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Verbascoside promotes lipolysis in 3T3-L1 hypertrophic cells through alteration in the expression of lipolysis-related genes

Verbascoside, 3T3-L1 hipertrofik hücrelerinde lipolizle ilişkili genlerin ekspresyonunda değişiklik yoluyla lipolizi artırır

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Abstract

Purpose: Recent studies have shown promising outcomes in the utilization of natural, non-toxic polyphenols with fat-burning properties to combat obesity. The aim of this study was to investigate the changes in the gene expression profile of hypertrophic 3T3-L1 adipocytes in response to Verbascoside (VB) treatment and the mRNA levels of 5' AMP-activated protein kinase (AMPK), peroxisome proliferator-activated receptor gamma (PPAR-γ), hormone-sensitive lipase (HSL), and lipid droplet-associated protein (perilipin1, PLIN1), which play an important role in fat metabolism.

Materials and methods: For this purpose, 3T3-L1 preadipocytes were differentiated and hypertrophied. Triglyceride accumulation in the cells was evaluated using Oil Red O staining. The hypertrophic 3T3-L1 cells were incubated with VB for 48 h.

Results: Microarray analysis at the end of this period showed that VB increased the mRNA levels of many proteins involved in lipolysis processes, but did not change the expression of PPARγ, AMPK, HSL and PLIN1. Therefore, we confirmed by RT-PCR that PPARγ, AMPK, HSL, and PLIN1 expression levels were altered by VB treatment. Our results showed that VB treatment caused a significant increase in the expression of genes involved in lipolytic processes compared to the control.

Conclusion: The findings of this study suggest that VB may play an active role through important intracellular proteins involved in lipolytic processes.

Keywords: Verbascoside, polyphenol, adipose, lipolysis, 3T3-L1.

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Öz

Amaç: Son zamanlarda yapılan çalışmalar, obezite ile mücadele için yağ yakıcı özelliklere sahip doğal, toksik olmayan polifenollerin kullanımında umut verici sonuçlar göstermiştir. Bu çalışmanın amacı, Verbascoside (VB) tedavisine yanıt olarak hipertrofik 3T3-L1 adipositlerin gen ekspresyon profilindeki değişiklikleri ve yağ metabolizmasında önemli rol oynayan 5' AMP ile aktive olan protein kinaz (AMPK), peroksizom proliferatör ile aktive olan reseptör gama (PPAR-γ), hormona duyarlı lipaz (HSL) ve lipid damlacığı ile ilişkili protein (perilipin1, PLIN1) mRNA seviyelerini araştırmaktır.

Gereç ve yöntem: Bu amaçla, 3T3-L1 preadipositleri farklılaştırıldı ve hipertrofiye edildi. Hücrelerdeki trigliserit birikimi Oil Red O boyaması kullanılarak değerlendirildi. Hipertrofik 3T3-L1 hücreleri 48 saat boyunca VB ile inkübe edildi.

Bulgular: Bu sürenin sonunda yapılan mikroarray analizi, VB'nin lipoliz süreçlerinde yer alan birçok proteinin mRNA seviyelerini artırdığını, ancak PPARγ, AMPK, HSL ve PLIN1 ekspresyonunu değiştirmediğini gösterdi. Bunu doğrulamak için yaptığımız RT-PCR'a göre PPARγ, AMPK, HSL ve PLIN1 ekspresyon seviyelerinin VB tedavisi ile değiştiğini belirledik. Sonuçlarımız, VB uygulamasının lipolitik süreçlerde yer alan genlerin ifadesinde kontrole kıyasla önemli bir artışa neden olduğunu göstermiştir.

Sonuç: Bu çalışmanın bulguları, VB'nin lipolitik süreçlerde yer alan önemli hücre içi proteinler aracılığıyla aktif bir rol oynayabileceğini göstermektedir.

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Anahtar kelimeler: Verbascoside, polifenol, adipoz, lipoliz, 3T3-L1.

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Introduction

Modern lifestyle, which is characterized by intensive consumption of high-calorie foods and little or no physical activity, is a major contributing factor to obesity. Obesity is an important risk factor involved in many pathophysiological processes, including type 2 diabetes mellitus (T2DM), heart disease, insulin resistance (IR), hyperglycemia, dyslipidemia, hypertension, and some types of cancer [1-3]. Global public health has been severely threatened by this condition, which has become a pandemic over the past 50 years [4]. A key feature of obesity is the excessive accumulation of dysfunctional adipose tissue [5]. In order to prevent and manage obesity, lifestyle changes such as diet and exercise are particularly crucial [6-8]. Despite the widespread use of pharmacological drugs and surgical methods to treat obesity [7, 9], these treatments may not be effective for everyone and can cause significant side effects [10, 11].

Phenylethanoid glycosides are water-soluble natural compounds isolated from plants [12]. There is increasing evidence that natural products containing phenylethanoid glycosides have potential anti-obesity effects [12]. Verbascoside (VB) is a polyphenol belonging to the phenylethanoid glycoside subgroup [13, 14]. As with many phenolic compounds, VB has been investigated for its favorable health properties and has been shown to have many beneficial effects, such as antioxidant, anti-inflammatory, anti-cancer, and anti-obesity properties [15-18].

Considering its anti-obesity properties, it has been reported that this substance can reduce fat absorption by decreasing the activity of pancreatic lipase [19], diminish high glucose-induced metabolic stress via 5' AMP-activated protein kinase (AMPK)-dependent mechanisms in insulin-resistant hypertrophic adipose cells [15], and decrease triglyceride (TG) accumulation in a dose-dependent manner [15, 20]. It also augments fatty acid (FA) oxidation and suppresses lipogenesis by PPARα upregulation and fatty acid synthase (FASN) downregulation through AMPK activation [15, 20]. In a study involving the administration of various extracts containing VB, serum TG and cholesterol levels and fatty liver were reduced in the hyperlipidemic mouse group, but weight gain and food consumption were the same as those in the control group [15]. In another study examining the effects of Metabolaid®, a combination of Lippia citriodora (lemon verbena, LC) (containing VB); Hibiscus flower (Hibiscus sabdariffa L.) (HS) extracts, it was reported that LC significantly reduced body weight and weight gain, caused a decrease in epididymal, retroperitoneal and total white adipose tissue weight, and reduced adipocyte size and lipid accumulation in high-fat-diet-induced obese mice [21].

