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In Vitro Anti-Obesity Effect of *Aloe vera* Extract Through Transcription Factors and Lipolysis-Associated Genes

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ABSTRACT

In recent years, obesity has been associated with heart diseases, type 2 diabetes, obstructive sleep apnea, certain types of cancer and osteoarthritis, and become a global health problem. Therefore, researchers seek to find functional drugs against obesity. For the past few decades, medicinal plants have been examined to for their anti-obesity effects, including *Aloe vera*. In this study, it was aimed to elucidate the inhibitory effect of *Aloe vera* extract on adipogenesis. Firstly, 3T3-L1 pre-adipocytes were stimulated so as to differentiate into mature adipocyte using adipogenic differentiation cocktail consisting of 10 µg/mL insulin, 0.5 mM isobutylmethylxanthine, 0.25 mM dexamethasone, and 100 ng/mL biotin on day 0. Various concentrations (10-50 µg/mL) of *Aloe vera* extract with no cytotoxic effect were applied to differentiated 3T3-L1 cells. Glyceraldehyde-3-phosphate dehydrogenase (GPDH) activity, Oil red O staining, intracellular triglyceride levels, and gene expressions of transcription factors and lipolysis-associated genes were examined in order to investigate the effect of *Aloe vera* extract on adipocyte differentiation. *Aloe vera* treatment caused a continuous decrease in cell size and intracellular triglyceride accumulation. Despite the fact that GPDH activity, mRNA levels of transcription factors and lipolysis-associated genes decreased in mature adipocytes treated with *Aloe vera* extract. These results suggest that *Aloe vera* may have a therapeutic effect in prevention and/or treatment of adipogenesis-related obesity.

Keywords: 3T3-L1, adipogenesis, *Aloe vera*, cell differentiation, transcription factors, lipolysis-associated genes

Introduction

Obesity is a condition in which body fat is accumulated more than required levels and is recognized as a disease related to various health problems (1, 2). It has been observed that the risk of developing diabetes and hypertension in adult obese individuals increased 10 and 4 times, respectively, compared to healthy individuals, and the overall mortality rate in obese individuals increased by 40% compared to their normal weight peers (3). According to the data of the World Health Organization released in 2016, there are approximately 2 billion overweight adults in the world which is accepted that 650 million of them are affected by obesity (4). This increasing trend makes it possible to estimate that the world's obese population will exceed half of the total population in 2025, and this problem is expected to become even more serious in developed countries (5). In order to treat and prevent obesity, it is important to understand the mechanisms of adipogenesis and lipid accumulation (6).

Adipogenesis is pre-adipocytes to mature adipocytes procedure including an across-the-board system with various transcription factors regulation, such as peroxisome proliferator activated receptor γ (PPARγ) which act as a central player through differentiation course whether *in vitro* or *in vivo* (7). When the preadipocytes are stimulated by inducer that differentiation seem started, CCAAT/enhancer-binding proteins β (C/EBPβ) and sterol, regulatory element-binding protein 1c (SREBP-1c) is actived during early stage of adipocytes

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differentiation, then they induce C/EBPα and PPARγ regulated a cross-talk to enhance and maintain adipocytes gene degree at later adipogenesis (8). Genes leading to the expression of adipocyte-specific genes are adipose triglyceride lipase (ATGL), Acetyl-CoA carboxylase (ACC), hormone sensitive lipase (HSL), fatty acid synthase (FAS), and adipocyte protein 2 (aP2) (9).

Complementary and alternative medicine is an attractive therapy for obesity and obesity related diseases because it causes fewer side effects, and less toxicity compared to medicinal therapy. Although, plant extracts have been used in folkloric medicine in the treatment of obesity for hundreds of years, there are very few *in vivo* or *in vitro* studies describing the activity and mechanism of these drugs (10-12). In the literature, *Hibiscus sabdariffa*, *Panax ginseng*, *Rosa canina*, *Camellia sinensis* and *Zingiber officinale* extracts have been shown to suppress the adipogenesis process by inhibiting the transcription factors (13-17).

