Ethanolic Fraction of *Terminalia tomentosa* Attenuates Biochemical and Physiological Derangements in Diet Induced Obese Rat Model by Regulating Key Lipid Metabolizing Enzymes and Adipokines

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ABSTRACT

The prevalence of overweight-obesity and associated comorbidities have reached alarming levels necessitating the need to explore effective therapeutics. In the present work, we demonstrated the promising antiobesity activity of ethanolic fraction of *Terminalia tomentosa* bark (EFTT) in diet induced obese rat model. High Fat Diet (HFD)-fed obese rats were orally administered with EFTT (50, 100 and 200 mg/kg body weight). Changes in body weight, body composition, bone mineral concentration, bone mineral density, plasma glucose, insulin, leptin, adiponectin, circulatory and tissue lipid profiles, and the activities of liver antioxidant enzymes, key lipid metabolic enzymes and mRNA expressions of fatty acid synthase (FAS), peroxisome proliferator-activated receptor gamma (PPAR-γ), leptin and tumor necrosis factor alpha (TNF-α) were assessed in experimental rats in the presence and absence of EFTT. At a dose of 200 mg/kg b.wt, EFTT has substantially attenuated body weight and related patho-physiological alterations in HFD-induced obese rats. These findings were correlated with histological observations of adipose tissue. The therapeutic activity of EFTT could be possibly through restoration of antioxidants status, regulation of key lipid metabolizing enzymes, expression of FAS, leptin, PPAR-γ and by synchronized control of energy metabolism in liver and adipose tissue.

Key words: Absorptiometry, body composition, dual-x-ray, HFD, histopathology, lipid profiles

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INTRODUCTION

Regulation of energy homeostasis as a means to overcome metabolic disorders is one of the most rapidly advancing subjects in biomedical research today.[1] Obesity is a metabolic disorder characterized by distorted body composition, dyslipidemia, increased insulin resistance, disturbances in adipocytokines and alterations in lipid, carbohydrate and protein metabolism. Changes in lifestyle, increased consumption of dietary fats and reduced physical activity have contributed to obesity epidemic all over the world.[2,3] Over weight and obesity leads to hormonal imbalances, increases the risk for hypertension, diabetes, cancer, and cardiovascular diseases,[4,5] all of which tend to decrease life expectancy.[6,7]

Breakthroughs in understanding the molecular mechanisms regulating body weight provide potential opportunities for therapeutic intervention and bring renewed hope and vitality for the development of antiobesity drugs.[8] However, considering the side effects posed by existing antiobesity drugs, there is a growing research focus on effective alternative therapeutics, which may be a part of diet or drug. Identifying such molecules can be useful in treating obesity ailments.

Terminalia tomentosa (Combretaceae) is a large tree found in deciduous forests and extensively disseminated in south East Asian countries including India and Burma. Many plants of the genus Terminalia have been reported to possess medicinal values such as antidiabetic, cardioprotective, anti inflammatory and antioxidant.[9-12] The common polypheolic compounds reported in many Terminalia species are: Ellagic acid, Dimethyl ellagic acid, Pentamethyl flavellagic acid, Trimethyl flavellagic acid and β-sitosterol.[3,11] Although, *Terminalia tomentosa* bark is used in Indian traditional and folklore medicine for wound healing, GI disorders and anti-inflammatory purposes,[13] it lacks adequate scientific evidences. Ramachandra Row and Subbarao (1962) reported the chemistry and constituents of *T. tomentosa*.[14] They isolated β-sitosterol, oleanolic acid, arjunolic acid, barringtogenol and tomentosic acid. Anjum Ghalaut *et al.* (2013)[15] developed and optimized a convenient, high throughput, and reliable UPLC-QTOFMS method to analyze crude water extract from *T. tomentosa*. They identified the presence of 5-Aminovaleric acid, Zeatin riboside, Thymine, 4-Methoxy cinnamic acid, Niacinamide, (-) Epigallocatechin, Indole-3-aldehyde, Resveratrol, Chlorogenic acid, (+)-Epicatechin, Quercetin-3-O-rhamnopyranoside, Quercetin, and Kynurenic acid.

However, with regard to biological activity of *T. tomentosa*, there are hardly any scientific reports in the literature except the ones observed by Alladi, *et al.*, (2012),[16] Sabu and Kuttan, (2009).[17] Therefore, we

aimed to evaluate the efficacy of ethanolic fraction of *T. tomentosa* bark in diet induced obese rat model, in terms of biochemical, physiological, histological and molecular aspects.

METHODS

Animals and diets

All experiments were carried out with male Sprague-Dawley rats (SD rats) weighing 180±10 g, obtained from National Institute of Nutrition (NIN), Hyderabad, India. The composition of normal diet and high fat diet are as mentioned as in our earlier studies.[2] After initial acclimatization, the animals were randomly divided in to different groups as mentioned below. All procedures involving laboratory animals were in accordance with the Institute Animal Ethical Committee regulations approved by the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA), (IAEC approval number: 36 a/2012(i)/a/ CPCSEA/ IAEC/SVU/MB; dt.01.07.2012).

Chemicals

To measure plasma glucose and insulin, kits were procured from Stanbio Laboratory USA, Bio-Merieux, RCS, Lyon, France, respectively. Plasma was analyzed for total cholesterol, triglycerides, phospholipids, free fatty acids, VLDL, HDL by colorimetric methods using kits (Nicholas Piramal India Ltd, Mumbai). Orlistat (Cat No 04139) was obtained from Sigma-Aldrich. All other reagents used in experiments were of analytical grade and of high purity.

