



Article

***Terminalia bellirica* Extract Attenuates Fat Accumulation Through Modulation of Obesity-Related Dysmetabolism in 3T3-L1 Adipocytes and High-Fat Diet-Induced Obese Mice**

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Abstract

Terminalia bellirica extract (TBE) has long been utilized in Ayurvedic medicine across Indian and surrounding regions for diverse therapeutic applications. Despite its traditional prominence, systematic investigations addressing the anti-obesity efficacy and underlying mechanisms remain limited. In this study, we evaluated the anti-obesity potential of TBE using both 3T3-L1 adipocyte and high-fat diet (HFD)-induced mice model. In vitro studies using 3T3-L1 adipocytes demonstrated that TBE significantly inhibited lipid accumulation and downregulated key genes involved in adipogenesis and lipogenesis, while upregulating genes promoted lipolysis and energy metabolism. To validate these cellular effects in a physiological context, mice were randomly assigned to six groups: normal control (NC), HFD-induced obese (C), HFD with metformin (100 mg/kg b.w., PC), and HFD with TBE at 50, 100, and 200 mg/kg b.w. Consistent with the in vitro findings, TBE supplementation significantly reduced body weight gain, adipose tissue mass, and adipocyte size in HFD-induced obese mice. Taken together, these results indicate that TBE exerts anti-obesity effects through modulation of adipose tissue metabolic pathways, highlighting its therapeutic potential for obesity management.

Keywords: *Terminalia bellirica*; obesity; adipogenesis; lipogenesis



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1. Introduction

Obesity is a chronic metabolic disorder characterized by a persistent positive energy balance, with excess energy stored as triglycerides (TGs) in adipose tissue [1]. This excessive lipid storage induces a chronic low-grade inflammatory state, contributing to the dysregulation of adipokines, cytokines, and other signaling molecules produced by adipose tissue. Given that obesity results from complex molecular and cellular changes during adipocyte differentiation, physiology, and morphological remodeling, a comprehensive understanding of these mechanisms is essential for developing effective strategies to regulate obesity and its associated metabolic complications [2–4].

Alterations in adipogenesis, lipogenesis, lipolysis, and energy metabolism underlie the disrupted metabolic equilibrium observed in obesity [5]. The enhancement of adipogenesis and lipogenesis plays a critical role in obesity by promoting the differentiation of preadipocytes into mature adipocytes and facilitating the synthesis of TGs and free fatty acid (FFA). These processes are related to key transcription factors and genes such as the CCAAT/enhancer-binding protein (C/EBP) family, sterol regulatory element-binding protein 1c (SREBP1c), and fatty acid synthase (FAS) [6–8]. In contrast, lipolysis refers to the breakdown of stored TGs into FFA and glycerol through the activation of cyclic AMP-dependent protein kinase A (cAMP-PKA). Hormone-sensitive lipase (HSL) phosphorylated by PKA translocates to the lipid droplet surface, inducing FFA and glycerol release [9,10]. Energy metabolism is subsequently regulated through fatty acid oxidation facilitated by carnitine palmitoyltransferase 1 (CPT1) and heat-generating system mediated by uncoupling protein 1 (UCP1) [11,12].

Terminalia bellirica extract (TBE) has been extensively utilized in Ayurvedic medicine across India and neighboring regions to treat various ailments, including constipation, diarrhea, diabetes, hypertension, and inflammation. This plant is characterized by a complex phytochemical composition comprising gallic acid, chebulagic acid, and ellagic acid [13–15]. Several studies demonstrate beneficial effects of TBE in regulating lipid metabolism, enhancing insulin sensitivity, and reducing oxidative stress [16–18]. Building on the existing evidence supporting anti-obesity effects of TBE and its constituent compounds, this study aimed to evaluate the effects of TBE using comprehensive approaches involving both 3T3-L1 adipocytes and high-fat diet (HFD)-induced obese mouse models, with a focus on the key metabolic pathways related to obesity.

2. Results

2.1. TBE Treatment Downregulated Adipogenesis and Lipogenesis in Adipocytes

We observed elevated intracellular TG levels in differentiated adipocytes of the control (C) group compared to the normal control (NC) group (Figure 1A). The C group also exhibited significantly increased ratio of phosphorylated MAPK to MAPK, along with significantly elevated adipogenic transcription factors, including SREBP-1c, C/EBP α , and PPAR- γ , as well as G6PDH (Figure 1B,C). Furthermore, the ratios of *p*-ACL/ACL and *p*-ACC/ACC were significantly suppressed, whereas the levels of FAS and LPL were significantly elevated in the C group. However, treatment with TBE during the differentiation of 3T3-L1 preadipocytes significantly reduced lipid accumulation, as indicated by decreased intracellular TG levels compared to the C group. TBE treatment effectively suppressed the differentiation-induced upregulation of adipogenesis and lipogenesis-related factors. Additionally, in differentiated adipocytes, TBE treatment upregulated adiponectin protein expression while downregulating leptin protein expression relative to the C group.

2.2. TBE Treatment Promoted Lipolysis and Energy Metabolism in Adipocytes

An increase in TG levels and Oil red O staining quantification and cAMP level were detected in the C group compared to the NC group (Figure 2A,D). Protein expressions of PKA and phosphorylated HSL were significantly reduced, whereas PDE3B and perilipin expression were markedly elevated in the C group relative to the NC group (Figure 2B,C). Additionally, energy metabolism-related proteins, including ratio of phosphorylated AMPK to AMPK, UCP-1, and CPT-1, were significantly decreased in the C group compared to the NC group. However, treatment with TBE promoted lipolysis and energy metabolism pathways relative to the C group, resulting in a decrease in intracellular TG levels and Oil red O staining quantification in the TBE group.