For decades, *Aloe vera* has been broadly applied as a medicinal plant to overcome various health problems (18). *Aloe vera* has anti-obesity activity as well as wound healing, analgesic, anti-inflammatory, antibacterial, antiviral, antifungal, antioxidant and antitumor activities (19-21). Aloe-emodin, an anthraquinone compound isolated from *Aloe vera* leaf, was shown to suppress the differentiation of 3T3-L1 pre-adipocytes to mature adipocytes and reduce lipid accumulation via inhibition of the transcription factors (22). Adipocyte differentiation was also nearly blocked with *Aloe vera* in a study using human mesenchymal stem cells (23). In another study, administration of dietary *Aloe vera* extract in obese mice reduced body weight and prevented adipocyte differentiation by suppressing PPARγ expression (24). Oral administration of isolated phytosterols from *Aloe vera* significantly reduced visceral fat weights than obese control in Zucker diabetic fatty rats. In the same study, it was observed that phytosterols isolated from *Aloe vera* show an antiobesity effect by suppressing the expression of transcriptional factors, gluconeogenic and lipogenic enzymes and inducing the expression of glycolysis and lipolysis-related enzymes (25). Nevertheless, the effect of *Aloe vera* extract on adipocyte differentiation has not been completely understood. Further studies of the effect of *Aloe vera* extract on adipocyte differentiation need to be carried out. This study aims to investigate the effect of *Aloe vera* extract on adipocyte differentiation through the transcriptional factors using pre-adipocyte cell line.

Materials and Methods

Reagents: All cell culture chemicals were purchased either from Sigma-Aldrich, Merck or other standard suppliers. Mouse Glycerol-3-phosphate dehydrogenase (GPDH) ELISA kit was commercially available from Elabscience. 3T3-L1 mouse embryo preadipocyte cell line was purchased from the Health Protection Agency (HPA) cell culture collection (Sigma 86052701). Specific PCR primers for *PPARγ*, *C/EBPa*, *SREBP-1c*, *ATGL*, *ACC*, *HSL*, *FAS*, *aP2*, and β-actin were designed using primer3web program (v. 4.1.0) and synthesized by PRZ BioTECH (Bilkent, Ankara, Turkey).

Preperation of Aloe Vera Extract: *Aloe vera* (L.) Burm. f. was purchased from a local Landscape Market in Mersin, Turkey. Leaves were washed, peeled, and dried. The dried *Aloe vera* leaves were then blended using an electric blender and boiled with ethanol 95% and then filtered. The filtrate was concentrated, obtaining thick gel-like extract of *Aloe vera* (26).

Cell culture: Frozen 3T3-L1 cells were immediately thawed and transferred into a 10 cm^2 culture dish with culture media (DMEM, 10% bovine calf serum (BCS), and 1% penicillin/streptomycin). The cells were incubated in a humidified cell culture incubator with 5% CO_2 at 37°C. The media was replaced the following day. When the 3T3-L1 cells reached 70% confluence, cells were subcultured by adding 1 mL Trypsin-EDTA solution into the culture dish and incubated for 5 minutes at 37°C. Culture media was added to the culture dish, and then cell suspension was transferred into a new 10 cm^2 culture dish, which included 10 mL of culture media (27). To determine the numbers of cells that needed to be seeded for experiments, 10 µL of the cell suspension was loaded onto a hemocytometer. Cells in the four squares of the hemocytometer were counted, averaged, and multiplied by 10^4 (28).

Preparation of Aloe Vera Solution in Different Concentrations: Before use, the 1 mg/mL *Aloe vera* stock solution was diluted with DMEM to create extracts at concentrations in the range 10-100 µg/mL.

Effect of Aloe Vera Extract Applications in Different Concentrations on Cell Viability: MTT test was performed to determine the non-cytotoxic concentrations of *Aloe vera* extract on adipocytes. Absorbance was measured at a

wavelength of 570 nm using VersaMax Microplate Reader (29). The treatments were performed in triplicates.