Preparation of ethanolic fraction

Bark of *Terminalia tomentosa* was collected from Seshachalam forests of Eastern Ghats and authenticated by a Taxonomist (Prof. N. Yasodamma), Department of Botany, Sri Venkateswara University, India, voucher number 2161, and a specimen has been preserved at the departmental herbarium. Bark was shade dried and pulverized to a coarse powder and sequentially extracted with ethyl acetate, ethanol and water. The respective filtrates obtained were evaporated to dryness in a rotary evaporator to obtain their dried extracts. Based on phytochemical analysis and biological activity,[5] ethanolic extract of bark was subjected to column chromatography to separate in to fractions and from them phytochemicals rich active fraction was used for further investigation.

HPLC analysis of ethanolic fraction of *T. tomentosa* bark

The isolated active ethanolic fraction of bark of *T. tomentosa* was subjected to reverse phase HPLC using the phenomenx column (C18, 250X4.60 mm, 4 μm). LC-20AT pump (Shimadzu), gradient controller (Shimadzu chromatpgraphy crop., Japan) and a Rheodyne injection value with a 20 μl fixed sample loop (Model 7725i, Rheodyne, Cotati, CA, USA). Separations were achieved at ambient temperature by isocratic elution at flow rate of 1.0 ml/min. The detection and quantification were carried out with a Shimadzu RF=10AXL fluorescence detector connected on line with a SPD-20A UV detector. The fluorescence excitation and emission (Ellagic acid, Dimethyl ellagic acid, Pentamethyl flavellagic acid, Trimethyl flavellagic acid and β-sitosterol) wavelengths were set to 254 to 450 nm.[8-11] Acquisition and processing of the chromograms were performed using LC solution software. The concentrations were determined as the peak-height measurement against external standards.

Acute toxicity studies

The acute oral toxicity study was performed in overnight fasted animals as per Organization for Economic Co-operation and Development

(OECD) guidelines. The animals did not show any sign of abnormal behavior or mortality up to a dose of 2000 mg/kg BW of EFTT. Hence, its 1/10th concentration was used as the Maximum therapeutic dose in the present study.

Experimental design

Group I –Normal control (Normal diet control)
Group II – High Fat Diet (HFD) control
Group III-HFD + Orlistat 5 mg/kg BW
Group IV-HFD + EFTT 50 mg/kg BW
Group V -. HFD + EFTT 100 mg/kg BW
Group VI-HFD + EFTT 200 mg/kg BW

Measurement of body weight, body composition, BMC and BMD in experimental rats

The body weight of each rat was measured every week. Body composition of experimental animals was assessed at the end of experiment by Dual-X ray absorptiometry (DXA), using body composition analysis system (halogen 1000 series). DXA data were used to compare levels of body adiposity, bone mineral concentration (BMC), and bone mineral density (BMD) between the control and experimental groups. Lean body mass, total body fat percent, bone mineral concentration and bone mineral density were calculated according to manufacturer's protocols.[2] For measurement of nutrient metabolites like food intake, water intake, urine volume and fecal weight, both control and experimental rats were placed in the metabolic cages for 72h (Techniplast, Italy).

Estimation of glucose, insulin and insulin resistance

At the end of the experiment, blood was collected from overnight fasted animals under inhalation of anesthesia by retro orbital puncture method, plasma was separated by centrifugation at 2500 rpm for 15 min. Plasma glucose was estimated using kit (Cat No 1060-500, Stanbio laboratory, USA). Plasma level of insulin was determined using kits from Bio-Merieux, RCS, Lyon, France. Insulin resistance was calculated using the homeostasis model assessment.

Estimation of leptin and adiponectin

Plasma leptin and adiponectin levels were measured by using enzyme-linked immunosorbent assay kits (Crystal Chem, Downer's Grove, IL, USA), performed in duplicate, as per the manufacturer's guidelines and expressed in ng mL^{-1}.

Assay of plasma amylase and lipase

Plasma amylase and lipase activities were determined by kinetic method using the commercial kits available at Labtest®, Minas Gerais, Brazil and Bioclin®, Minas Gerais, Brazil, respectively.

Oral glucose tolerance test (OGTT)

OGTT was performed at the end of the experiment, after overnight fasting, glucose was administered orogastrically at a dose of 2.0 g/kg BW and blood samples were collected from supra orbital sinus at 0, 30, 60, 90 and 120 min. Glucose levels were estimated at all intervals.[5]

Plasma lipid analysis

For estimation of lipid profiles, blood samples were centrifuged at 2500 rpm/min for 15 min to separate plasma, which was then stored at -80°C for further biochemical analysis. Total cholesterol, HDL, LDL and triacylglyceride levels were estimated by CHOD-PAP method and GPO-PAP method.[11]

Tissue lipid analysis

Tissue lipids were extracted from experimental animals by the method of Floch *et al.*, (1957)[18] using a chloroform-methanol mixture (2:1, v/v). The liver tissue was homogenized in cold chloroform-methanol (2:1, v/v) and contents were extracted after 24 h. The extraction was repeated four times. The combined filtrate was washed with 0.7% KCl and the aqueous layer was discarded. The organic layer was made up to a known volume with chloroform and used for tissue lipid analysis.