VB has several known beneficial effects on lipid metabolism. However, its impact on the gene expression profile is not well understood. This study is the first to investigate how the gene expression profile changes in hypertrophic 3T3-L1 adipocytes in response to VB treatment. Second, we aimed to investigate the effect of VB on the mRNA expression levels of genes such as AMPK and PPAR-γ, which play an important role in adipose tissue metabolism. Finally, we investigated the effect of VB on direct lipolysis-related genes, such as hormone-sensitive lipase (HSL) and lipid droplet-associated protein (perilipin1, PLIN1), which have not yet been studied to our knowledge.

Materials and methods

Differentiation and establishment of the 3T3-L1 hypertrophic adipocyte model

In this study, we used the 3T3-L1 cell line (ATCC® CL173™), a fibroblast cell line derived from a mouse (Mus musculus) embryo known as a preadipocyte. Preadipocytes were propagated and differentiated according to previously established procedures [22]. Briefly, cells were cultured in a low-glucose medium containing bovine calf serum and antibiotics (penicillin and

streptomycin) to create experimental groups. The cells were cultured in the same medium until they reached confluence. When the cells reached approximately 80% density in the culture dishes, a medium containing a differentiation cocktail of 3-Isobutyl-1-methylxanthine (IBMX, Sigma I5879, USA), dexamethasone (DEX, Sigma D4902, USA), insulin (INS) (Sigma I6634, USA), and fetal bovine serum (FBS, Biowest, South America) was added to the medium to induce the differentiation of preadipocytes into adipocytes, and the cells were incubated in this medium (MD1) for 48 hours. After incubation, the medium containing (Gibco, Waltham, MA, USA) high glucose FBS and INS (MD2) was changed every other day for 10 days for maturation and 18 days for hypertrophy.

Oil Red O staining

To confirm the transformation of preadipocytes into mature (day 10) and hypertrophic (day 18) cells, they were analyzed microscopically using the Oil Red O staining kit [Biovision Lipid (Oil Red O) Staining Kit (Catalog # K580-24)]. Fat deposition in the cells was analysed using the Oil Red O staining method. Briefly, differentiated 3T3-L1 adipocytes were fixed with 10% formalin in Phosphate Buffered Saline (PBS, Wisent, Saint-Jean-Baptiste, Canada) for 1 h. The formalin was removed, and the cells were gently washed cells 2X with distilled water. After that, 60% isopropanol was added to each well and incubated for 5 min. The isopropanol was removed, and the Oil Red O working solution was added for 30 min. The working solution was then removed, and the cells were washed 2-5X with distilled water. A microscope was used to observe TG accumulation at 10X and 20X magnifications.

Administration of verbascoside

Following confirmation of the model's success, we administered VB to hypertrophied adipocytes at 50 and 100 µM concentrations for 48 h on day 18 after differentiation. Details of the experimental timeline are shown in Figure 1.

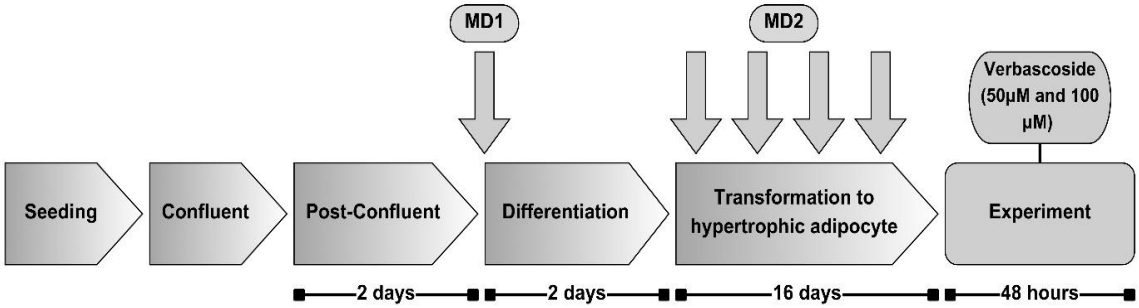


Figure 1. Illustration of experimental design.

Verbascoside was administered 50 -100 µM dose. For medium 1, DMEM containing high glucose (4.5 g/L) was prepared by adding 0.5 mM IBMX, 1 µM DEX, 10 µg/mL INS, 10% FBS, and 1% P/S solution. For medium 2, DMEM containing high glucose (4.5 g/L) was prepared by adding 10µg/ml INS, 10% FBS, and 1% P/S solution. MD1; medium 1; MD2; medium 2; IBMX; 3-Isobutyl-1-methylxanthine, DEX; dexamethasone, INS; insulin, FBS; fetal bovine serum, P/S; penicillin/streptomycin, DMEM; Dulbecco's Modified Eagle Medium

Microarray analysis

Total RNA was extracted from cells that received VB treatment and control cells 48 h after treatment using TRIzol LS Reagent (Invitrogen, USA) and Hybrid-R (GeneAll), following the manufacturer's instructions. RNA concentrations and purity values were determined by spectrophotometry (NanoDrop 2000c, Thermo Scientific). To ensure the quality of the isolated RNA, samples with a purity ratio of 1.8 to 2 were selected and included in the study. The GeneChip™ Human Gene 2.0 ST Array platform developed by Macrogen was used for analysis. cDNA was synthesized using a GeneChip WT (Whole Transcript) amplification kit. For cleavage and labeling, Sens cDNA was fragmented, and biotin was labeled with terminal deoxynucleotidyl transferase (TdT) using the GeneChip WT Terminal labeling kit. Approximately 5.5 µg of labeled DNA target was hybridized at 45°C for 16 h using the Afymetrix GeneChip Array. Hybridized arrays were washed and dyed on a GeneChip Fluidics Station 450 and scanned

on a GCS3000 Scanner (Afymetrix). Probe cell density data calculations were performed using Afymetrix GeneChip Command Console Software (AGCC). Software (Afymetrix Power Tools, R 3.3.3) was used for the analysis. Data were summarized for raw data preparation and statistical analysis and normalized using the robust Robust MultiAverage (RMA) method implemented in Afymetrix Power Tools (APT). The resultant gene levels were exported for RMA analysis. For a DEG set, using full connectivity as a measure of similarity and Euclidean distance, a hierarchical set analysis was performed. Gene enrichment and functional annotation analysis of the key probe list were performed using Gene Ontology (http://geneontology.org). All data analyses were performed using R 3.3.2 (www.r-project.org).