Differentiation of 3T3-L1 Cells to Mature Adipocytes: The cells were then seeded at 50,000 cells/mL in 6-well, 12-well, 96-well plates with culture media, and this is day 0 of the experiment. The cells were incubated in the cell culture incubator. On day 2, when the cells reached 100% confluence, the culture media was switched to differentiation media (DMEM, 10 % fetal bovine serum (FBS), 1% penicillin/streptomycin, 10 µg/mL insulin, 0.5 mM IBMX, 0.25 mM dexamethasone, and 100 ng/mL biotin) including 10, 20, 30, 40, and 50 µM of *Aloe vera* extracts (non-cytotoxic concentrations) were provided to the cells and then cells were incubated in cell culture incubator. As negative control, only differentiation media was applied to the cells. On day 4, the differentiation media was removed and replaced with post-differentiation media (DMEM, 10% FBS, 1% penicillin/streptomycin, and 1 µg/mL insulin). The post-differentiation media was refreshed every other day until day 18 (27).

Lactate Dehydrogenase (LDH) Release Assay: At the end of the differentiation period, LDH release assay was performed to determine the cytotoxic effects of *Aloe vera* extracts in mature adipocytes using LDH Cytotoxicity Detection Kit (Roche Diagnostics, Mannheim, Germany) (Catalog No: 11644793001). The percentage of cytotoxicity was calculated using the following formula (30);

Cytotoxicity (%) = [(Sample absorbance - Control absorbance) / (Maximal absorbance / Control absorbance)] x 100

Oil Red O Staining: During the differentiation process, lipids are accumulated as a single lipid droplet in cytosol of the cell. This indicates that white pre-adipocytes have developed into mature white adipocytes. To examine whether the treatment of *Aloe vera* in the different growth stages of 3T3-L1 cells affects the differentiation process, lipid droplets in fully differentiated mature white adipocytes (day 18) were stained using Oil Red O. Images of the stained lipids were taken with an Euromex inverted microscope at 10x, 20x and 40x magnification. After the imaging, stained oil droplets were dissolved in isopropyl alcohol, and relative triglyceride content was quantified by reading the absorbance at 490 nm on a microplate reader (31).

Glycerol-3-Phosphate Dehydrogenase (GPDH) Activity Assay: GPDH activity was measured using a commercial kit (Elabscience Biotechnology Inc., Texas, USA) (Catalog No: E-EL-M0424) and expressed as units/mg of protein (32).

Estimation of Total Protein: Protein content in cell lysate was determined using Bradford's dye. Briefly, 100 µL cell lysate was reacted with 1 mL of Bradford reagent (2 parts stock Bradford reagent and 7 parts deionized water) and absorbance was recorded at 595 nm. Protein concentration was expressed as bovine serum albumin (BSA) equivalent/mL (33).

RNA Preparation and Quantitative Real-Time PCR: Total RNA was extracted using TriPure RNA isolation kit (Roche Diagnostics, Mannheim, Germany) (Catalog No: 11667165001), according to the manufacturer's protocol. The total RNA (2 µg) was used for cDNA synthesis using First Strand cDNA synthesis kit (Promega Corporation, Wisconsin, USA) (Catalog No: E6560S). Then mRNA expression was quantitatively determined by Roche LightCycler 480 II Real-Time PCR device using Invitrogen Universal EXPRESS SYBR GreenER qPCR SuperMixes and Two-Step qRT-PCR kit. Real-Time PCR was performed according to the methods of Lasa et al. (34) and Eseberri et al. (35). To quantify the results from real-time PCR, the threshold cycle (Ct) values from the amplified target genes were incorporated into the $2^{-\Delta\Delta Ct}$ formula. β-actin was used as the housekeeping gene. The primers used in this experiment are shown in Table 1 (36-42).

Statistical Analysis: Statistical analyzes were done in SPSS for Windows v16.0 package program. ANOVA was used to test the statistical differences between the groups for all characteristics and Tukey multiple comparison test was used for subgroup comparisons. Cell viability data were corrected with Abbott's formula and the IC_{10} value was calculated according to concentration versus Probit regression analysis with non-overlap of the 95 % confidence intervals. Mean ± standard deviation values were given as descriptive statistics and error bar graph was drawn for variables. Differences with a value of $p<0.05$ were considered statistically significant.