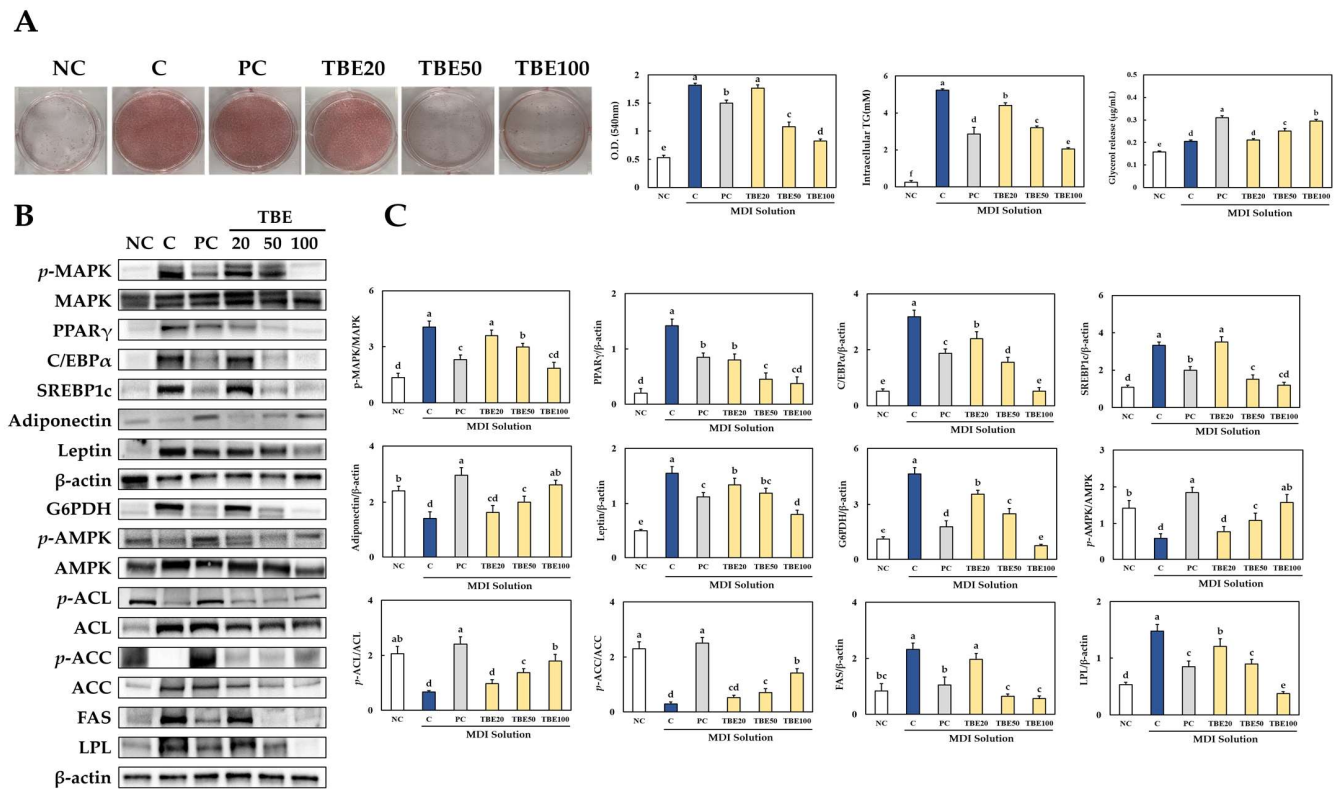


Figure 1. The Effect of TBE treatment on the triglyceride accumulation (A), adipogenesis and lipogenesis (B), band image; (C) quantification in 3T3-L1 cells after differentiation. NC, undifferentiated 3T3-L1 cells; C, differentiated 3T3-L1 cells; PC differentiated 3T3-L1 cells treated with metformin 1 mM; TBE20, differentiated 3T3-L1 treated with *Terminalia bellirica* extract (TBE) 20 μ g/mL; TBE50, differentiated 3T3-L1 treated with TBE 50 μ g/mL; TBE100, differentiated 3T3-L1 treated with TBE 100 μ g/mL. Values are presented as mean $\pm$ standard deviation ($n = 3$ biological replicates, representing three independent wells per condition), and different superscript letters indicate significance at $p < 0.05$. The molecular weights of the detected proteins are as follows: *p*-MAPK, 42, 44 kDa; MAPK, 42, 44 kDa; PPAR γ , 53, 57 kDa; C/EBP α , 28, 42 kDa; SREBP1c, 110–130 kDa; adiponectin, 27 kDa; leptin, 19 kDa; G6PDH, 59 kDa; *p*-AMPK, 62 kDa; AMPK, 62 kDa; *p*-ACL, 125 kDa; ACL, 125 kDa; *p*-ACC, 280 kDa; ACC, 280 kDa; FAS, 273 kDa; LPL, 36, 53 kDa; β -actin 42 kDa.

2.3. TBE Supplementation Prevented HFD-Induced Obesity and Modulated Biochemical Parameters

The initial body weights did not differ significantly among all groups, indicating comparable baseline conditions prior to dietary intervention. However, at the end of the experimental period, the HFD control (C) group exhibited a significant increase in body weight gain, as well as liver and adipose tissue weights, compared to the normal control (NC) group. Dietary supplementation with TBE significantly reduced body weight gain and adipose tissue weight in HFD-fed mice relative to the C group, suggesting that TBE supplementation effectively mitigates HFD-induced obesity. Food intake was significantly increased in the C group, while TBE supplementation resulted in a significant reduction in food intake compared with the C group. However, no significant differences in food efficiency ratio (FER) were observed among the HFD-fed groups (Table 1) ($p < 0.05$). A significant increase in adipose tissue mass was observed in mice fed HFD compared to the NC group (Figure 3d). Additionally, adipocyte size was markedly larger in the C group relative to the NC group (Figure 3g). However, supplementation with TBE significantly reduced both adipose tissue mass and lipid droplet diameter compared to the C group.

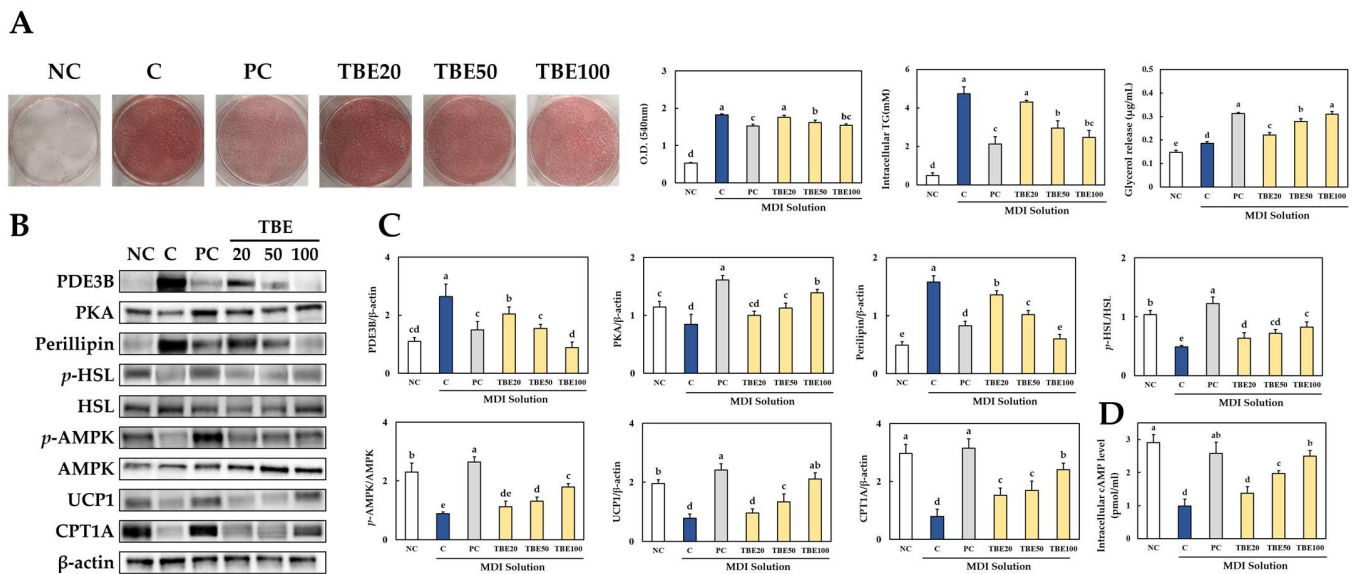


Figure 2. The effect of TBE treatment on the triglyceride accumulation (A) and lipolysis and energy metabolism (B), band image; (C) quantification and cAMP level (D) in 3T3-L1 cells after differentiation. NC, undifferentiated 3T3-L1 cells; C, differentiated 3T3-L1 cells; PC differentiated 3T3-L1 cells treated with metformin 1 mM; TBE20, differentiated 3T3-L1 treated with *Terminalia bellirica* extract (TBE) 20 µg/mL; TBE50, differentiated 3T3-L1 treated with TBE 50 µg/mL; TBE100, differentiated 3T3-L1 treated with TBE 100 µg/mL. Values are presented as mean ± standard deviation ($n = 3$ biological replicates, representing three independent wells per condition), and different superscript letters indicate significance at $p < 0.05$. The molecular weights of the detected proteins are as follows: PDE3B, 115 kDa; PKA, 42 kDa; perilipin, 62 kDa; p -HSL, 81, 83 kDa; HSL, 38, 86, 88 90 kDa; p -AMPK, 62 kDa; AMPK, 62 kDa; UCP1, 33 kDa; FABP4, CPT1A, 88 kDa; β -actin 42 kDa.