Cell viability assay

Cell viability was determined using the Enhanced Cell Counting Kit 8 (WST-8/CCK8) from Elabscience (E-CK-A362). To determine viability, 5.000 hypertrophied 3T3-L1 cells were cultured in high-glucose Dulbecco's Modified Eagle Medium (DMEM) at 37°C and 5% CO_2 for 48 h, followed by treatment with 50 and 100 μM VB. After 48 h of exposure to the agents, cell viability was determined by adding 10 μL of WST-8 Buffer to each well and incubating for 2 h. A Thermo Fisher Scientific™ Multiskan™ GO microplate spectrophotometer was used at 450 nm to measure the absorbance. All graphs represent the results of at least three separate experiments.

Measurement of adipocyte size

Digital images of hypertrophied 3T3-L1 adipocytes with or without VB treatment were taken under a microscope after oil red staining, and then their diameters were measured using Adiposoft software (Ver.1.3, NIH).

qRT-PCR analysis

We performed qRT-PCR analysis to confirm differentially expressed mRNAs with a high fold change and to validate the gene expression in our research hypothesis (AMPK, PPARγ, HSL, and PLIN1). RNA (1 ng) was reverse transcribed to complementary DNA (cDNA) using a commercially available VitaScript™ FirstStrand cDNA Synthesis Kit (PROCOMCURE). qRT-PCR was performed using 2×Magic SYBR mix (Procomcure Biotech, Austria), and the relative change of gene expressions was analyzed according to the GAPDH reference/housekeeping gene. Primer sequences used in real-time PCR were shown in Table 1.

Table 1. Primer sequences used in the experiment for qRT-PCR

Gene	NCBI RefSeq	Primer sequence	Product length (bp)
GAPDH	NM_001289726.2	F:AGGTCGGTGTGAACGGATTTG	95
		R:GGGGTCGTTGATGGCAACA	
PPARγ	NM_011146.4	F:GCCCTTTGGTGACTTTATGGA	170
		R:GCAGCAGGTTGTCTTGGATG	
HSL	NM_010719.5	F:GCTCATCTCCTATGACCTACGG	142
		R:TCCGTGGATGTGAACAACCAGG	
AMPKα1	NM_001013367.3	F:GGTGTACGGAAGGCAAAATGGC	151
		R:CAGGATTCTTCCTTCGTACACGC	
PLIN1	NM_175640.2	F:GAGAAGGTGGTAGAGTTCCTCC	143
		R:GTGTGTCGAGAAAGAGTGTTGGC	

Statistical analysis

All experiments were performed in triplicate and repeated independently to confirm the results. Data were analyzed using SPSS 25.0 (IBM SPSS Statistics 25 software (Armonk, NY: IBM Corp.) package program). One-way ANOVA was used to calculate the statistical differences between two groups using Dunnett's post hoc test and two-way ANOVA with Tukey's comparison test and Student's t-test. Statistical results with $*p<0.05$ were considered statistically significant. The changes in folds between the patient and control groups were examined as a result of the analysis. The lower limit was determined as ≤−1.5, and the upper limit was ≥1.5-fold, so that the increasing and decreasing expression changes of lncRNAs could be determined. Negative values (0) indicate upregulation. GO and KEGG pathway analyses were performed to analyze the functions of mRNAs determined by microarray and to determine which biological processes, molecular function, and biochemical pathways are involved.

Results

OIL RED O staining

To validate the experimental model, Oil Red O staining was conducted on three types of adipocytes: preadipocytes, mature adipocytes, and hypertrophic adipocytes. The stained samples were then examined qualitatively under a microscope. The model's effectiveness was confirmed by observing distinct characteristics among the three adipocyte types. The findings revealed a progressive increase in lipid accumulation from the preadipocyte stage to the hypertrophic adipocyte stage. As this process occurred, the cells transformed into an oval shape and expanded in diameter due to the accumulation of lipids (Figure 2).

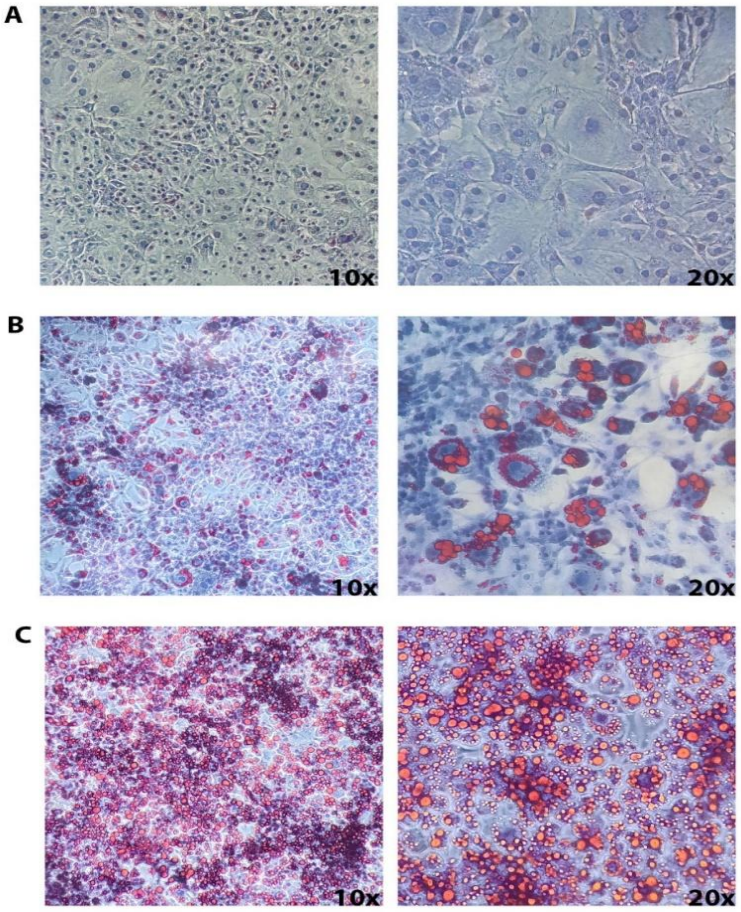


Figure 2. Demonstration of lipid accumulation during the differentiation of 3T3-L1 cells from preadipocytes to hypertrophic adipocytes. A) Pre-adipocytes before differentiation (day 0); B) mature adipocytes (day 10); C) hypertrophic adipocytes (day 18). Staining shows neutral lipids in red and nuclei in blue