Assay of key lipid metabolizing enzymes

Enzyme activities were analyzed as per the procedures described in respective kits. Carnitine palmitoyl transferase (CPT) was estimated by ELISA Kit (Cat No MBS705997 Mybiosource), total Acetyl-CoA carboxylase (ACC) was measured by ELISA Kit (Cat No 7996 Path Scan). Total fatty acid synthase (FAS) was assayed by ELISA Kit (Cat No 7689 MAK107 Sigma Aldrich, USA). Lecithin-cholesterol acyltransferase (LCAT) (Roar Biomedical, Inc.), Lipoprotein lipase (LPL) (Cat No STA-610 Cell biolabs INC, USA) and HMG-CoA reductase activity (Cat No CS1090 Sigma Aldrich, USA) were measured by kit methods.[19]

Liver antioxidants analysis

After the completion of experimental period rats we anesthetized and sacrificed. Liver was excised, rinsed in ice cold normal saline followed by ice-cold 10% KCl solution, blotted, dried and weighed. A 10% w/v homogenate was prepared with ice-cold KCl solution and centrifuged at 2000 rpm for 10 min at 4°C. The supernatant thus obtained was used for the estimation of thiobarbituric acid reactive substances (TBARS),[20] assay of catalase (CAT),[21] reduced glutathione (GSH), superoxide dismutase (SOD),[22] and glutathione peroxidase (GpX).[23]

Semi quantitative RT-PCR analysis

Total RNA was isolated from the adipose tissue by using tri-reagent (Sigma-Aldrich, USA) according to manufacturer's protocol and reverse transcribed to obtain cDNA using DNA synthesis kit (Applied Biosystems, Foster City, USA). Twenty nanogrames of cDNA was used for semi quantitative PCR. The PCR amplification was performed for 38 cycles using the following cycling conditions: 30 sec of denaturation at 94°C, 30 sec of annealing at 59°C and 1 min of extension at 72°C, with following primers: *FAS* (F:ATGTGGTACGGAAGGTGGAG; R:TGGCTACCTTCGTCTGTGTG), *PPAR-γ* (F: CTGACCCAATGGT TGCTGATTAC; R:GGACGCAGGCTCTACTTTGATC), leptin (F: GAGACCTCCTCCATCTGCTG; R: CATTCAGGGCTAAGGTCCAA) and *TNF-α* (F: GTCGTAGCAAACCACCAAG; R: AGAGAACCTGGGA

GTAGATAAG)β-Actin(F:GGCACCACACTTTCTACAAT;R:AGGTCT CAAACATGATCTGG).

Histopathological examination

Adipose tissues from all groups of rats were removed and kept in 10% formalin buffer solution. A small piece of tissue was sectioned with microtome, fixed on slides, stained with haematoxilin and eosin (H *and* E) and observed under optical microscope.

Statistical analysis

Results are expressed as mean ± SD (standard deviation). The statistical analysis of results was done by using t-test and one-way analysis of variance (ANOVA) followed by Ducan's. Values with $p < 0.05$ were considered to be statistically significant.

RESULTS

Preliminary phytochemical analysis

Phytochemical analysis of different extracts of *Terminalia tomentosa* (TT) was carried out according to Harborne methods with slight modifications. The presence of flavonoids, saponins, glycosides, terpenoids, amino acids, alkaloids, carbohydrates, phenolic compounds, and proteins was noticed. Based on phytochemical analysis and toxicological evaluation, ethanolic fraction of *T. tomentosa* bark was used for further investigation.

HPLC analysis of EFTT

HPLC analysis of EFTT revealed the presence of Ellagic acid, Dimethyl ellagic acid, Pentamethyl flavellagic acid, Trimethyl flavellagic acid and β-sitosterol based on their retention times (13.7 min, 14.7 min, 17.3 min, 21.1 min and 18.1 min, respectively) as represented in Figure 1.

Consumption of HFD for 18 weeks produced a substantial increase in body weight, fat free mass, total fat, fat % and decrease in bone mineral concentration (BMC) and bone mineral density (BMD) of experimental rats as summarized in Table 1. While the fat % of normal diet control rats was 5.2%, HFD-fed rats showed 21.6%, indicating 4 fold increase. However, oral administration of EFTT in dose dependent manner (50, 100 and 200 mg/kg BW) for 6 weeks, caused significant ($p < 0.05$) decrease in body weight, fat free mass, fat % but increased BMC and BMD [Figure 2a], but there was no significant change in food intake, water intake and urine volume as depicted in Table 1. Among different doses administered 200 mg/kg BW of EFTT showed more promising activity.
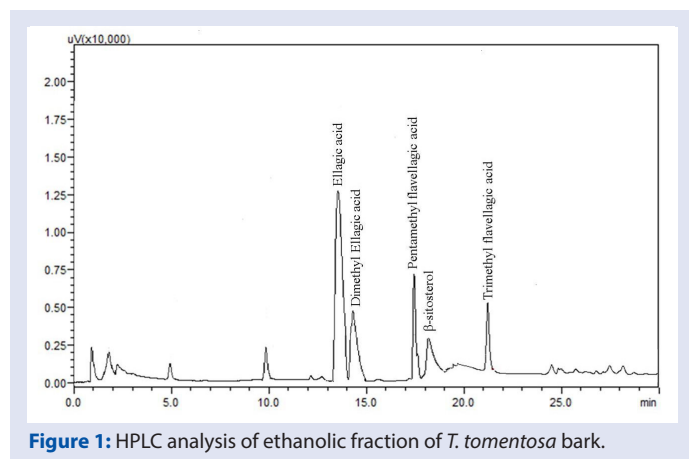

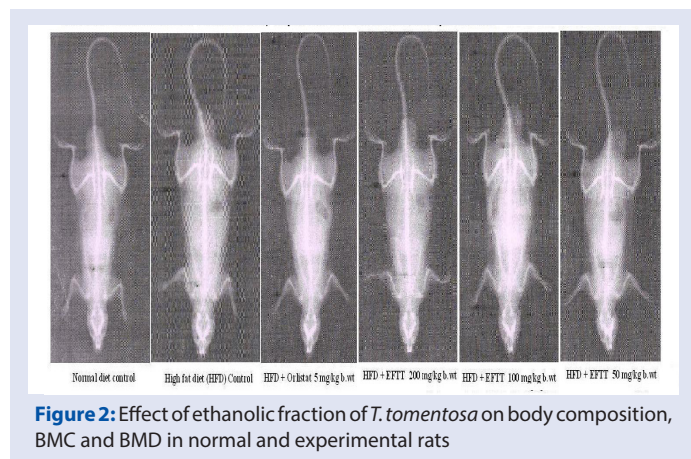**Figure 1:** HPLC analysis of ethanolic fraction of *T. tomentosa* bark.

