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Anti-Lipase and Anti-Obesity Activities of *Terminalia paniculata* Bark in High Calorie Diet-Induced Obese Rats

Ramgopal Mopuri and Balaji Meriga

Tissue Culture and Molecular Biology Laboratory, Department of Biochemistry,
Sri Venkateswara University, Tirupati-517502, A.P, India

Abstract: Our aim in the present study was to evaluate the anti-lipase and anti-obesity activities of *Terminalia Paniculata* in high caloric diet (HCD) induced obese rats. Different solvent extracts (Hexane, ethyl acetate, ethanolic and aqueous) were prepared from the bark of *T. paniculata* and tested for their lipase inhibiting and anti-obesity activities. Among the extracts tested, ethanolic extract showed the highest anti-lipase activity (75%). For evaluating anti-obesity activity, HCD-fed groups were orally administered with 200 mg/kg b. wt of different solvent extracts. We have noticed that ethanolic extract of *Terminalia paniculata* (TPEE) showed effective weight reducing and anti-hyperlipidemic activity when compared to other solvent extracts. And the efficiency of TPEE is comparable to that of orlistat (30mg/kg b.wt), a standard anti-obesity drug. Although food consumption was moderately increased in HCD-fed rats, TPEE administration significantly reduced weight gain in them. Serum total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL) and very low density lipoproteins (VLDL) levels were significantly ($P< 0.05$) lowered, while high density lipoproteins (HDL) increased in TPEE administered HCD groups. Moreover, elevated fecal cholesterol levels were noticed in HCD+ TPEE treated groups, compared to HCD controls. Based on our results we demonstrate that TPEE has potential anti-lipase and anti-obesity activities.

Key words: *Terminalia Paniculata* · High Calorie Diet · Lipid Profiles · Fecal Cholesterol

INTRODUCTION

Obesity has become one of the serious health problems in the modern world. Accumulation of high fat in body causes obesity which leads to insulin resistance, hypertension, cardiovascular diseases, arthritis and certain types of cancers [1-3]. Prevention and treatment of obesity, dislipidemia are important for a healthy life [4]. Obesity therapies include reduction of nutrient absorption and administration of drugs that affect lipid mobilization and utilization. In order to reduce dietary fat absorption, strategies aimed to inhibit pancreatic lipase have been developed to fight obesity [5]. A good number of anti-hyper mghlipidemic drugs such as orlistat, simvastatin, lovostatin and atarvostatin are available in the market, however, their usage is limited by their side effects [6]. Therefore, more recent drug trials have focused on screening for natural sources that have multiple targets/ways to control obesity and hyperlipidemic condition with minimal side effects.

Terminalia paniculata belongs to Combretaceae family. It is widely distributed in western and southern parts of India. The bark part of the plant is brown with shallow fissures and blaze whitish with brown grains. The extracts prepared from bark and flowers of this plant are reported to be used in vitiated conditions of kapha, pitta, bronchitis, diabetes, skin disease, leprosy, inflamed parotid glands and menstrual disorders in the traditional system of medicine [7-10]. However, there are scanty reports on the anti-obesity activity of this plant. Hence, in the present study we have prepared different solvent extracts of *T. paniculata* bark and evaluated the anti-lipase and anti-obesity activities in high caloric diet-induced obese rats.

Corresponding Author: Balaji Meriga, Department of Biochemistry Sri Venkateswara University, India.
Tel: +91-98490-86856.

MATERIALS AND METHODS

Collection of Plant Material and Preparation of Plant Extracts: The bark of *Terminalia paniculata was* collected from Seshachalam forests spread around Tirupati andhra Pradesh, India. Plant was authenticated by Dr. Madhavachetty, Dept of Botany, Sri Venkateswara University, Tirupati, voucher number 136 and a specimen has been preserved at the departmental herbarium. The bark of *T. paniculata* was shade dried, pulverized to a coarse powder and sequentially extracted based on their polarity with hexane, ethyl acetate, ethanol and water. The filtrates obtained were evaporated to dryness at 50-65°C in a rotary vacuum evaporator to obtain a dark colored molten mass.