Microarray analysis result

We conducted a whole-gene microarray analysis to assess changes in the expression of 52,141 genes, and our results showed that VB treatment led to changes in the gene expression profiles. Between-group differences in gene expression were examined using clustering analyses. VB-treated groups were different from the control group (Figure 3A), and the heat map showed significant changes in gene expression in the VB-treated groups (Figure 3B). According to the microarray data analysis, the number of probes displayed based on 1.5-fold and 2-fold changes and statistical test p-values compared to the control group is shown as a bar plot (Figure 4). After administering VB, 185 genes were upregulated, and 250 genes were downregulated in the V50 group compared to those in the control group. In the V100 group, 126 genes were upregulated, and 220 genes were downregulated compared to the control group. There was no difference in gene expression between the VB groups (V50 and V100).

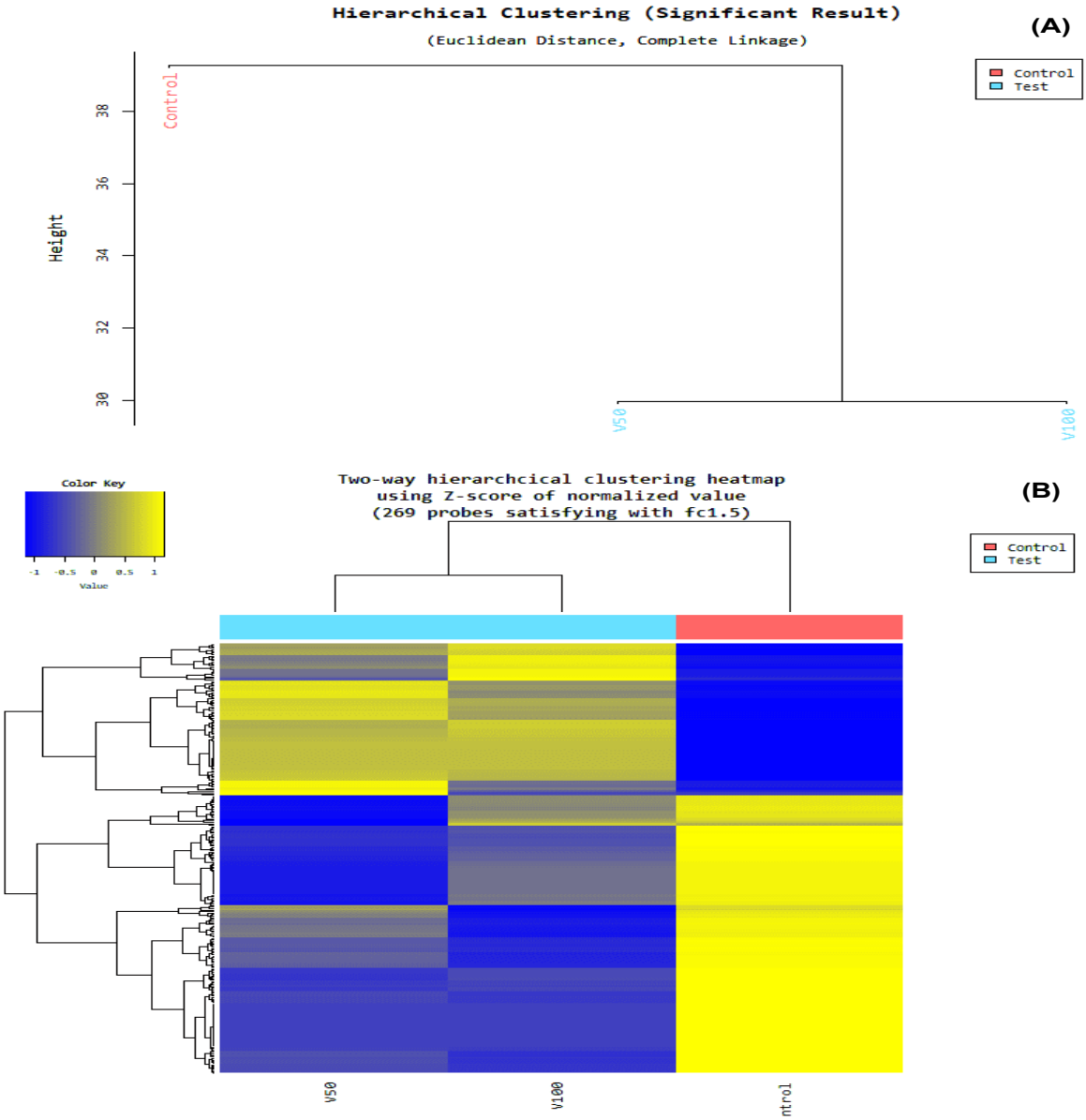


Figure 3. Cluster analysis of microarray results. A) Hierarchical clustering clusters samples in several groups that possess high expression similarities using z-score of normalized value. B) Heatmap shows results of hierarchical clustering analysis (Euclidean Distance, Complete Linkage) which clusters the similarity of probe sets and samples by expression level (normalized value) from the significant list