Figure 2: Effect of ethanolic fraction of *T. tomentosa* on body composition, BMC and BMD in normal and experimental rats

Table 1: Effect of EFTT on body composition, BMC, BMD and nutrient metabolites in experimental rats

Parameters	Body weight (g)	Lean Mass (g)	Fat (%)	BMC (g)	BMD (g/sq/cm)	Water intake (ml/day)	Food intake (g)	Urine volume (ml)
Normal Control	285±13.5	270±4.9	5.2±0.4	7.8 ±0.61	0.16±0.001	18.9±2.1	12.3±1.1	6.9±0.2
High Fat Diet Control	487±11.9[a*]	381.6±11.2[a*]	21.6±1.4[a*]	5.4±0.21[a*]	0.11±0.002[a*]	19.1±2.3	13.1±1.4	7.1±0.4
HFD+Orlistat 5mg/kg	321±9.8[b*]	290.8±7.2[b*]	9.4±1.1[b*]	6.9±0.71[b*]	0.12±0.001	17.2±3.2	13.3±1.2	6.8±0.5
HFD+EETB 50mg/kgb.wt	391±13.2[b*]	338.9±7.9	13.3±1.5	6.9±0.01[b*]	0.13±0.003	19.1±1.1	13.5±1.8	7.2±0.4
HFD+ EETB 100 mg/kg b.wt	364±7.6[b*]	326.7±11.2	10.4±0.8[b*]	7±0.03[b*]	0.14±0.001	18.8±2.6	13.7±1.1	6.7±0.1
HFD+ EETB 200 mg/kg b.wt	345±14.2[b*]	309±6.7[b*]	10.2±1.2[b*]	7.1±0.02[b*]	0.17±0.002[b*]	18.7±1.8	13.9±1.4	6.6±0.4

Values are mean ± SD, n = 6; Values are statistically significant at *p<0.05; [a] significantly different from normal control; [b]Significantly different from HFD control.

Effect of EFTT on glucose, insulin and insulin resistance in experimental rats

There was a marked elevation in plasma glucose, insulin and insulin resistance in HFD-induced obese rats when compared to normal control group. The levels of plasma glucose, insulin and insulin resistance in control and experimental obese rats are shown in Table 2. However, oral administration of EFTT (200 mg/kg BW) significantly ($p < 0.05$) attenuated the above alterations.

Effect of EFTT on leptin, adiponectin and amylase and lipase in experimental rats

The levels of leptin, adiponectin and the activities of amylase and pancreatic lipase of normal and experimental obese rats are represented in Figure 3. There was a significant ($P < 0.05$) decrease in adiponectin level but increase in leptin, amylase and pancreatic lipase activities in HFD-fed groups when compared to normal control rats. Administration of 200 mg/kg BW of EFTT to obese rats significantly alleviated the above alterations.

Effect of EFTT on oral glucose tolerance test

Figure 4 summarizes the results of oral glucose tolerance test (OGTT) of the control and experimental obese rats. In normal control rats, maximum elevation in blood glucose level was observed at 60 min after glucose load and declined to near basal level at 120 min, whereas, in HFD-induced obese rats, the peak increase in blood glucose level was noticed even after 60 min and remained high over the next 60 min. Interestingly, oral administration of EFTT obese rats at a dose of 200 mg/kg BW elicited a significant ($p< 0.05$) decrease in blood glucose level at 60 min and beyond when compared with HFD control rats.

Effect of EFTT on organs weight

The changes in organs weight in control and HFD group of animals during the experiment are depicted in Table 3. Consumption of HFD produced a significant ($p < 0.05$) increase in weights of liver, fat pads (retraperitonoial, mesenteric and epididymal fat), kidney and heart compared to normal control group. Oral administration of EFTT significantly ($p < 0.05$) mitigated raise in organ weights in contrast to HFD control group, and the most profound effect was noted with 200 mg/kg BW of EFTT.

Effect of EFTT on circulatory and tissue lipid profiles

Table 4 represents the plasma levels of total cholesterol, FFAs, TGs, PLs, HDL, LDL and VLDL and liver lipids (total cholesterol, FFAs, TGs and PLs) of control and HFD-fed rats. HDL level was found to be decreased,

Table 2: Estimation of plasma glucose, insulin, insulin resistance in experimental rats

Parameters	Glucose (mg/dl)	Insulin (µU/ml-1)	Insulin resistance
Normal Control	79.1±4.9	4.1±0.9	3.9±0.07
High Fat Diet Control	166.7±9.8a*	11.3±1.5 a*	6.7±0.04 a*
HFD+Orlistat 5 mg/kg	113.4±7.9 b**	7.2±1.1 b*	4.1±0.01 b*
HFD+ EFTT 50 mg/kg b.wt	132.2±11.2 b*	10.1±0.4b*	6.3±.009b*
HFD+ EFTT 100 mg/kg b.wt	121.1±4.6	9.6±0.6	5.2±0.02
HFD+ EFTT 200 mg/kg b.wt	116.7±6.1 b*	7.9±0.4 b*	4.7±0.03 b*