Assay of Lipase Inhibitory Activity: Lipase inhibitory activity of *T. paniculata* bark extracts (Hexane, ethyl acetate, ethanolic and aqueous extracts) was determined using a modified assay method [11, 12]. Briefly, a suspension containing 1% (v/v) triolein and 1% (v/v) Tween 40 in 0.1 M phosphate buffer (pH 8) was prepared and emulsified. Assay was then initiated by adding 800µl of the triolein emulsion to 200µl of porcine pancreatic lipase (0.5 gm pancreatin in 15ml of 0.1 M phosphate buffer at pH 8.0) and 200µl of plant extract. The contents were mixed and absorbance measured immediately at 450 nm and designated as T0. The test tubes containing reaction mixture were incubated at 37°C for 30 min and then the absorbance was recorded at 450 nm and designated as T30. The variation in absorbance = [A450 (T0) - A450 (T30)] was calculated for both control and test groups and the % inhibition was calculated using the following formula.

$$\% \text{ inhibition} = \frac{\Delta A450 \text{ Control} - \Delta A450 \text{ Extract}}{\Delta A450 \text{ Control} \times 100}$$

Animals and Feed: Male Sprague-Dawley rats were obtained from National institute of nutrition, Hyderabad, India. The rats were housed under 22±2ºC temperature, 40-60% humidity and 12-12±1 h light-dark cycle. During the course of obesity induction, rats weighing 160-170g were taken for the study and they were broadly divided in to two groups. Group one was normal control (normal pellet diet was fed) and six groups were fed with high calorie diet (HCD) to induce obesity and water *ad libetum*. Experimental protocols were followed as per institutional animal ethical committee guidelines (Resolution No: 36/2012-2013/ (i)/a/CPCSEA/IAEC/ SVU/MB-MRG).

Composition of High Fat Diet: High calorie diet (HCD) was obtained from National centre for laboratory animal sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, India. Diet composition was as follows; Casein (342g), Cystine (30g), Starch (172g), Sucrose (50g), Cellulose (50g), G.N.Oil (25g), Thallow (190g), Mineral mixture (35g), Vit. Mixture (10g).

Experimental Design: All rats were randomly divided in to seven groups (*n=6*) and fed with normal diet or high fat diet.

Group 1: Normal control group (NC)
Group 2: High calorie diet group (HCD)
Group 3: HCD + Orlistat 30mg/kg b.wt
Group 4: HCD + TPHE 200mg/kg b.wt
Group 5: HCD + TPEtE 200mg/kg b.wt
Group 6: HCD + TPEE 200mg/kg b.wt
Group 7: HCD + TPAE 200mg/kg b.wt

Determination of Body Food Intake: Each rat was fed with 25g/day of diet. The total amount of food consumed by each rat was monitored the next day. Consumption of feed = total quantity of feed given to rat - leftover feed.

Determination of Body Weight: Throughout the 42 days of study period, the body weight of each rat was measured once in a week by using standard weighing machine (Docbel Group of Industries, New Delhi, India). Net weight gain was calculated as: Net weight gain (W) = final weight (W_1) - initial weight (W_0).

Collection of Serum: For serum analysis, rats were fasted overnight and blood collected by retro-orbital puncture. Serum was separated by centrifugation at 3000 rpm for 10 min and serum samples were stored at -80ºC for further analysis.

Determination of Lipid Profiles: Serum cholesterol and triacylglyceride levels were measured by CHOD-PAP method. Serum HDL level was estimated by GPO-PAP method [13]. LDL level was calculated by the method of Johnson *et al.* [14]. The atherogenic index was calculated by using the method described by Muruganandan *et al.* and Suanarunsawat *et al.* [15, 16].

Fecal Lipid Estimation: Feces were collected from experimental rats before and after treatment with plant extracts, dried and powdered. From this, fecal lipids were

extracted with chloroform and methanol (2:1) and dissolved in 1% triton X 100 and estimated by standard kit method [17].

RESULTS

Lipase Inhibitory Activity: Hexane, ethyl acetate, ethanol and aqueous extracts of *T. paniculata* bark were tested for pancreatic lipase inhibitory activity and the results are shown in Table 1. Among the extracts tested, ethanolic extract exhibited highest inhibitory activity (75%).

Food Intake And Body Weight: High calorie diet substantially increased body weights of rats. When compared to normal control group, HCD-fed rats consumed more feed. However, food consumption was reduced in HCD+ plant extract administered groups when compared to HCD group (Fig. 1A). Among different plant extracts tested, TPEE substantially prevented weight gain in HCD groups as shown in Figure 1B.

Serum Lipid Profiles: S.D rats fed with HCD showed increased levels of serum triglycerides, LDL, VLDL and total cholesterol and decreased HDL levels. However, when compared to other extracts of *T. paniculata*, oral administration of TPEE significantly (P<0.05) suppressed the raise in lipid profiles. HDL level was increased by TPEE indicating healthy atherogenic index (Fig. 2).