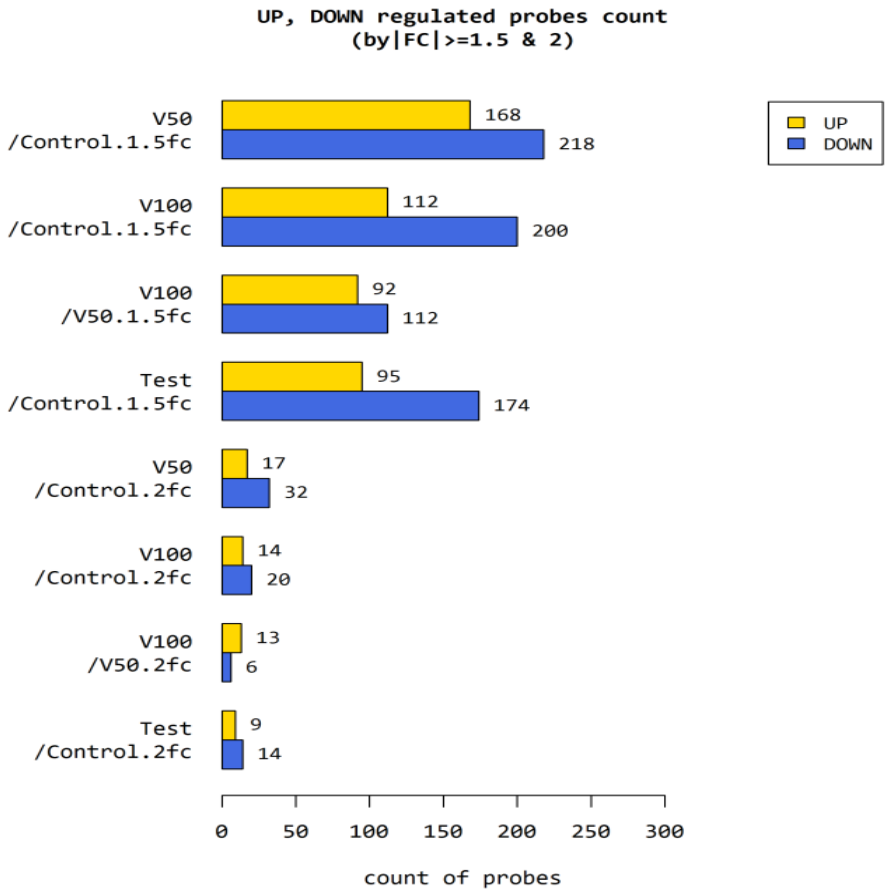


Figure 4. Shows the number of up and down regulated probe sets based on fold change of comparison pair

Effect of VB doses used in the experiment on cell viability

VB was administered to cells at a dose of 50 and 100 µM, and the test was carried out at the end of 48 h. While no significant change was noted at a dose of 50 µM compared with the control group, a significant decrease in viability was observed at dose of 100 µM compared with the control group (Figure 5). Therefore, we decided to perform the confirmation of the genes to be investigated only in the V50 group.

Effect of VB on adipocyte diameter

Microscopic examination at 100X magnification revealed a decrease in adipocyte diameter in the VB group when compared to the control group. Representative micrographs of the control and V50 groups stained with Oil Red O and viewed at 100X magnification are displayed in Figure 6A. As illustrated in Figure 6B, a statistically significant reduction in adipocyte diameter was observed in the V50 group compared to the control group. The VB treatment group exhibited smaller adipocyte sizes compared to the control group (p<0.05). To elucidate the mechanisms by which VB influences hypertrophic adipocytes, we assessed the mRNA expression levels of genes associated with lipogenesis and lipolysis in a hypertrophic adipocyte cell model.

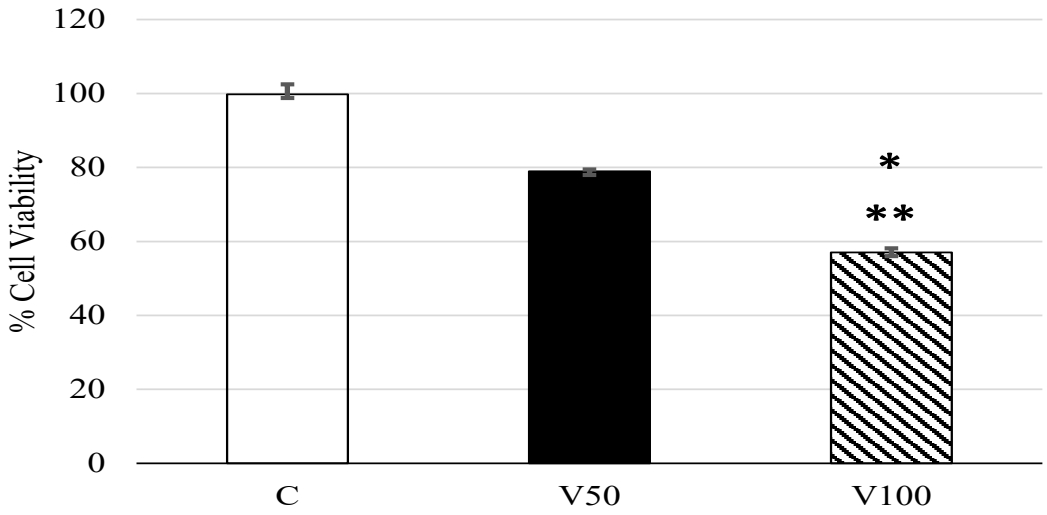


Figure 5. Cytotoxicity of VB at 50 and 100 µM in hypertrophied 3T3-L1 cells using the WST8 assay. Hypertrophied 3T3-L1 cells were incubated with 50 and 100 µM of VB for 48 h. ** *p*=0.0012 when compared with C group; # *p*=0.0275 when compared with V50 group. C, untreated hypertrophied 3T3-L1 cells; V50, hypertrophied 3T3-L1 cells treated with 50 µM VB; V100, hypertrophied 3T3-L1 cells treated with 100 µM VB. All data is presented here as quantified summaries of the findings from three separate tests, one-way ANOVA (post hoc: Tukey's test) was used, F (2, 7)=12.24, mean ± standard deviation (C: 99.77±2.70; V50: 78.96±0.50, V100: 57.03±1.11)