Table 1. The effect of *Terminalia bellirica* extract on body weight, weight gain, and organ and adipose tissue weight in mice with HFD-induced obesity.

Variables	Groups		HFD				
	NC	C	PC	TBE50	TBE100	TBE200	
Initial Body Weight (g)	20.27 ± 1.04 ^{NS*}	20.62 ± 1.72	20.91 ± 1.28	21.44 ± 0.21	21.23 ± 0.32	20.38 ± 0.41	
Final Body Weight (g)	30.66 ± 2.51 ^e	46.53 ± 2.07 ^a	35.83 ± 2.31 ^d	43.05 ± 2.32 ^b	40.22 ± 2.73 ^{bc}	38.90 ± 2.11 ^c	
Weight Gain **	10.40 ± 1.51 ^e	25.91 ± 1.96 ^a	14.92 ± 1.06 ^d	21.61 ± 2.42 ^b	18.99 ± 2.49 ^c	18.52 ± 2.27 ^c	
FER ***	6.06 ± 0.7 ^b	7.21 ± 0.52 ^a	7.20 ± 0.52 ^a	7.22 ± 0.59 ^a	7.26 ± 0.78 ^a	7.27 ± 0.76 ^a	
Food Intake (g/day)	2.55 ± 0.20 ^a	2.49 ± 0.30 ^a	2.31 ± 0.16 ^{cd}	2.36 ± 0.14 ^{bc}	2.24 ± 0.12 ^{cd}	2.19 ± 0.10 ^d	
Energy Intake (kcal/day)	9.43 ± 0.74 ^d	13.03 ± 1.58 ^a	12.08 ± 0.83 ^{bc}	12.36 ± 0.71 ^{bc}	11.72 ± 0.63 ^{bc}	11.47 ± 0.51 ^{cd}	
Organ Weight (g)							
Liver	1.18 ± 0.14 ^c	1.71 ± 0.22 ^a	1.46 ± 0.27 ^{ab}	1.57 ± 0.22 ^{ab}	1.52 ± 0.20 ^{ab}	1.42 ± 0.07 ^b	
Kidney	0.34 ± 0.01 ^{NS}	0.37 ± 0.02	0.37 ± 0.02	0.36 ± 0.03	0.35 ± 0.03	0.35 ± 0.02	
Spleen	0.09 ± 0.02 ^{NS}	0.10 ± 0.02	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.01	0.09 ± 0.02	
Adipose Tissue Weight (g)							
Total WAT	1.33 ± 0.34 ^e	6.36 ± 0.41 ^a	3.90 ± 0.66 ^d	5.87 ± 0.74 ^a	5.24 ± 0.18 ^b	4.65 ± 0.12 ^c	
Subcutaneous WAT	0.51 ± 0.12 ^e	2.77 ± 0.15 ^a	1.57 ± 0.25 ^d	2.62 ± 0.34 ^{ab}	2.43 ± 0.07 ^b	2.14 ± 0.15 ^c	
Visceral WAT	0.26 ± 0.09 ^d	1.29 ± 0.17 ^a	0.76 ± 0.14 ^c	1.02 ± 0.20 ^b	0.95 ± 0.10 ^b	0.85 ± 0.15 ^{bc}	
Epididymal WAT	0.57 ± 0.15 ^d	2.30 ± 0.21 ^a	1.57 ± 0.29 ^c	2.23 ± 0.26 ^a	1.86 ± 0.09 ^b	1.67 ± 0.08 ^{bc}	
BAT	0.11 ± 0.04 ^c	0.24 ± 0.04 ^a	0.14 ± 0.04 ^{bc}	0.22 ± 0.08 ^a	0.19 ± 0.02 ^{ab}	0.17 ± 0.02 ^{bc}	

NC, normal diet control; C, 60% high-fat diet (HFD)-induced obesity control; PC, metformin 100 mg/kg body weight (b.w.) in 60% HFD; TBE50, *Terminalia bellirica* extract (TBE) 50 mg/kg b.w. in 60% HFD; TBE100, TBE 100 mg/kg b.w. in 60% HFD; TBE200, TBE 200 mg/kg b.w. in 60% HFD; WAT, white adipose tissue; BAT, brown adipose tissue. Values are presented as mean ± standard deviation ($n = 8$), and different superscript letters indicate significance at $p < 0.05$. * NS: not significant. ** Weight gain (g/16 weeks) = final body weight (g) – initial body weight (g). *** FER (food efficiency rate) = weight gain (g)/total food consumption (g) × 100.