Fecal Lipids: Fecal metirial was collected from the rectum and wet weight of the feces was mesured in all groups. In our obesarvation among all expermintal groups, Decreased fecal matter weights were noticed in HCD +TPEE adminsitered groups when compared to HCD group. When fecal matter was assessed for metabolic fate of unabsorbed lipids, increased lipids were found in TPEE+ HCD groups than HCD-fed group, indicating that TPEE might interfere in lipid digestion, absorption and transportation (Table 2).

DISCUSSION

Obesity is becoming one of the biggest complications to global health in this millennium [18]. It is well known that dietary fat sources strongly influence several biochemical variables both in plasma or serum (Increased TC, TG, LDL, VLDL and decreased HDL) and biological membranes [19-21]. 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 [18]. Accumulation of more and more adipocytes in the form of fat pads in various parts of the body leads to obesity.

Table 1: Lipase inhibitory effect of *T. paniculata* extracts

Plant name/drug	Family	Solvent extract	Inhibition (%)
Terminalia paniculata	Combretaceae	Hexane	0.00
		Ethyl acetate	19.02
		Ethanolic	75.10
		Aqueous	12.04
Orlistat	-	-	89.14

Table 2: Effect of *T. paniculata* extracts on fecal lipid profiles of rats

Parameters	NC	HCD	Orl	TPHE	TPEtE	TPEE	TPAE
Fecal mat weight (g)							
21st day	0.9±0.21	1.4±1.23	1.4±0.98	1.4±1.18	1.4±1.28	1.4±1.20	1.4±1.12
42nd day	1.2±0.89	1.9±3.52	1.1±2.10*	1.8±2.15	1.9±1.09	1.2±2.10*	1.75±1.08
Fecal lipids (mg/g)							
21st day	2.6±1.84	7.5±1.3	7.8±1.2	7.7±1.2	8.1±0.95	7.9±0.95	7.8±1.02
42nd day	2.9±0.58	8.8±2.12	12.1±1.1*	8.0±2.45	9.2±2.05	11.5±0.68*	8.9±0.86

The data are given as mean ±S.D (n=6). * Indicates significant differences (P <0.05) compared to HCD control group. NC: Normal control group, HCD: high calorie diet group, Orl: Orlistat (30mg/kg b.wt) TPHE: *T. paniculata* hexane extract (200mg/kg b.wt), TPEtE: *T. paniculata* ethyl acetate extract (200mg/kg b.wt), TPEE: *T. paniculata* ethanolic extract (200mg/kg b.wt), TPAE: *T. paniculata* aqueous extract (200mg/kg b.wt)

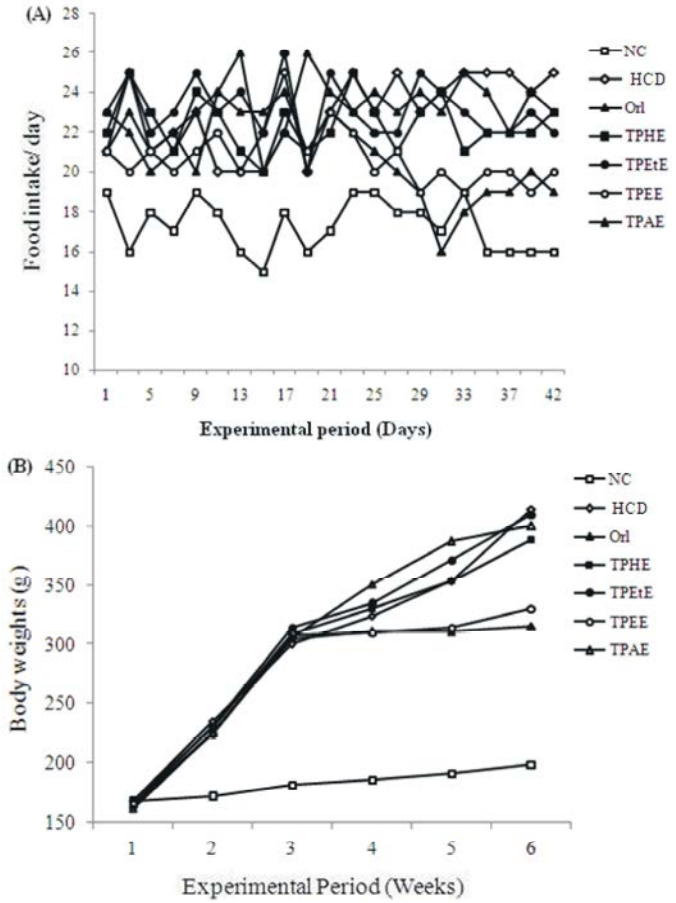

Fig. 1: Effect of *T. paniculata* extracts on food intake and body weight gain of S.D rats. (A) Food intake, (B) Body weights. The data are given as mean ±S.D (*n*=6). NC: Normal control group, HCD: High calorie diet group, Orl: Orlistat (30mg/kg b.wt), TPHE: *T. paniculata* hexane extract (200mg/kg b.wt), TPEtE: *T. paniculata* ethyl acetate extract (200mg/kg b.wt), TPEE: *T. paniculata* ethanolic extract (200mg/kg b.wt), TPAE: *T. paniculata* aqueous extract (200mg/kg b.wt)