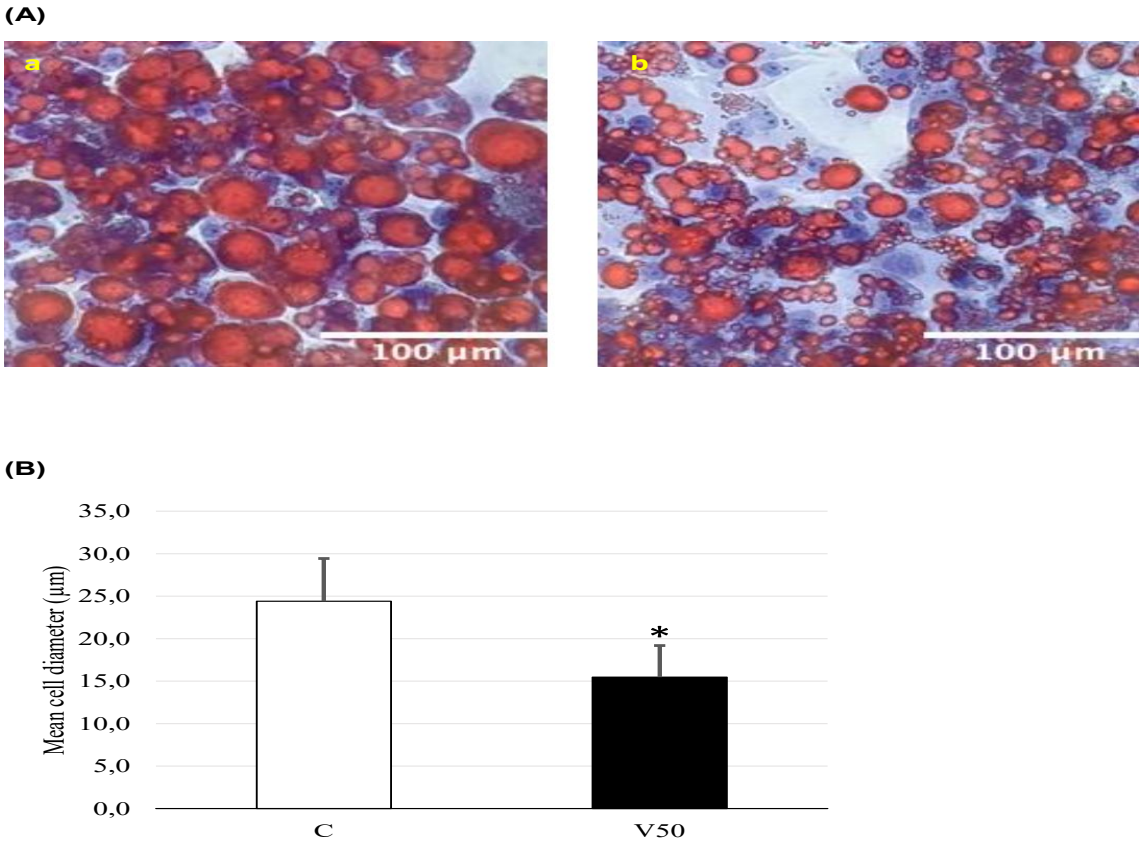
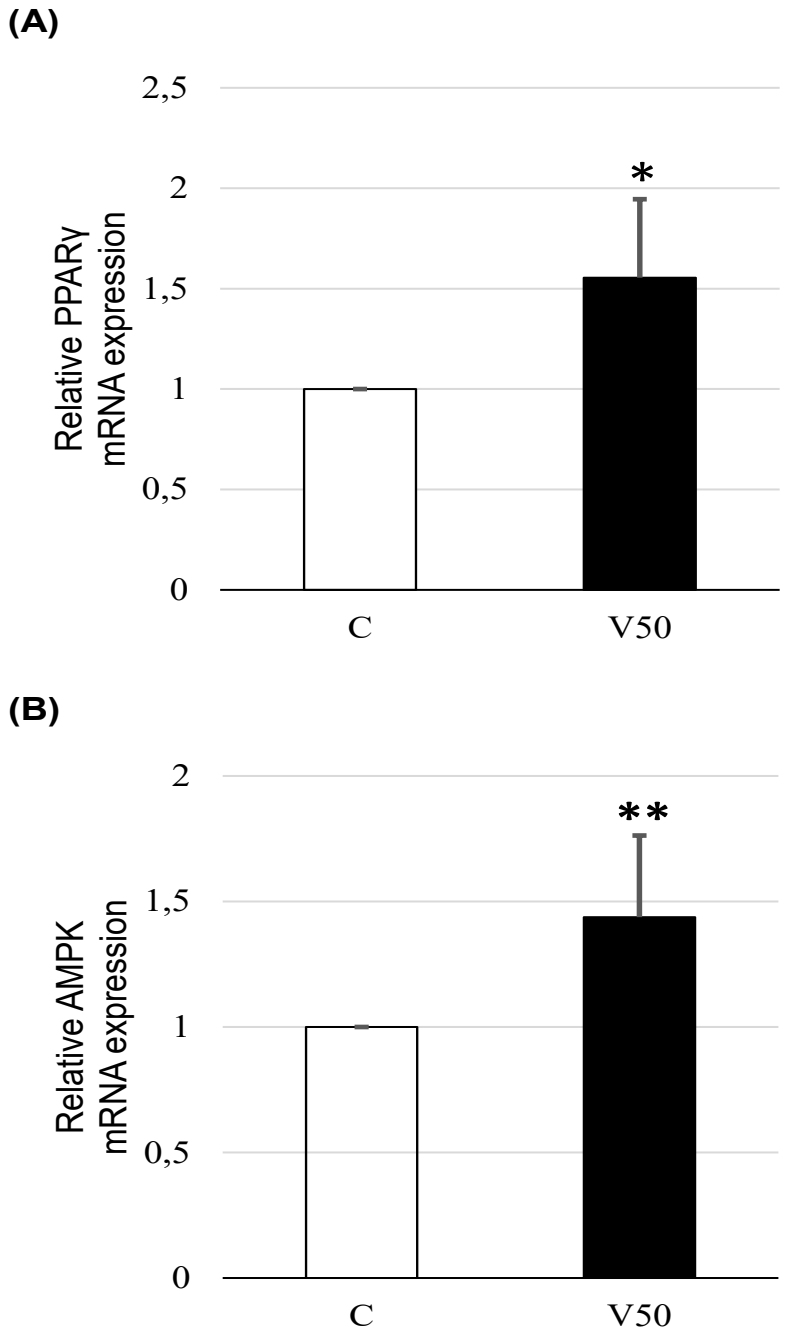


Figure 6. Representative images (A) and diameter (B) of hypertrophied 3T3-L1 cells. a: C, untreated hypertrophied 3T3-L1 cells; b: V50, hypertrophied 3T3-L1 cells treated with 50 µM VB. Student's t-test was used, t(150)=12.49, ****p*=0.0001 when compared with C group, mean ± standard deviation (C: 24.40±5.08, n=64; V50: 15.47±3.75, n=88)

qRT-PCR validation of selected mRNAs

Our results showed that although microarray analysis did not detect a significant fold increase in these genes, qRT-PCR showed a statistically significant upregulation of PPARγ, AMPK, HSL, and PLIN1 gene expression after VB treatment compared to control in hypertrophic 3T3-L1 cells. Primer sequences used in real-time PCR were shown in Table 1. The mRNA expression levels of PPARγ (Figure 7A), AMPK (Figure 7B), HSL (Figure 7C), and PLIN1 (Figure 7D) were significantly increased in the V50 group compared to the control group. These findings are consistent with those of previous research comparing mRNA gene expression using RT-PCR and DNA microarray methodologies, with RT-PCR providing more accurate results [23]. These findings support the idea that RT-PCR is the most appropriate approach to measure mRNA expression.