Values are mean ± SD, n = 6. Values are statistically significant at *p <0.05. [a] Significantly different from normal control. [b]Significantly different from HFD control.
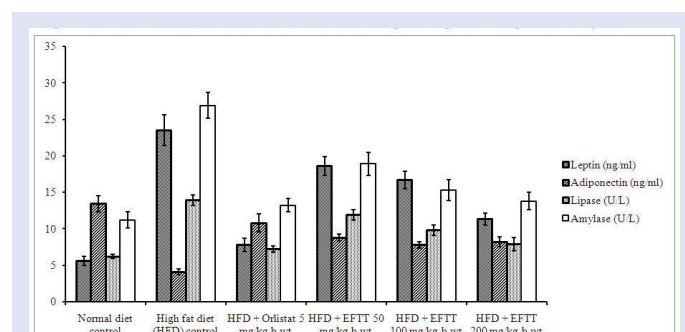
Figure 3: Effect of ethanolic fraction of T.tomentosa bark on Leptin, Adiponectin, Lipase and Amylase

Values are mean± SD, n=6; Values are statistically significant at *p<0.05; asignificantly different from normal control; significantly different from HFD control

while all other lipids were increased in plasma of HFD-fed rats. The concentrations of liver lipids were significantly increased in obese rats when compared to normal rats. Treatment with 200 mg/kg BW of EFTT significantly ($p < 0.05$) reduced the concentrations of plasma (Except HDL) and liver lipids in obese rats to near normal level.

Effect of EFTT on liver antioxidants

Figure 5 shows the level of antioxidants in control and experimental obese rats. There was a significant ($p < 0.05$) elevation in TBARS and decrease in SOD, CAT, GSH and GPx activities in HFD-fed rats when compared with control rats. Oral administration of EFTT (200 mg/kg BW) brought back the above alterations to near normal levels.

Table 3: Effect of EFTT on organ weights

Parameters	Liver (g)	Fat (g/100 g b.wt fat)			Kidney (g)	Heart (g)	Testis (g)
		RF	MF	EF			
Normal Control	8.4±1.3	1.7±0.12	0.6±0.01	1.1±0.03	2.4±0.07	0.9±0.01	1.8±0.04
High Fat Diet Control	15.2±1.8a*	3.9±0.21a*	1.2±0.02a*	1.9±0.12a*	3.6±0.12 a*	1.5±0.02a*	2.9±0.03a*
HFD+Orlistat 5 mg/kg	10.2±0.9b*	1.9±0.31b*	0.7±0.04 b*	1.2±0.03 b*	2.5±0.01 b*	1.1±0.01 b*	2.1±0.01 b*
HFD+ EFTT 50 mg/kg b.wt	13.8±1.1b*	3.1±0.14b*	0.9±0.12 b*	1.7±0.01 b*	2.6±0.01 b*	1.3±0.02 b*	2.2±0.04 b*
HFD+ EFTT 100 mg/kg b.wt	12.1±1.7	2.8±0.17	0.9±0.11	1.4±0.01	2.7±0.03	1.2±0.01	2.2±0.01
HFD+ EFTT 200 mg/kg b.wt	10.8±0.7 b*	2..1±0.25b*	0.7±0.22 b*	1.3±0.02 b*	2.5±0.01 b*	1.1±0.04 b*	2.0±0.02 b*

Values are mean ± SD, n = 6. Values are statistically significant at *p <0.05. aSignificantly different from normal control. bSignificantly different from HFD control.
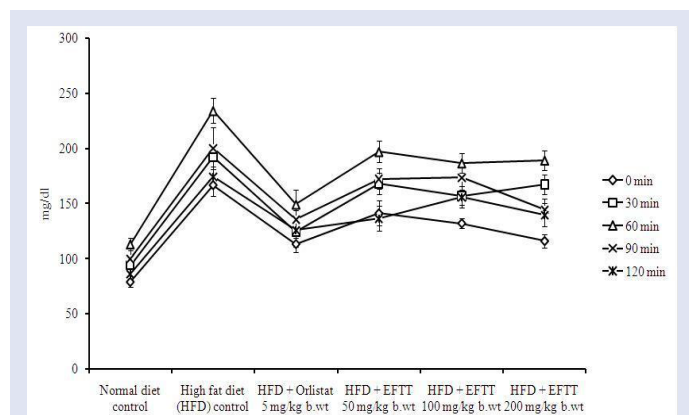

Figure 4: Effect of ethanolic fraction of T.tomentosa bark on glucose tolerance test in normal and experimental rats

Values are mean± SD, n=6; Values are statistically significant at *p<0.05; asignificantly different from normal control; bignificantly different from HFD control.

Figure 5: Effect of ethanolic fraction of T.tomentosa bark on liver anti oxidants in normal and experimental rats

Values are mean± SD, n=6; Values are statistically significant at *p<0.05; asignificantly different from normal control; bignificantly different from HFD control.
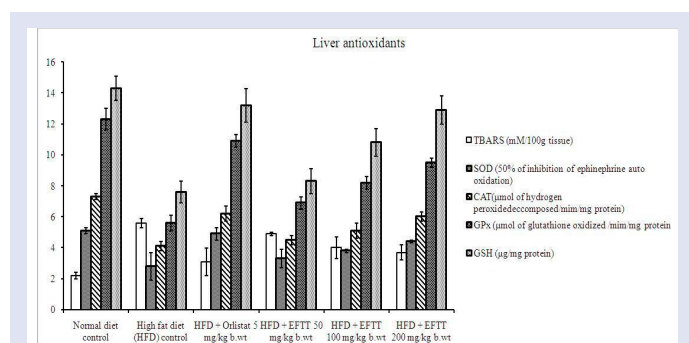
Effect of EFTT on lipid metabolic marker enzymes