Results

The cell viability was defined by MTT assay. To assess cytotoxic effect of *Aloe vera*, cell viability in 3T3-L1 preadipocytes was evaluated using MTT assay. The effects of different concentration of *Aloe vera* extracts (10-100 µg/mL) on cell viability were shown in Figure 1. *Aloe vera* treatment at concentrations of 10-50 µM for 48 hours had no remarkable inhibitory effect on

Table 1. Primers for PCR Amplification of each studied gene

Gene Name	Forward Primer	Reverse Primer
PPARγ (36)	5'- ACC CCC TGC TCC AGG AGA T -3'	5'- TGC AAT CAA TAG AAG GAA CAC GTT -3'
C/EBPα (37)	5'- CGC AAG AGC CGA GAT AAA GC -3'	5'- GCG GTC ATT GTC ACT GGT CA -3'
SREBP-1c (38)	5'- GAC GCT CAT TGG CCT GG -3'	5'- CTC TGG AGG CAG ACG ACA AG -3'
HSL (38)	5'- GCT GGG CTG TCA AGC ACT GT -3'	5'- TAC CGT CGG ATG GGT CAA TG -3'
ATGL (39)	5'- GGATGAAAGAGCAGACGGGTAG -3'	5'- CGCAAGACAGTGGCACAGAG -3'
ACC (40)	5'- CTT GGG GGC ATC CAG ATT AT-3'	5'- AGG ATC ATA TGG GGC CTT TG -3'
FAS (40)	5'- CTC TGA TCA GTG GCC TCC TC -3'	5'- TGC TGC AGT TTG GTC TGA AC -3'
aP2 (41)	5'- AGCCTTTCTCACCTGGAAGA -3'	5'- TTGTGGCAAAGCCCACTC -3'
β-actin (42)	5'- AGG TCA TCA CTA TTG GCA AC -3'	5'- ACT CAT CGT ACT CCT GCT TG -3'

(**PPARγ:** Peroxisome proliferator-activated receptor γ, **C/EBPα:** CCAAT-enhancer-binding proteins alpha, **SREBP-1c:** sterol regulatory element-binding protein-1c, **HSL:** hormone sensitive lipase, **ATGL:** adipose triglyceride lipase, **ACC:** Acetyl-CoA carboxylase, **FAS:** fatty acid synthase, **aP2:** adipocyte protein 2)

cell viability (≤10% cytotoxicity). For the concentrations of 60-100 μg/mL *Aloe vera* extract showed an inhibition activity on cell growth in 48 hours (>10% cytotoxicity). The IC_{10} value of *Aloe vera* extract on 3T3-L1 cells was found to be 43.83 μg/mL.

LDH release assay was performed to determine cytotoxicity after differentiation process. The effects of different concentration of *Aloe vera* extract on LDH release was shown in Figure 2. Cytotoxic effect of *Aloe vera* extract in mature adipocytes was statistically higher than preadipocytes. Treatment with *Aloe vera* extract at concentrations of 30-50 μg/mL decreased the cytotoxicity compared with untreated mature adipocytes (p<0.05). The other concentrations of *Aloe vera* extract also decreased the cytotoxicity however this reduction was not statistically significant.

Microscopic image of 3T3-L1 cells after Oil Red O staining was shown in Figure 3. As a result of the morphological examination, *Aloe vera* extract applications caused a continuous decrease in cell size and intracellular triglyceride accumulation in a dose-dependent manner. Relative intracellular triglyceride contents were shown in Figure 4. Although relative intracellular triglyceride contents exhibited reduction for all *Aloe vera* concentrations compared with untreated mature adipocytes, this decrease was statistically significant only for concentrations of 40 μg/mL and 50 μg/mL (p<0.05)

GPDH activity was measured in order to determine the terminal differentiation after the differentiation process. GPDH activities were shown in Figure 5. GPDH activity of mature adipocytes was statistically higher than preadipocytes. *Aloe vera* treatments at concentrations of 30-50 μg/mL decreased the GPDH activity compared with untreated mature adipocytes (p<0.05). Aloe vera extract tratment at concentrations of 10 and 20 μg/mL also decreased the GPDH activity however these decreases were not statistically significant .

Gene expressions of *PPARγ*, *C/EBPa*, and *SREBP-1c* were shown in Figure 6. *PPARγ*, *C/EBPa*, and *SREBP-1c* mRNA levels of mature adipocytes were statistically higher than preadipocytes. The application of *Aloe vera* at different concentrations showed a dose dependent lowering effect on expression of these genes. However, this decrease was significant in groups which were treated with the *Aloe vera* extract at concentrations of 40-50 μg/mL and 30-50 μg/mL for *PPARγ* and *SREBP-1c* mRNA levels and for *C/EBPa* mRNA levels respectively (p<0.05). The other concentrations of *Aloe vera* extract also decreased the gene expressions however these decreases were not statistically significant.