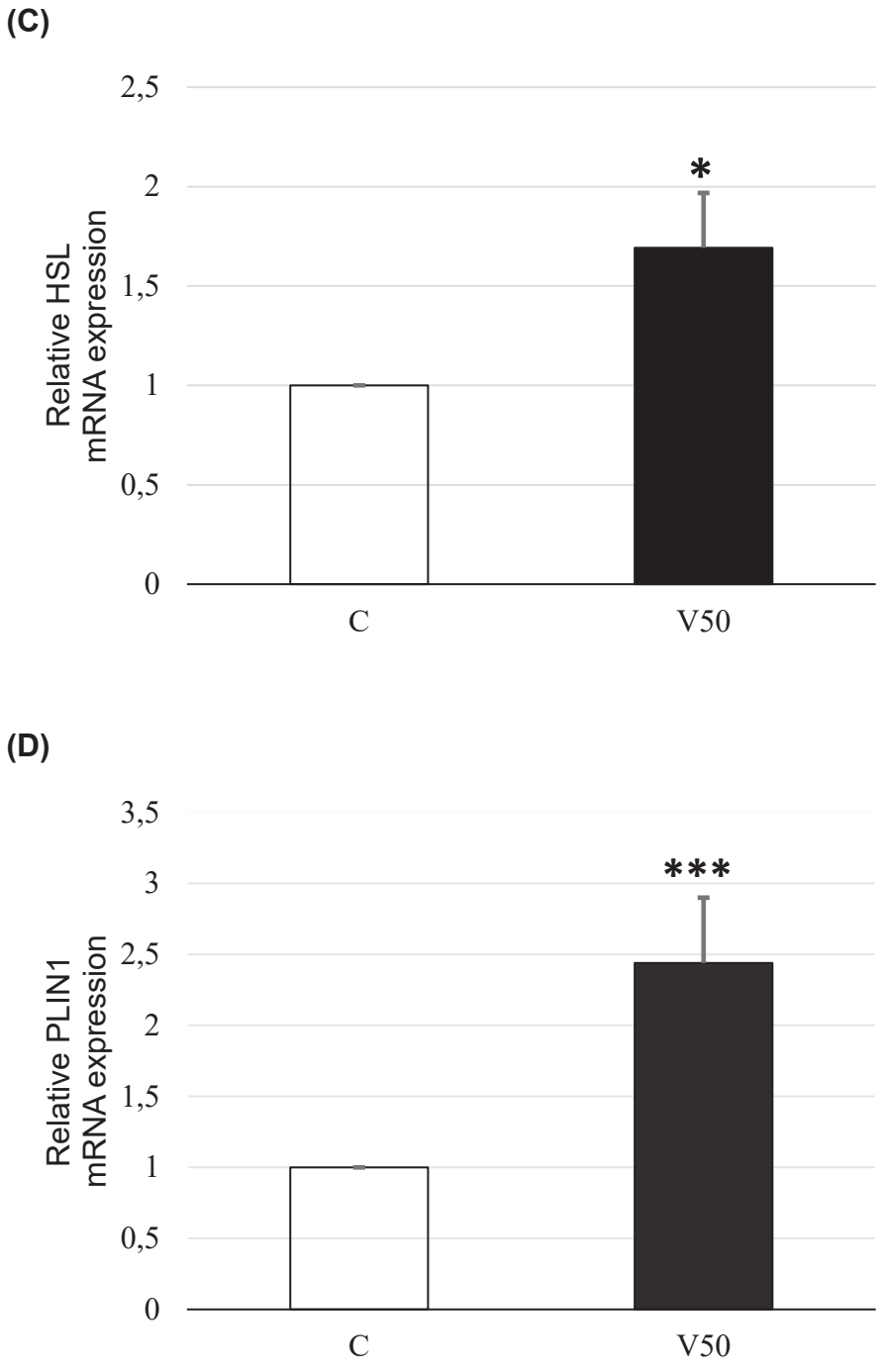


Figure 7. Relative changes of (A) PPARγ t(12)=2.29, *p=0.041 difference between the groups, mean ± standard deviation (C: 1±0; V50: 1.554±0.391), (B) AMPK t(12) = 3.25, **p=0.007 difference between the groups, mean ± standard deviation (C: 1±0; V50: 1.437±0.325), (C) HSL t(11)=2.78, *p=0.018 difference between the groups, mean ± standard deviation (C: 1±0; V50: 1.692±0.276) and (D) PLIN1 t(12) =6.65, ***p=0.001 difference between the groups mean ± standard deviation (C: 1±0; V50: 2.440±0.460) mRNA expression. Student's t-test was used, C untreated hypertrophied 3T3-L1 cells, V50 hypertrophied 3T3-L1 cells treated with 50 μM VB, n=6-7

Discussion

A contemporary lifestyle, characterized by the frequent consumption of calorie-rich meals and a dearth of physical activity, is a major contributor to the prevalence of obesity. This issue is particularly important because of the pathological development of excessive dysfunctional adipose tissue, which is commonly observed in cases of obesity. Obesity is well recognized as a significant risk factor for several diseases, including T2DM, cardiovascular disease, IR, elevated blood glucose levels (hyperglycemia), abnormal lipid levels (dyslipidemia), hypertension, and certain forms of cancer [1-3]. In recent years, there has been an increasing focus on the study of adipocyte biology owing to the growing prevalence of obesity-related health issues on a global scale.