Figure 6 depicts the levels of CPT-1, ACC, FAS, LCAT, LPL, and HMGR of control and experimental obese rats. Significantly ($p < 0.05$) decreased levels of CPT-1, LCAT, LPL and concomitant increase in the levels of ACC, FAS, HMGR was observed in tissues of HFD control rats when compared with normal control rats. However, EFTT (200 mg/kg BW) upon oral administration significantly ($p < 0.05$) increased the activity of CPT-1, LCAT, LPL but decreased the activity of ACC, FAS, HMGR in tissues of treated obese rats.
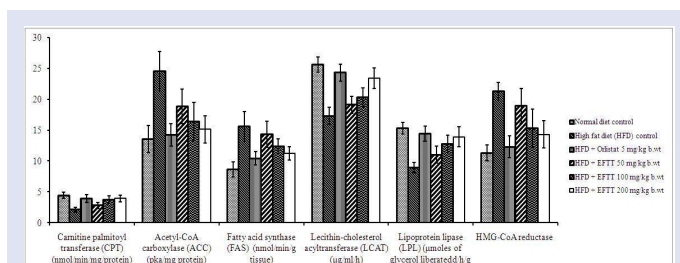

Figure 6: Effect of ethanolic fraction of T.tomentosa bark on lipid metablosim enzymes

Values are mean± SD, n=6; Values are statistically significant at *p<0.05; asignificantly different from normal control; bsignificantly different from HFD control.

Effect of EFTT on molecular expression of obesity associated genes

Figure 7 represents the mRNA expression levels of PPAR γ, FAS, leptin and TNF- α of control and experimental obese rats. Increased expression levels of PPAR γ, FAS, leptin and TNF- α were observed in HFD control rats when compared with normal control rats. Oral administration of EFTT considerably decreased the expression level of the above genes in obese rats in a dose dependent manner.

Effect of EFTT on adipocytes size and volume

High fat diet consumption caused apparent alterations in adipose tissue architecture. It increased adipocytes' size and volume in HFD-fed control group when compared to normal control diet fed group as shown in Figure 8. Interestingly, oral administration of EFTT at doses of 100 and 200 mg/kg BW for 42 days has considerably reduced the adipocytes size, volume and other aspects even better than orlistat.

DISCUSSION

Diet-induced obesity (DIO) in rodent model would closely imitate the accepted metabolic features of human obesity, therefore, helps to find effective functional foods or therapeutics for preventing and/or reducing obesity.[24-26] Apart from genetic factors, dietary factors, particularly the consumption of high fat/caloric diets are important predisposing factors for development of obesity ailments.

In the present study, enhanced body weight, total body fat, fat % and decreased bone mineral concentration and bone mineral density were observed in HFD-fed rats, which are the hallmarks of obesity. Development of obesity in response to HFD feeding could be explained by the ability of HFD to induce a positive-energy balance that results in an increased bodyweight and plasma comorbidity factors.[4,11-13] It might be due to consumption of a diet rich in energy in the form of saturated fats (beef tallow), and its accumulation in various body parts and fat pads [27] leads to excessive growth of adipose tissue. Comparable effects have been

Table 4: Effect of EFTT on circulatory and tissue lipid profiles

	Plasma lipid Profiles (mg/dl)					Liver lipid profiles (mg/g/dl)			
	TC	TG	HDL	LDL	VLDL	TC	TG	Phospholipids	FFA
Normal Control	155.7±9.3	167.8±12.3	54.2±2.1	51.4±4.5	45.3±3.2	84.3±5.6	56.9±4.6	49.6±4.5	64.3±4.6
High Fat Diet Control	289.9±16.4 a*	324.5±9.8 a*	29.8±3.5 a*	167.8±8.9 a*	102.3±5.7 a*	156.3±6.1 a*	97.8±6.6 a*	129.9±3.3 a*	102.4±6.7 a*
HFD+Orlistat 5 mg/kg	195.3±10.7 b*	201.1±10.2 b*	46.7±3.3 b*	82.2±5.4 b*	57.3±6.7 b*	91.3±7.8 b*	78.9±6.3 b*	61.4±5.5 b*	73.2±3.4 b*
HFD+ EFTT 50 mg/kg b.wt	267±12.4 b*	284.1±15.6 b*	29.1±2.2 b*	154.3±8.9 b*	81.2±5.4 b*	126.3±9.9 b*	81.5±5.6 b*	92.3±4.6 b*	89.2±5.4 b*
HFD+ EFTT 100 mg/kg b.wt	233±11.3	241.6±13.4	39.9±5.4	126.2±7.8	77.3±10.9	115.6±14.5	96.5±9.8	73.4±6.7	80.3±10.9
HFD+ EFTT 200 mg/kg b.wt	206.7±13.4 b*	217.7±14.4 b*	44.5±3.3 b*	80.8±10.2 b*	60.3±6.2 b*	91.1±9.1 b*	75,9±9.4 b*	67.2±3.4 b*	76.3±6.2 b*

Values are mean ± SD, n = 6. Values are statistically significant at *p <0.05. [a] Significantly different from normal control. [b] Significantly different from HFD control.
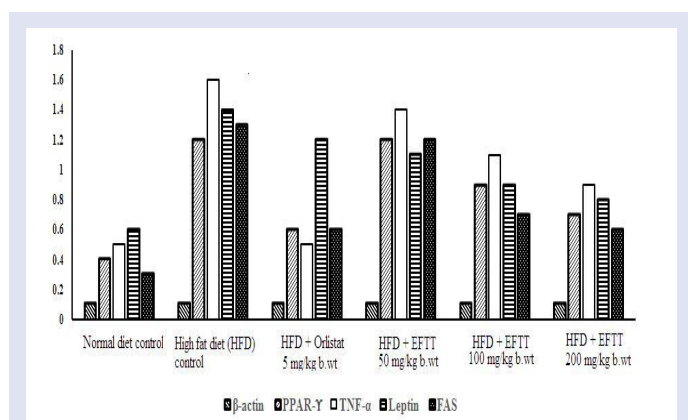