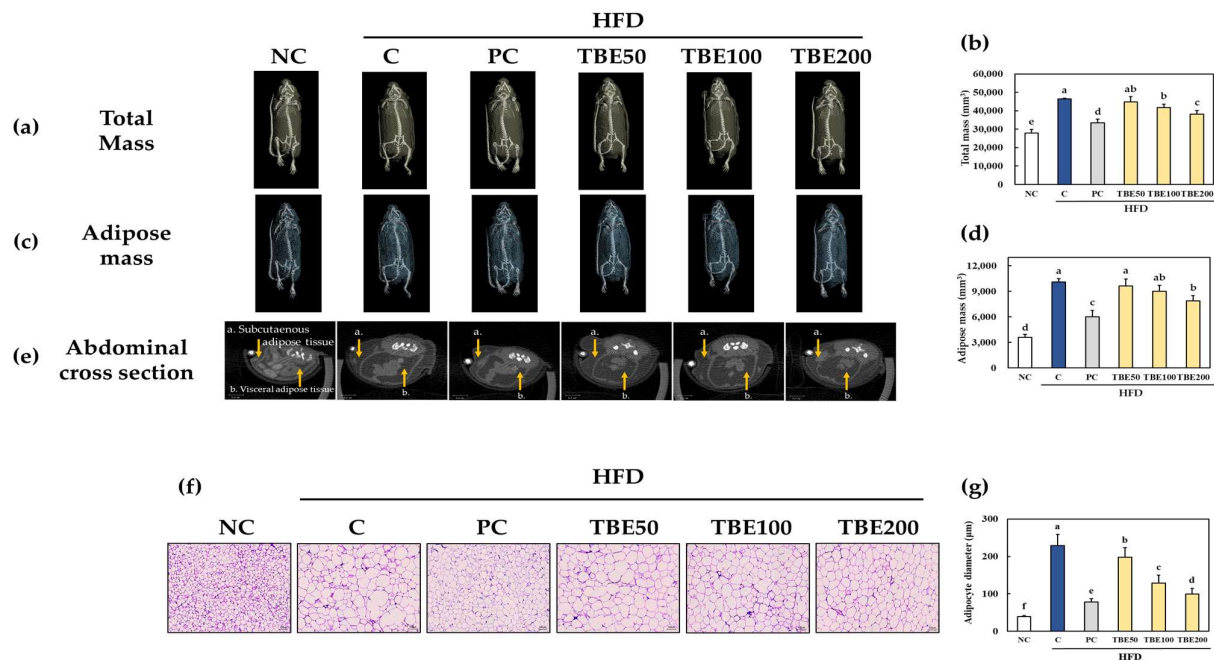


Figure 3. The effect of *Terminalia bellirica* extracts on micro-CT analysis and lipid droplet size in HFD-induced obese mice. (a) Micro-CT image, (b) quantification of total mass, (c) micro-CT image, (d) quantification of adipose mass, (e) abdominal cross section, (f) lipid droplet size, and (g) quantification of epididymal WAT. NC, normal diet control; C, 60% high-fat diet (HFD)-induced obesity control; PC, metformin 100 mg/kg body weight (b.w.) in 60% HFD; TBE50, *Terminalia bellirica* extract (TBE) 50 mg/kg b.w. in 60% HFD; TBE100, TBE 100 mg/kg b.w. in 60% HFD; TBE200, TBE 200 mg/kg b.w. in 60% HFD. Values are presented as mean $\pm$ standard deviation ($n = 8$), and different superscript letters indicate significance at $p < 0.05$.

Supplementation with HFD resulted in significantly elevated serum levels of TGs, TC, LDL-C, and HDL-C, as well as AST and ALT, compared to the NC group. Fecal TG and TC levels also increased significantly in the C group. Both the PC- and TBE-treated groups showed significant reductions in serum TG, TC, LDL-C, HDL-C, AST, and ALT levels compared to the HFD group. Moreover, TBE supplementation significantly increased fecal levels of TGs and TC compared to the C group (Table 2) ($p < 0.05$).

Table 2. The effects of *Terminalia bellirica* extract on lipid profiles; ALT and AST levels in mice with HFD-induced obesity.

Variables	Groups		HFD				
	NC	C	PC	TBE50	TBE100	TBE200	
Serum							
ALT (mU/mL)	43.06 $\pm$ 3.02 ^c	56.85 $\pm$ 10.57 ^a	45.44 $\pm$ 3.73 ^{bc}	52.87 $\pm$ 9.55 ^{ab}	51.46 $\pm$ 7.12 ^{ab}	50.88 $\pm$ 5.68 ^{ab}	
AST (mU/mL)	32.38 $\pm$ 5.24 ^c	53.89 $\pm$ 5.49 ^a	47.18 $\pm$ 6.31 ^b	49.32 $\pm$ 6.24 ^{ab}	48.73 $\pm$ 5.66 ^{ab}	47.66 $\pm$ 3.08 ^b	
Triglyceride (mM)	4.89 $\pm$ 0.98 ^d	11.41 $\pm$ 1.65 ^a	5.81 $\pm$ 1.01 ^d	10.04 $\pm$ 1.31 ^b	8.98 $\pm$ 1.32 ^b	7.27 $\pm$ 1.35 ^c	
Total Cholesterol (μ g/ μ L)	1.45 $\pm$ 0.37 ^d	2.66 $\pm$ 0.32 ^a	1.91 $\pm$ 0.24 ^c	2.48 $\pm$ 0.35 ^{ab}	2.29 $\pm$ 0.34 ^b	2.13 $\pm$ 0.37 ^{bc}	
LDL-Cholesterol (μ g/ μ L)	0.05 $\pm$ 0.03 ^e	0.28 $\pm$ 0.02 ^a	0.10 $\pm$ 0.02 ^d	0.17 $\pm$ 0.03 ^b	0.14 $\pm$ 0.01 ^c	0.12 $\pm$ 0.03 ^{cd}	
HDL-Cholesterol (μ g/ μ L)	0.16 $\pm$ 0.05 ^c	0.40 $\pm$ 0.05 ^a	0.25 $\pm$ 0.07 ^b	0.26 $\pm$ 0.08 ^b	0.25 $\pm$ 0.11 ^b	0.25 $\pm$ 0.04 ^b	
LDL/HDL Ratio	0.29 $\pm$ 0.14 ^c	0.71 $\pm$ 0.10 ^a	0.39 $\pm$ 0.10 ^c	0.70 $\pm$ 0.20 ^a	0.64 $\pm$ 0.25 ^{ab}	0.49 $\pm$ 0.13 ^{bc}	
Feces							
Triglyceride (mM)	4.07 $\pm$ 1.44 ^e	7.73 $\pm$ 1.37 ^d	9.79 $\pm$ 1.36 ^c	10.96 $\pm$ 1.99 ^c	12.86 $\pm$ 1.92 ^b	14.87 $\pm$ 2.57 ^a	
Total Cholesterol (μ g/ μ L)	3.02 $\pm$ 1.32 ^d	5.39 $\pm$ 2.13 ^c	9.21 $\pm$ 1.66 ^b	9.50 $\pm$ 2.55 ^b	10.69 $\pm$ 2.00 ^b	13.04 $\pm$ 2.44 ^a	

NC, normal diet control; C, 60% high-fat diet (HFD)-induced obesity control; PC, metformin 100 mg/kg body weight (b.w.) in 60% HFD; TBE50, *Terminalia bellirica* extract (TBE) 50 mg/kg b.w. in 60% HFD; TBE100, TBE 100 mg/kg b.w. in 60% HFD; TBE200, TBE 200 mg/kg b.w. in 60% HFD. Values are presented as mean $\pm$ standard deviation ($n = 8$), and different superscript letters indicate significance at $p < 0.05$.

2.4. TBE Supplementation Downregulated Adipogenesis and Lipogenesis in HFD-Induced Obese Mice

To confirm whether TBE supplementation affects adipogenesis- and lipogenesis-related factors in HFD-induced obese mice, we examined these factors in white adipose tissue. Compared with the NC group, the C group showed a significantly increased *p*-MAPK, PPAR γ , C/EBP α , SREBP1c. In addition, the FAS and LPL protein levels were markedly increased in the C group, indicating enhanced adipogenic and lipogenic activity. In contrast, TBE supplementation significantly attenuated these obesity-induced molecular alterations. Specifically, TBE supplementation resulted in a pronounced downregulation of *p*-MAPK and adipogenic transcription factors, including PPAR γ , C/EBP α , SREBP1c, compared with the C group. Moreover, the elevated protein levels of FAS and LPL were significantly suppressed by TBE supplementation. Collectively, our study demonstrated that TBE supplementation resulted in downregulation of protein levels involved in adipogenesis and lipogenesis (Figure 4).

