	:	Body Mass Index
gm/kg	:	Gram per kilogram
WHR	:	Waist Hip Ratio
IAEC	:	Institutional Animal Ethics Committee
p.o.	:	Per oral
RET	:	Retroperitoneal
EPI	:	Epididymal
MES	:	Mesenteric
WAT	:	White Adipose Tissue
LDL	:	Low density lipoproteins
HDL	:	High density lipoproteins
TBARS	:	Thiobarbituric acid reactive substance
DTNB	:	5, 5 dithiobis (2-nitrobenzoic acid)
SE	:	Standard Error
TC	:	Total cholesterol
TG	:	Triglycerides
Kcal	:	Kilo calories
PL	:	Pancreatic lipases
FAs	:	Fatty acids
MGs	:	Monoglycerides
VLDL	:	Very low density lipoprotein
ROS	:	Reactive oxygen species

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