Figure 7: Effect of ethanolic fraction of *T. tomentosa* bark on molecular expression of obesity associated genes. β-actin, PPAR-Y, TNF-α Leptin, FAS

Figure 8: Effect of ethanolic fraction of T.tomentosa bark on adipocyte in normal and experimental rats

reported by earlier studies on HFD induced obesity in rats. HFD caused rodents to develop visceral adiposity, hyperglycemia, dyslipidemia, hyper insulinemia and hepatic steatosis, which are distinctly linked with human obesity.[28] Fascinatingly, oral administration of EFTT had significantly reversed HFD induced alterations in a dose dependent manner.

Diet induced obesity in animals revealed the relationship between obesity and poor bone quality, as resolute by suppressed micro architectural and biomechanical properties.[29] Extreme obesity has been shown to have negative impact on bone health, which is associated with profound increase in the levels of obesity-related factors like TNF-α and IL-6.[30-32] In the present study, the elevated level of TNF-α was observed in HFD induced obese rats. Numerous scientific reports suggested the importance of chronic inflammation in the development of obesity, which can be detrimental to bone.[32] Oral administration of EFTT 100 and 200 mg/kg BW enhanced bone mineral density and bone mineral content in obese rats as shown in Figure 2b and Table 1. This is in line with previous report.[30] On the contrary, a few reports suggested that individuals with increased body weight also have enhanced BMD. So, the relationship between osteoblasts and adipocytes is still controversial. This contradiction could mainly be due to difference in the extent of obese state, dietary pattern and the age at which obesity is attained or the age at which obesity onsets.

Rodents who were fed a high fat diet showed fat accumulation, low grade inflammation and insulin insensitivity in peripheral tissues, including the liver.[31] It is well known that insulin can indirectly inspire leptin secretion through its collective effects on the metabolism of nutrients,
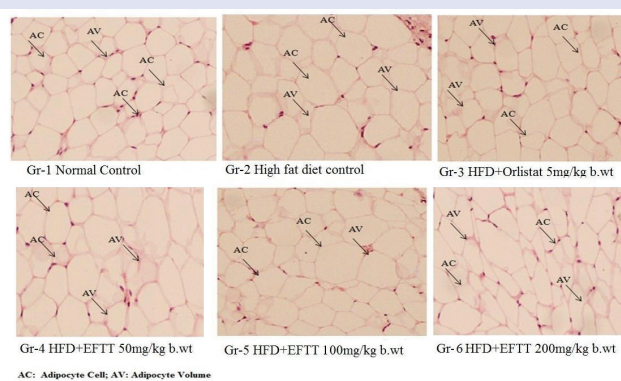
especially on glucose utilization in adipocytes. In the present study, HFD-fed rats developed a hyperglycemic state associated with insulin resistance and/or glucose intolerance. The fact that slight hyperglycemia evolved in spite of the marked hyperinsulinemia indicates a successful induction of insulin resistance in HFD-induced obese rat model.[33] In addition, diminished hepatic and muscular uptake of glucose produced hyperlipidemia due to increased fat mobilization from adipose tissue and resistance to the antilipolytic actions of insulin. In the present study, we observed that EFTT treatment substantially alleviated the HFD-induced alterations in plasma insulin, blood glucose, insulin resistance and OGTT level in a dose dependent manner, suggesting that EFTT could do this possibly by regulating the cell energy metabolism or reducing free fatty acids.[29]

Adipose tissue plays a key role not only in fat storage but also as an endocrine tissue. Adipose tissue secretes adipokines (e.g. leptin, adiponectin, resistin, visfatin, tumour necrosis factor and interleukin-6), which play a role in energy regulation.[34-36] Leptin and adiponectin are the two most important adipocyte-secreted hormones known to play vital roles in the regulation of metabolic homeostasis.[31] During the development of obesity, elevated level of leptin and concomitant decreased level of adiponectin were observed.[16-18] Their secretion is positively interrelated with the extent of the triglyceride stores in adipocytes. In the present study, increased leptin and decreased adiponectin levels were observed in plasma of HFD control group when compared with normal control group. The results of the current study agree with those previously reported.[29] Oral administration of EFTT (200 mg/kg BW)

lowered leptin and increased adiponectin in plasma of HFD induced obese rats. Several polyphenols have been shown to exert profound effect on leptin levels, lipid catabolism and decreasing insulin resistance thus may play essential roles in regulating obesity. In the present study, it is promising that *Terminalia tomentosa* suppressed the body weight development possibly through improving leptin sensitivity in the treated groups, which would enhance energy expenditure and reduced body weight. The role of phytoconstituents mentioned in Figure 1 may be well attributed to this therapeutic effect.

Amylase and lipase are vital digestive enzymes in digestion of carbohydrates and lipids, and determining factors for the functional efficiency of pancreas. Quite often, such enzymes are used as targets for drug design in an attempt to treat several metabolic disorders including obesity and diabetes.[31] The potential of polyphenols as inhibitors of such digestive enzymes has been recently reviewed.[35] In the present study, pancreatic lipase and amylase levels were found to be increased in HFD-fed rats, which were reduced upon EFTT (200 mg/kg BW) administration [Figure 5]. It is quite possible that this fraction may possess bioactive factors that inhibit pancreatic lipase and α-amylase, which would hinder the digestion and absorption of lipids and carbohydrates leading to lesser caloric intake and weight loss in EFTT treated groups.