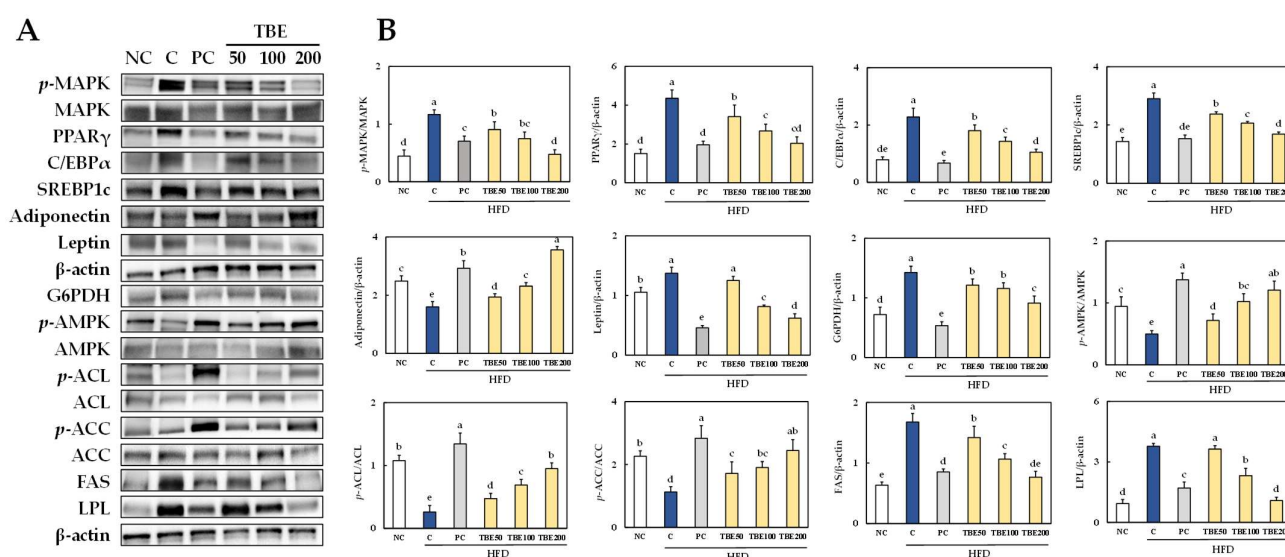


Figure 4. The effects of *Terminalia bellirica* extracts on the expression of protein-related adipogenesis and lipogenesis (A) band image; (B) quantification in white adipose tissue from HFD-induced obese mice. NC, normal diet control; C, 60% high-fat diet (HFD)-induced obesity control; PC, metformin 100 mg/kg body weight (b.w.) in 60% HFD; TBE50, *Terminalia bellirica* extract (TBE) 50 mg/kg b.w. in 60% HFD; TBE100, TBE 100 mg/kg b.w. in 60% HFD; TBE200, TBE 200 mg/kg b.w. in 60% HFD. Values are presented as mean $\pm$ standard deviation ($n = 3$), and different superscript letters indicate significance at $p < 0.05$. The molecular weights of the detected proteins are as follows: *p*-MAPK, 42, 44 kDa; MAPK, 42, 44 kDa; PPAR γ , 53, 57 kDa; C/EBP α , 28, 42 kDa; SREBP1c, 110–130 kDa; adiponectin, 27 kDa; leptin, 19 kDa; G6PDH, 59 kDa; *p*-AMPK, 62 kDa; AMPK, 62 kDa; *p*-ACL, 125 kDa; ACL, 125 kDa; *p*-ACC, 280 kDa; ACC, 280 kDa; FAS, 273 kDa; LPL, 36, 53 kDa; β -actin 42 kDa.

2.5. TBE Supplementation Promoted Lipolysis and Energy Metabolism in HFD-Induced Obese Mice

To investigate the impact of TBE on modulation of lipolysis and energy metabolism, Western blot analysis was performed. In WAT, the C group exhibited a marked suppression of lipolytic signaling compared with the NC group. Specifically, the protein expression levels of key lipolysis-related factors, including *p*-HSL and ATGL, were significantly reduced in the C group. In contrast, TBE supplementation significantly restored lipolytic activity in WAT, as evidenced by increased *p*-HSL and elevated ATGL protein expression compared with the C group. In BAT, the C group showed a significant downregulation of proteins

involved in energy metabolism, including *p*-AMPK, UCP1, and CPT1A, relative to the NC group. In contrast, TBE supplementation markedly increased the expression levels of these proteins. Our results revealed that TBE supplementation significantly upregulated the protein levels associated with lipolysis and energy metabolism-related factors compared to the C group (Figure 5).

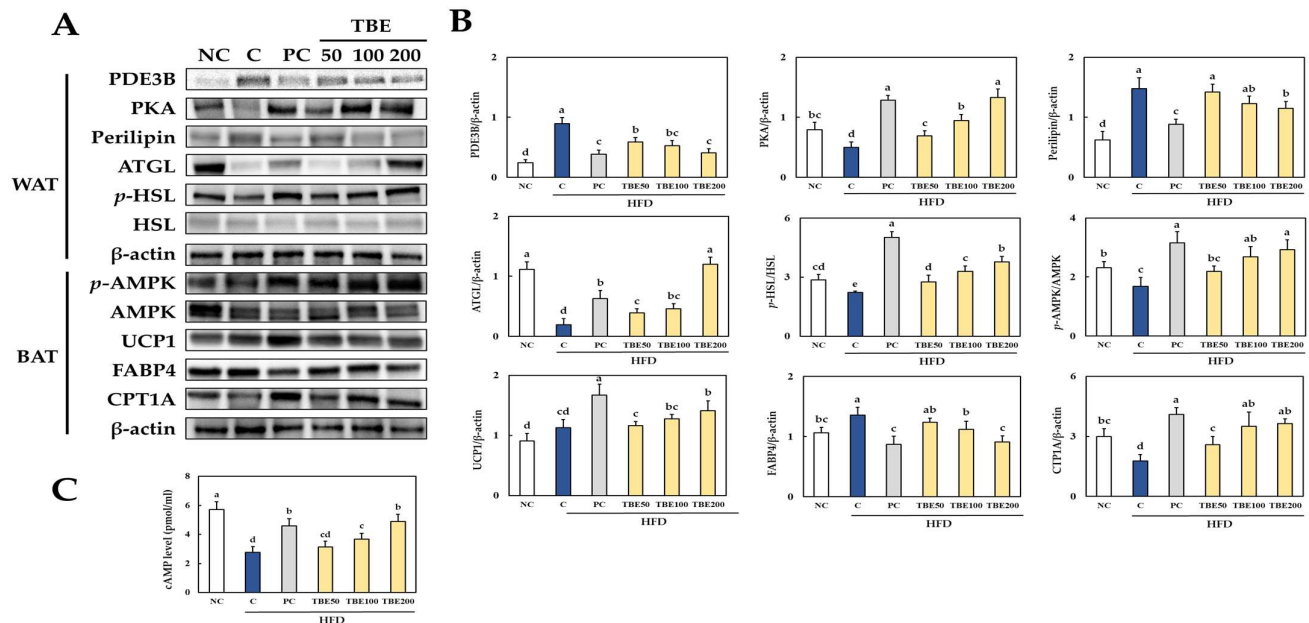


Figure 5. Effects of *Terminalia bellirica* extracts on the expression of protein-related lipolysis and energy metabolism (A) band image; (B) quantification; and (C) levels of cAMP in white adipose tissue and brown adipose tissue from HFD-induced obese mice. NC, normal diet control; C, 60% high-fat diet (HFD)-induced obesity control; PC, metformin 100 mg/kg body weight (b.w.) in 60% HFD; TBE50, *Terminalia bellirica* extract (TBE) 50 mg/kg b.w. in 60% HFD; TBE100, TBE 100 mg/kg b.w. in 60% HFD; TBE200, TBE 200 mg/kg b.w. in 60% HFD. Values are presented as mean $\pm$ standard deviation ($n = 3$), and different superscript letters indicate significance at $p < 0.05$. The molecular weights of the detected proteins are as follows: PDE3B, 115 kDa; PKA, 42 kDa; perilipin, 62 kDa; ATGL, 54 kDa; *p*-HSL, 81, 83 kDa; HSL, 38, 86, 88 90 kDa; *p*-AMPK, 62 kDa; AMPK, 62 kDa; UCP1, 33 kDa; FABP4, 15 kDa; CPT1A, 88 kDa; β -actin 42 kDa.