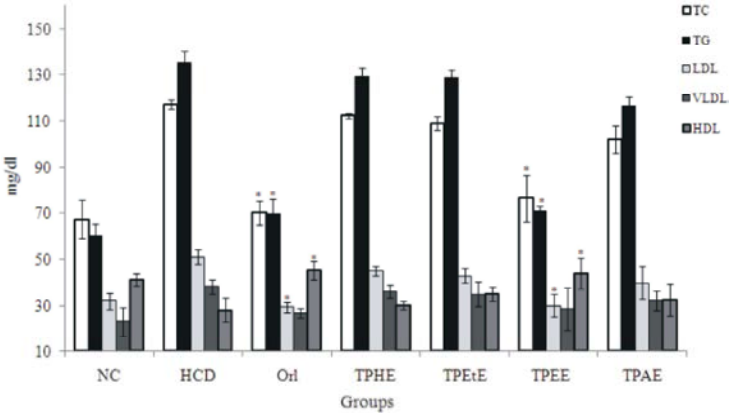

Fig. 2: Effect of *T. paniculata* extracts on serum lipid profiles of S.D rats. (A) Food intake. The data are given as mean ±S.D (*n*=6). * Indicates significant differences (P <0.05) compared to HCD control group. NC: Normal control group, HCD: high calorie diet group, Orl: Orlistat (30mg/kg b.wt) TPHE: *T. paniculata* hexane extract (200mg/kg b.wt), TPEtE: *T. paniculata* ethyl acetate extract (200mg/kg b.wt), TPEE: *T. paniculata* ethanolic extract (200mg/kg b.wt), TPAE: *T. paniculata* aqueous extract (200mg/kg b.wt)

Phytochemicals such as tannins, flavonoids and alkaloids present in *T. paniculata* exert a wide spectrum of biological activities in animals and humans. Previous reports also demonstrate that bark extract of *T. paniculata* decreases inflammation, type-2 diabetes and hepatic damages in toxic conditions. The present study provides the first line of evidence of the anti-obesity effect of ethanolic extract of *T. paniculata* bark. It is well known that increasing consumption of high calorie foods is considered as a major reason to induce adipogenesis and obesity which lead to some chronic diseases such as hypertension, hyperlipidemia, diabetes and fatty liver.

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 useful medications for the treatment of hyperlipidemic condition in animal models [22]. The human lipase enzymes include the pre-duodenal (lingual and gastric) and the extra-duodenal (pancreatic, hepatic, lipoprotein and endothelial) lipases plays major role in digestion of dietary triacylglycerol and phospholipids [23]. Pancreatic lipase (PL) is the key enzyme for lipid absorption that hydrolyses triacylglycerols in the gastrointestinal tract [24]. 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 [25]. Therefore, research is focused on natural products, with special emphasis on pancreatic lipase inhibitors to combat obesity [22, 26-29].

In the present work ethanolic extract of *T. paniculata* (TPEE) has shown maximum reduction in weight gain of HCD-fed rats when compared to other solvent extracts of *T. paniculata*. This could be due to lesser digestion and transport of dietary lipids in TPEE treated groups. This is evident from the highest lipase inhibitory activity of TPEE. In other words, the effective inhibition of intestinal lipolysis is possible if some of the molecules present in TPEE could bind to rat lipase or colipase. In this study, serum lipid profiles of TPEE-administered groups showed very good anti-hyperlipidemic efficacy of TPEE, as evidenced by a healthy atherogenic index. Food consumption pattern of TPEE-administered group indicates that TPEE could influence leptin receptors which might cause decreased food intake in them. Alternatively TPEE might have a role in regulating secretion of adiponectin like adipokines which have a role in obesity development. In addition, we also noted the presence of high level of fecal lipids in fecal matter of TPEE-administered groups supports the view that impaired lipid digestion and absorption has occurred in them vindicating the lipase-inhibiting activity of TPEE. Based on our results we demonstrate that ethanolic extract of *T. paniculata* (TPEE) possesses potential anti-lipase and anti-obesity activity. The role of TPEE in transcriptional regulation of certain obesity associated genes is under study.

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Conflict of interest.

Authors declare that there is no conflict of interest.

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