An anomaly in lipid metabolism is a principal reason for dyslipidemia, which is a major risk factor for metabolic syndrome including obesity.[14] Elevated levels of LDL-cholesterol is the risk feature for heart disease, where as increased HDL-C is supportive in transporting excess cholesterol to the liver.[18,19] In the present study, HFD-induced obese rats showed anomalies in plasma and tissue biomarkers related to lipid metabolism, which were mitigated by TTE administration [Table 4]. During fed state, increment of chylomicrons synthesis and absorption is a common factor, which leads to an increase in TCs, TGs level and endogenous VLDL production. Even though the majority of circulating FFAs take place from outside the intra-abdominal region, FFAs released from adipose tissues serve as an indicator for insulin resistance,[7,8] and also it may be due to the development of leptin resistance. TGs elevation was due to dietary cholesterol that had been shown to reduce fatty acid oxidation, which in turn increased the levels of hepatic and plasma TGs, and the excessive accumulation of TGs in the lipid stores is associated with a number of metabolic complications,[2,37] In this study, the elevated level of phospholipids in HFD control group may be due to the elevated levels of FFA and total cholesterol.[38] Amplified free fatty acid (FFA) flux from adipose tissue to non adipose tissues especially liver ensuing from abnormalities of fat metabolism, participates in increased synthesis and secretion of VLDL. The positive mechanism of lipid profile caused by EFTT might be due to its influences in intestinal fat absorption, transportation and deposition. Our findings are supported who worked on *In vitro* and *In vivo* models of obesity. In the present study, oral supplementation of *Terminalia tomentosa* significantly lowered TC, TGs, FFAs and PLs in both plasma and liver and LDL-C levels in plasma of HFD-fed rats. In another study,[33] it was reported that supplementation of certain phytoconstituents to fed animals lead to hypolipidemic state by decreasing cholesterol absorption and secretion from the intestine, which lead to the lowered availability of FFAs to the liver.

Experimentally induced obese condition leads to increased ROS production and reduction of antioxidant defense system. The combination of both leads to cellular and tissue damage promoted in various metabolic complications.[37,39] Free radicals may also be formed via the auto-oxidation of unsaturated fatty acids in plasma and membrane lipids and react with other polyunsaturated fatty acids of membrane leading to lipid peroxidation that will in turn result in elevated production of free radicals. Previous findings reported that

free radicals enhanced lipid peroxidation and associated impairment of tissue antioxidant marker enzymes in obese animals. Thus, reduction in lipid peroxidation and improved antioxidant defense system could be one of the possible mechanisms by which a drug can contribute to the cure and prevention of obesity and related complications. In the present study, oral administration of EFTT exactly did the same as evident from the reduced levels of TBARS and hydroperoxides, which were elevated in obese animals. Similarly, oral treatment of EFTT (200 mg/kg BW) boosted the cellular antioxidant defense by restoring the derangement of antioxidant markers (induced by free radicals during obesity) such as SOD, catalase, and GPx in liver tissue.

HMG Co-A reductase, ACC, LCAT, CPT, FAS, LPL are important enzymes in lipid metabolism. Hydroxy-3-methylglutaryl CoA reductase (HMGR) catalyses the rate-limiting step in cholesterol biosynthesis and its activity was found to be significantly increased in the liver of diabetic rats.[32] LCAT, a glycoprotein with a high content of carbohydrate residues, is synthesized mainly in the liver. The esterification of cholesterol on circulating lipoproteins is mediated by LCAT, and this phenomenon is critically important in facilitating the stability of HDL particle and its maturation.[36] LPL, a key enzyme in the regulation of the flux of fatty acids, mainly exist in tissues such as adipose tissue, skeletal muscle and heart, and it should be noted that the heart has the highest amount of LPL activity when compared to other tissues in the body. Phosphorylated AMPK phosphorylates (inactivates) ACC and lowers the intracellular malonyl-CoA level, which is the substrate for fatty acid synthesis and at the same time, the inhibitor of CPT-1, the rate-limiting enzyme of mitochondrial fatty acid oxidation. Suppression of ACC leads to the inhibition of endogenous FFAs. In the present study, elevated level of ACC, LCAT, HMGR and declined level in CPT, FAS and LPL were observed in HFD-fed rats. Administration of EFTT significantly attenuated all these enzymes profile near to normal level.

Obesity is connected with lipid accretion not only in adipose tissues but also in non adipose tissues, such as the liver and skeletal muscle.[32] Skeletal muscle is a major site of fatty acid catabolism and abnormal fat accumulation in skeletal muscle causes metabolic disorders including obesity. It is well known that during obesity PPARγ is activated by SREBP1c that leads to decreased FFA oxidation, increased FAS activity and increased lipogenesis.[34] The ROS produced during inflammation is considered as a predisposing factor, and increased mitochondrial ROS production during hyperglycemia may be central to much of the pathology of obesity[39-40] there by elevating the level of TNF-α. In the present study, there was an elevated mRNA level of PPAR-γ, FAS, leptin and TNF-α in adipose tissue of HFD induced obese rats, which were down regulated with oral administration of EFTT (200 mg/kg BW).

CONCLUSION

Our study demonstrates that EFTT could substantially attenuate obesity related biochemical, physiological and molecular alterations in HFD-induced obese rats, possibly through regulation of key lipid metabolizing enzymes, leptin and adiponectin levels and through down regulation of PPAR-γ, leptin, TNF- α and FAS. We suggest that ethanolic fraction of *T. tomentosa* bark may be considered as a potential candidate to develop antiobesity drugs.

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Conflicts of interest

There are no conflicts of interest.

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