3. Discussion

Obesity independently causes or exacerbates numerous health complications, including type 2 diabetes mellitus, coronary heart disease, respiratory disorders, and osteoarthritis. This issue has prompted unprecedented research interest globally [19,20]. Previous studies on *Terminalia bellirica* demonstrate that its extract exhibits promising anti-obesity effects by reducing body weight, fat accumulation, and adipocyte differentiation [16,21]. In particular, the study by Hiroko et al. using spontaneously obese type 2 diabetic model mice supplemented with *Terminalia bellirica* reports a significant attenuation in body weight gain. The extract effectively suppresses fat accumulation by reducing visceral, subcutaneous, and mesenteric fat deposits compared to the control group [16]. While these findings are encouraging, further investigation is warranted to elucidate the precise mechanisms of action, optimal dosage, and potential side effects of *Terminalia bellirica*. The present study aimed to evaluate the effects of TBE on lipid accumulation-related mechanisms using 3T3-L1 adipocytes and obese mouse models.

TGs are primarily stored in WAT, which serves as a major energy reservoir and plays a central role in systemic energy homeostasis [22]. In this study, TBE treatment significantly reduced intracellular TG accumulation and Oil Red O staining in adipocytes.

TBE supplementation in HFD-induced obese mice markedly decreased adipose tissue mass, including subcutaneous, visceral, and epididymal WAT. Although food intake was reduced in the TBE-treated groups, the FER did not differ significantly among HFD-fed groups. This indicates that the relationship between energy intake and body weight changes was comparable across treatments, suggesting that the observed reduction in body weight cannot be explained solely by differences in food consumption. Furthermore, TBE supplementation not only reduced TG and TC levels but also increased fecal TG and TC excretion. Because enhanced fecal lipid excretion has been reported for dietary fiber and bioactive compounds that inhibit lipid digestion and absorption, these results suggest that TBE may modulate lipid metabolism through the regulation of lipid absorption [23].

Adipocyte differentiation is mainly controlled by the transcription factors involved in the MAPK pathway. PPAR γ and C/EBP α , increased by MAPK cascade, are mutually activated and promote the expression of adipose-specific genes like adiponectin [21]. Our findings demonstrated that MDI solution induced the upregulation of phosphorylated MAPK, as well as adipogenesis-related transcription factors in mature adipocytes and the WAT of obese mice. Both TBE treatment in adipocytes and TBE supplementation in HFD-induced obese mice resulted in reduced protein expression of phosphorylated MAPK, C/EBP α , and PPAR γ involved in adipogenesis pathway.

Lipogenesis refers to the metabolic process responsible for fat accumulation through the conversion of acetyl CoA to triglycerides. Among the various lipogenesis-related pathways, SREBP1c is closely associated with the acetyl-CoA pathway. Throughout lipogenesis, coordinated shifts in enzyme activities and gene expression occur, including increased G6PDH activity alongside modulation of AMPK, ACC, and ACL, wherein AMPK functions as a key energy sensor that suppresses lipogenic gene expression and enzyme function [24]. These collective alterations result in elevated FAS levels, thereby promoting enhanced TG synthesis [25]. In this study, both treatment with TBE in adipocytes and TBE supplementation in high-fat diet-induced obese mice significantly reduced the protein expression of lipogenesis-related genes—including G6PDH, ACL, ACC, FAS, and LPL—that were otherwise elevated in the control groups.

Lipolysis and energy metabolism are precisely regulated by hormonal and enzymatic mechanisms that govern lipid mobilization and utilization. During periods of high energy demand, lipolysis is primarily activated by various hormones. Hormonal binding to receptors initiates a stimulatory G protein (Gs)-mediated signaling cascade, which activates adenylyl cyclase (AC) and elevates intracellular levels of the second messenger cAMP [26]. The increase in cytoplasmic cAMP activates PKA, which phosphorylates HSL and facilitates its translocation from the cytosol to the surface of lipid droplets. This process leads to the acute activation of TG hydrolysis in cooperation with ATGL, thereby promoting lipid breakdown and energy release [27,28]. In this study, both treatment with TBE in adipocytes and TBE supplementation in HFD-induced obese mice upregulated PKA, phosphorylated HSL, cAMP and decreased PDE3B, perilipin.

In this manuscript, the term “energy metabolism” refers specifically to molecular processes involved in lipid mobilization and mitochondrial fatty acid oxidation rather than whole-body energy expenditure. BAT dissipates chemical energy via uncoupled respiration, producing heat by converting stored energy into thermal energy [29]. This thermogenic activity is tightly regulated by AMPK, an essential regulator of energy metabolism in adipocytes. AMPK influences not only lipogenesis factors but also the cellular mechanisms responsible for heat generation in BAT. Upon an increase in the intracellular AMP:ATP ratio, AMPK becomes activated and subsequently modulates key metabolic mediators such as UCP1 and CPT1A. UCP1 facilitates heat generation system by dissipating the proton gradient generated by the electron transport chain during mitochondrial respiration,

thereby converting chemical energy into heat [30–32]. Additionally, fatty acids released are transported by FABP4 and utilized for β -oxidation, a process regulated by CPT1A that facilitates their uptake into mitochondria [33]. We observed increased FABP4, a marker of lipid accumulation, which further supports the association between fat accumulation and impaired energy metabolism. In addition, TBE modulated the expression of energy-regulating proteins in both 3T3-L1 adipocytes and HFD-induced obese mice.

The present study has several limitations that should be acknowledged. A limitation of this study is that the concentrations used in the in vitro experiments may exceed physiologically attainable systemic levels in humans. Therefore, the relevance of the cell-based findings to human physiology should be interpreted with caution. The in vitro assays were intended as exploratory mechanistic screenings rather than direct representations of human exposure. In the present study, TBE supplementation attenuated body weight gain in HFD-fed mice; however, this effect should be interpreted with consideration of food intake-related factors. Although the food efficiency ratio (FER) did not differ significantly among HFD-fed groups, both absolute food intake and estimated energy intake were significantly lower in the TBE-treated groups compared with the HFD control. Thus, reduced food intake likely contributed, at least in part, to the observed resistance to weight gain, and the body weight-lowering effect of TBE cannot be attributed exclusively to intake-independent mechanisms.

Nevertheless, several metabolic outcomes observed in the TBE-treated groups suggest that mechanisms beyond reduced food intake may also be involved. Notably, TBE supplementation significantly increased fecal lipid excretion and improved circulating lipid profiles, including reductions in serum triglyceride and total cholesterol levels. These findings imply that TBE may modulate intestinal lipid absorption and/or systemic lipid metabolism, thereby contributing to reduced lipid accumulation independently of total caloric intake. It should also be noted that TBE was administered mixed into the diet, and a formal palatability assessment was not conducted. Therefore, it cannot be excluded that changes in diet palatability influenced voluntary food consumption. This represents an important limitation of the current study. Future investigations employing pair-feeding designs or dedicated palatability studies will be necessary to clearly distinguish intake-dependent effects from intrinsic metabolic actions of TBE. Furthermore, the present study did not directly assess energy expenditure, physical activity levels, or the contribution of non-adipose tissues such as liver and skeletal muscle. Given that these factors play critical roles in whole-body energy balance, additional studies incorporating indirect calorimetry and tissue-specific metabolic analyses are warranted to more fully elucidate the mechanisms underlying the anti-obesity effects of TBE.