Established cell lines, including 3T3-L1, are often used to investigate adipocyte proliferation, differentiation, adipokine secretion, and gene and protein expression in vitro. Oil Red O staining confirmed the maturation (day 10) and hypertrophy (day 18) of the preadipocytes, thus validating our cell line model. In our study, we investigated the effects of VB on the gene expression profile of hypertrophied 3T3-L1 cells and, secondly, on the expression levels of genes involved in lipolysis pathways such as PPARγ, AMPK, HSL, and PLIN1.

Interestingly, microarray results of genes known to have important roles in adipose tissue metabolism (AMPK, PPARγ, HSL, and PLIN1), for which we wanted to investigate how they changed with VB administration, showed that none of their mRNA expression levels were changed. A common feature of these genes is that they have various roles in adipose tissue metabolism. It is reported in the literature that VB alone or various herbal extracts containing VB stimulate lipolytic processes [15, 24, 25]. Therefore, we searched a database (Uniprot.org) based on gene ontology to identify genes that play a role in the regulation of lipid metabolism. According to our microarray results, the expression of many genes involved in lipid metabolism was increased by 1.5-2.5 times by VB treatment. Therefore, we wanted to confirm PPARγ, AMPK, HSL, and PLIN1 gene expression by RT-PCR, in which we did not observe any changes in their expression following VB administration according to our microarray results.

Because of the cytotoxicity caused by 100 μM VB, we investigated how AMPK, PPARγ, HSL, and PLIN1 genes were altered by RT-PCR only at a dose of 50 μM VB (V50 group). Our RT-PCR results showed that VB administered at a dose of 50 μM in hypertrophic 3T3-L1 cells significantly upregulated the expression of all these genes compared to the control group. Although this finding did not confirm our microarray results, we believe that RT-PCR results are both more reliable and consistent with the literature information listed below [23]. Additionally, although this may seem like a contradiction, it has been reported in the literature that RT-PCR is more sensitive and reliable than microarray in detecting hypothesis-based gene expression changes [26]. For example, our indirect finding of lipolysis in 3T3-L1 cells with a 50 μM VB-induced decrease in diameter is compatible with the increase in PPARγ mRNA, which is mainly expressed in adipose tissue and found to be increased by RT-PCR. Although it is not known in the literature how PPARγ mRNA changes with VB, this increase can be explained by considering the mechanism of the effects of polyphenols on adipocytes. The effects of polyphenols are mediated by adrenergic membrane receptors, especially β_3-adrenergic receptors (β_3-ARs), which are most dominant in white and brown adipocytes and thus stimulate lipolysis [27, 28]. In addition, many intracellular signaling mechanisms mediate lipolysis induced by stimulation of β_3-Ars [29]. One of these intracellular pathways is the increase in many PPAR ligands, especially PPARγ [27]. Since polyphenols are thought to act via β_3-ARs, VB may cause an increase in PPARγ expression. Similarly, rosiglitazone, an agonist of PPARγ, has been shown to increase lipolysis [30]. Therefore, the decrease in hypertrophied 3T3-L1 cell diameter, which we thought to be due to lipolysis, may have been caused by VB increasing the PPARγ mRNA levels.

Another intracellular signaling protein with increased expression in the V50 group compared to that in the control group was AMPK. AMPK, a critical regulator of cellular energy balance, plays an important role in adipose tissue,

liver, hypothalamus, and skeletal muscle [31]. AMPK activates pathways that increase energy production, such as glucose transport and fatty acid oxidation, and inhibits energy-consuming pathways such as lipogenesis, protein synthesis, and gluconeogenesis [32]. Similar to other polyphenols, AMPK is activated in adipocytes in response to adrenergic stimulation [33].

Our findings support these effects of AMPK, and studies in the literature show increases in AMPK expression following VB administration. In accordance with the effects of polyphenols via adrenergic stimulation, we found that VB administration caused a statistically significant increase in the expression of HSL, a key enzyme for lipolysis, in the V50 group compared to that in the control. HSL is activated by cAMP-dependent protein kinase A (PKA) in response to adrenergic/noradrenergic stimulation [34]. Phosphorylation of HSL by PKA causes its translocation from the cytosol to the lipid droplet surface and the hydrolysis of triacylglycerols (TAGs) [35]. Our finding that VB administration decreased the diameter of hypertrophied 3T3-L1 cells is consistent with the possible effect of VB via β_3-ARs. In this study, we showed for the first time that VB causes an increase in HSL mRNA levels in adipocytes.

The most interesting finding of our study was the statistically significant increase in PLIN1 mRNA expression, which encodes the perilipin protein, with VB administration compared to the control. Perilipin, also known as lipid droplet-associated protein, perilipin 1, or PLIN, is encoded by the PLIN gene in humans [36]. PLIN1s are vital for efficient storage of TAGs in adipose tissues. HSL expression increases in lipid droplets during cold, starvation, and exercise, whereas the expression of perilipins in lipid droplets increases to promote fat storage under basal conditions such as rest and satiety [37]. While 3T3-L1 cells do not contain perilipin when they are in the preadipocyte form, perilipin expression increases when 3T3-L1 cells are transformed into mature adipocytes, causing TAGs storage and the formation of numerous lipid droplets in the cytoplasm. This causes hypertrophied 3T3-L1s to store 6-30 times more TAG than the control cells [38]. Although this protein is known to have functions in lipid storage, as summarized above, it has also been reported that PLIN1 plays a facilitating role in the transport of the aquaporin 7 (AQP7) water channel to the membrane. AQP7 mediates the transport of glycerol, whose intracellular levels increase due to lipolysis, out of the cell [39]. Therefore, the increase in PLIN1 mRNA expression levels, despite the lipolysis caused by VB administration, may prevent intracellular glycerol accumulation.

In conclusion, we demonstrated that VB administration to hypertrophic 3T3-L1 cells significantly altered the gene expression profile. In addition, in line with our hypothesis, we found that VB has an increasing effect on the mRNA expression of AMPK, PPAR-γ, HSL, and PLIN1 VB, which play a key role in lipid metabolism. The findings obtained from this study may provide valuable information regarding the potential of VB as a tool in the fight against obesity. We think that further studies should be carried out on the subject.

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