This study revealed that TBE possesses dual functions, simultaneously suppressing key factors involved in adipogenesis and lipogenesis while enhancing lipolysis and energy metabolism. While the current results are promising, their application to human weight loss strategies demands rigorous clinical trials. Furthermore, the specific bioactive component responsible for these effects in TBE has not yet been identified, emphasizing the necessity of continued research in this area.

4. Materials and Methods

4.1. Preparation of TBE

The TBE (*Terminalia bellirica* extract) was prepared using the dried fruit rind sourced from India and supplied by Daehan Chemtech Co., Ltd., Gwacheon, Republic of Korea, as the starting material. The rind was cleaned, ground, and sieved through a 10 mm mesh to obtain particles of uniform size. The powdered material was extracted with water in a multi-function extractor at a material-to-solvent ratio of 1:4. The extraction was carried

out at 40–45 °C for 8 h. The combined extracts were filtered through a 15–20 µm filter and concentrated under reduced pressure (≥ 550 mm Hg) at 40–60 °C until the solid content reached 20–25%. The concentrated solution was dried using a spray dryer operated at an inlet temperature of 180–190 °C and an outlet temperature of 90–110 °C for 4–8 h. The dried material was then ground to pass a 30-mesh sieve, passed through magnets ($\geq 12,000$ Gauss) to remove traces of metal, and sieved again for uniformity. The content of gallic acid, a marker compound, was determined through high-performance liquid chromatography (HPLC) using NANOSPACE (Osaka Soda Co., Osaka, Japan) for standardization purposes (Figure 6). A gallic acid reference standard (phyproof[®], absolute purity 97%) was purchased from PhytoLab GmbH & Co. KG (Vestenbergsgreuth, Germany; product No. 89798). The TBE was standardized to contain $\geq 9\%$ gallic acid.

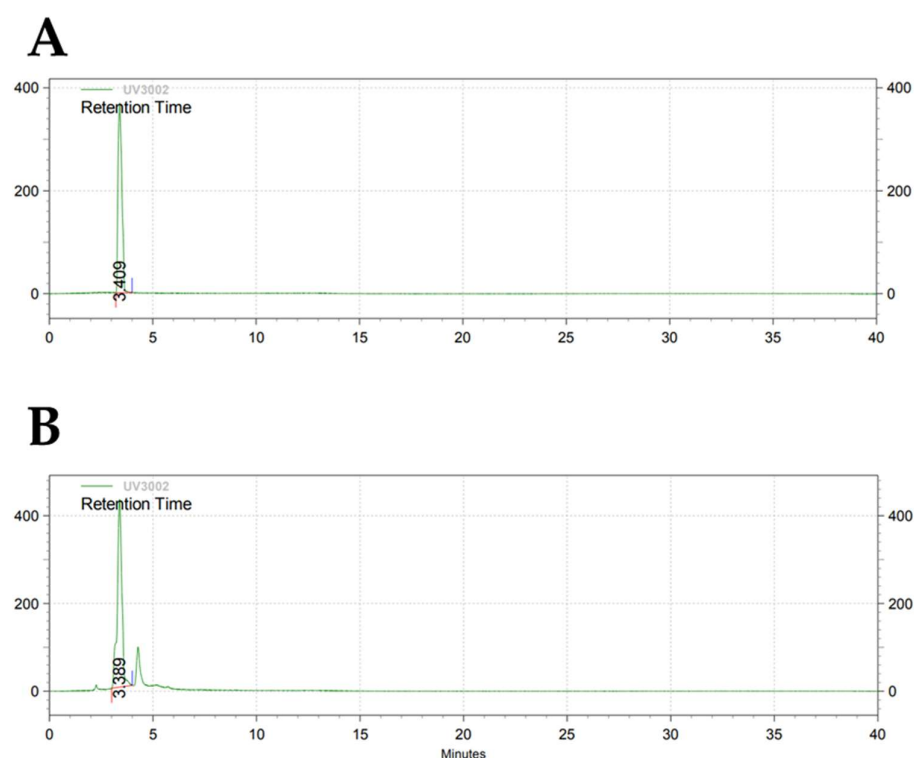


Figure 6. High-performance liquid chromatography (HPLC) analysis of gallic acid in TBE: standard (A) and TBE sample (B).

4.2. Cell Culture and Treatment

3T3-L1 preadipocyte cell line was obtained from the American Type Culture Collection (Rockville, MD, USA) and cultured under standard conditions of 95% air and 5% CO₂ at 37 °C. The cells were maintained in high-glucose Dulbecco's modified eagle medium (DMEM; Hyclone Laboratories, Logan, UT, USA) supplemented with 10% newborn calf serum (NCS; Hyclone Laboratories), 1% penicillin/streptomycin, 1% L-glutamine, and 1% sodium pyruvate (all from Hyclone Laboratories). For adipocyte differentiation, the cells were seeded into six-well plates at a density of 3×10^5 cells per well. Upon reaching near 100% confluence, the culture medium was replaced with differentiation medium containing 10% fetal bovine serum (FBS) instead of NCS, supplemented with an adipogenic cocktail comprising 0.5 mM 3-isobutyl-1-methylxanthine (IBMX), 1 µM dexamethasone, and 10 µg/mL insulin (all from Sigma-Aldrich, St. Louis, MO, USA) (Figure 7). Stock solutions of TBE were prepared in DMSO and diluted with culture medium to the indicated concentrations. The final concentration of DMSO in all treatments was adjusted to 1%

(v/v), and all experimental groups, including the control group, were treated with the same vehicle concentration.

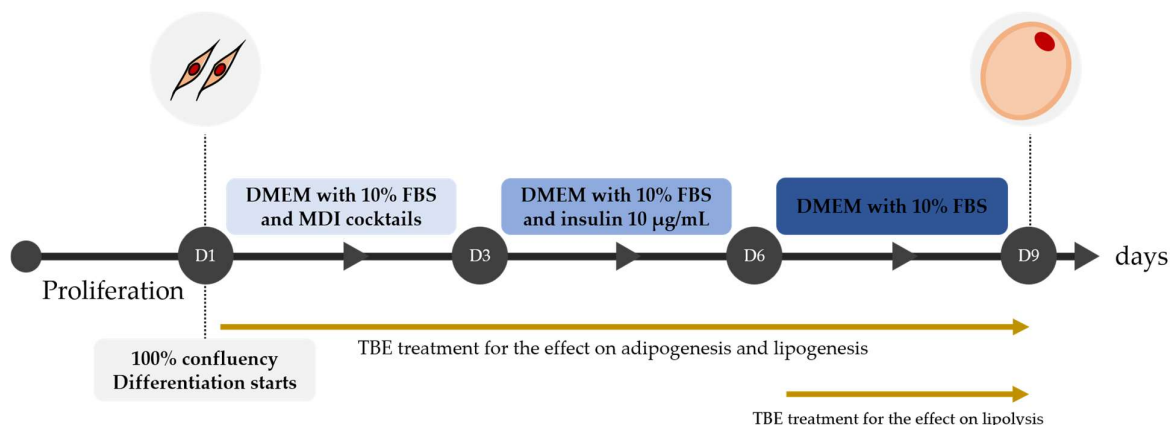


Figure 7. Schematic illustration of differentiation into mature adipocyte and TBE treatment protocol.

4.3. Lipid Accumulation Quantification

Lipid accumulation in differentiated adipocytes was assessed through Oil Red O staining and TG quantification. For Oil Red O staining, cells treated with TBE for the specified period were washed with phosphate-buffered saline (PBS) and fixed in 4% formaldehyde at room temperature for 30 min. After fixation, the cells were stained with 0.5% Oil Red O solution (Sigma-Aldrich) for 1 h and then extensively rinsed with distilled water. The stained lipid droplets were eluted using isopropanol, and absorbance was measured at 490 nm with a microplate reader to quantify lipid accumulation.

For TG quantification, differentiated adipocytes were lysed in Triton X-100 buffer and centrifuged at 19,326 g and 4 °C for 10 min. The supernatant was collected, and intracellular TG content was measured using a commercial TG assay kit (Abcam, Cambridge, UK) following the manufacturer's instructions.

4.4. Measurement of Free Glycerol

Free glycerol released from TG hydrolysis was quantified using the glycerolphosphate oxidase (GPO)-Trinder enzyme reaction protocol (Sigma-Aldrich) as previously described [34]. Following the completion of 3T3-L1 cell differentiation, culture medium was harvested and mixed with the free glycerol reagent according to the manufacturer's instructions. The mixture was incubated, and optical density was determined at 540 nm using a microplate reader. The concentration of free glycerol in each sample was calculated from a standard curve generated using a glycerol standard solution (Sigma-Aldrich).

4.5. High-Fat Diet-Induced Obese Mice Model

Male C57BL/6J mice (4 weeks old, weighing approximately 22 ± 1 g) were obtained from Saeron Bio (Uiwang, Republic of Korea). The animals were housed in environmentally controlled conditions with a temperature of 22 ± 2 °C, 55% relative humidity, and a 12 h light/dark cycle. After a 1-week acclimation period, the mice were randomly assigned to one of six experimental groups: normal control (NC), fed a standard AIN93G [35] diet (D10012G, Research Diets Inc., New Brunswick, NJ, USA); control group (C), receiving a 60% HFD (D12492, Research Diets INC.); positive control (PC), treated with metformin at 100 mg/kg body weight (b.w.) on a HFD; and three treatment groups supplemented with TBE at doses of 50, 100, and 200 mg/kg b.w. in the HFD. Diets were formulated based on the AIN93G diet standard, and the samples were incorporated into a 60% HFD at the designated concentrations and fed ad libitum for 16 weeks. At the end of the experimental

period, mice were euthanized under anesthesia, and tissues and blood samples were collected for further analysis. All procedures were approved by the Institutional Animal Care and Use Committee of Kyung Hee University (KHGASP-24-574, 2025-03-18), ensuring ethical standards and validity of the experiment.

4.6. Micro-CT Analysis

The day before the end of the animal experiments, the mice were anesthetized via intraperitoneal injection and subjected to whole-body and abdominal micro-computed tomography (micro-CT) scans under optimized conditions (voxel size: 150 μm ; energy: 45 kVp; intensity: 110 μA ; field of view/diameter: 79.8 mm; and integration time: 160 ms) using a VIVA CT 80 system (Scano Medical AG, Wangen-Brüttisellen, Switzerland). Image analysis was conducted with the micro-CT Evaluation Program V6.6. The Hounsfield unit (HU) thresholds were set at -200 , -30 , and 190 , with fat tissue quantified within the HU range of -200 to -30 .

4.7. Histological Analysis

Epididymal WAT was collected from mice and fixed in 10% neutral-buffered formaldehyde solution for 24 h to preserve tissue morphology. Following fixation, the samples were rinsed with phosphate-buffered saline and dehydrated through a graded ethanol series (100% to 70%). The tissues were then embedded in paraffin wax and sectioned into 5 μm thick slices. These sections were stained with hematoxylin and eosin (H&E), washed with distilled water, and examined under an optical microscope to visualize adipocyte morphology for histological analysis.

4.8. Biochemical Parameters in Serum and Feces

Blood samples were collected and centrifuged at $25,188 \times g$ for 20 min at 4°C to obtain serum. Fecal serum samples were collected from the cages before sacrifice. Dried fecal samples were homogenized in chloroform/methanol (2:1, *v/v*) and centrifuged at $2500 g$ for 10 min according to the method of Folche et al. [36]. All samples were stored at -80°C until use. Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), TGs, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) in serum and feces were measured using commercial ELISA kits obtained from Abcam. All assays were conducted following the manufacturers' protocols.

4.9. Measurement of cAMP Level

The cAMP levels in the cells and WAT of mice were measured using an ELISA kit from Enzo Biochem, Inc. (Farmingdale, NY, USA) following the manufacturer's instructions. For cells and WAT samples, samples were homogenized in 0.1 M HCl provided in the kit and centrifuged at $1000 \times g$ for 10 min, and the supernatants were collected.

4.10. Western Blot

Proteins were extracted from cells, as well as from white and brown adipose tissues of mice, and analyzed for the expression levels of *p*-mitogen-activated protein kinase (MAPK) (Erk1/2), MAPK, SREBP1c, peroxisome proliferator-activated receptor (PPAR- γ), C/EBP α , leptin, adiponectin, glucose-6-phosphate dehydrogenase (G6PDH), *p*-ATL citrate lyase (ACL), ACL, *p*-acetyl CoA carboxylase (ACC), ACC, FAS, lipoprotein lipase (LPL), PKA, phosphodiesterase 3B (PDE3B), *p*-HSL, HSL, perilipin, adipose triglyceride lipase (ATGL), *p*-AMP-activated protein kinase (AMPK), AMPK, UCP1, CPT1A, fatty acid-binding protein 4 (FABP4), and β -actin, following previously described methods [37].

Abbreviations

The following abbreviations are used in this manuscript:

ACC	acetyl CoA carboxylase
ACL	ATL citrate lyse
ALT	alanine aminotransferase
AST	aspartate aminotransferase
C/EBP	CCAAT/enhancer-binding protein
cAMP	cyclic adenosine monophosphate
CPT1A	carnitine palmitoyltransferase 1A
DMEM	Dulbecco's modified eagle medium
FABP4	fatty acid-binding protein 4
FAS	fatty acid synthase
FBS	fetal bovine serum
FFA	free fatty acid
G6PDH	glucose-6-phosphate dehydrogenase
H&E	hematoxylin and eosin
HDL-C	high-density lipoprotein cholesterol
HFD	high-fat diet
HSL	hormone-sensitive lipase
LDL-C	low-density lipoprotein cholesterol
MAPK	mitogen-activated protein kinase
PDE3B	phosphodiesterase 3B
PPAR	peroxisome proliferator-activated receptor
SREBP1c	sterol regulatory element-binding protein 1c
TC	total cholesterol
TG	triglyceride
UCP1	uncoupling protein 1
WAT	white adipose tissue

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