HSL, *ATGL*, *ACC*, *FAS*, and *aP2* gene expressions were shown in Figure 7. *HSL*, *ATGL*, *ACC*, *FAS*, and *aP2* mRNA levels of mature adipocytes were statistically higher than preadipocytes. The application of *Aloe vera* at different concentrations showed a dose dependent lowering effect on expression of these genes. However, this decrease was found significant in groups that was treated with the *Aloe*

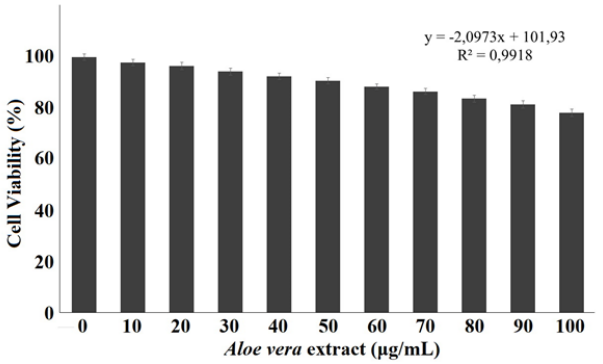

Fig. 1. Effect of *A. vera* extract administration at different concentrations on cell viability in undifferentiated 3T3-L1 preadipocytes

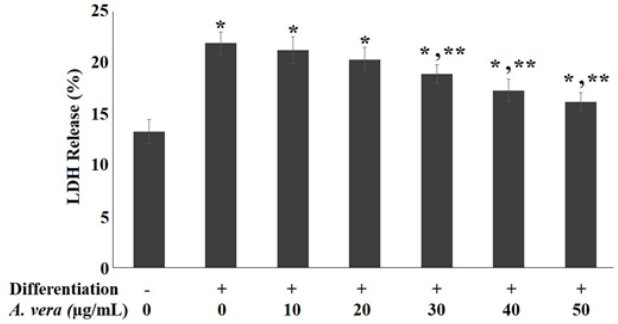

Fig. 2. Effect of *A. vera* extract administration at different concentrations on LDH release in 3T3-L1 cells
* p<0.05 in comparison to undifferentiated - untreated cells
** p<0.05 in comparison to differentiated - untreated cells

vera extract at concentrations of 30-50 µg/mL, 20-50 µg/mL and 40-50 µg/mL, for *HSL, ATGL, ACC* mRNA levels for *FAS* mRNA levels, and for *aP2* mRNA levels, respectively (p<0.05). The other concentrations of *Aloe vera* extract also decreased the gene expressions however these decreases were not statistically significant.

Discussion

Obesity is the excessive or abnormal accumulation of fat or adipose tissue in the body that impairs health via its association to the risk of development of diabetes mellitus, cardiovascular disease, hypertension, and hyperlipidemia. Mature adipocytes, the main cellular component of white adipose tissues, are uniquely equipped to function in energy storage and balance under tight hormonal control. Therefore, the differentiation of adipocytes and inhibition of fat accumulation can be an important strategy for improving obesity and also obesity-related diseases such as type II diabetes, hypertension and coronary heart disease (43, 44). Currently, studies related to

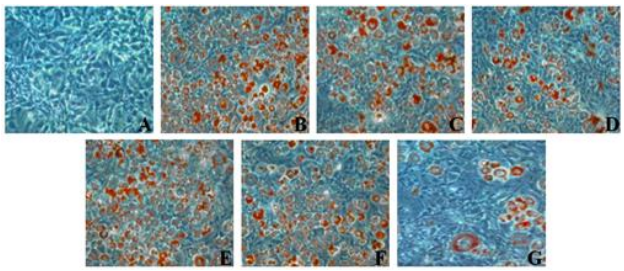

Fig. 3. Effect of *A. vera* extract administration at different concentrations on intracellular triglyceride storage in 3T3-L1 cells by Oil Red O staining. The cells stained with Oil Red O were visualized at ×100 magnification.
(**A**; Undifferentiated - untreated cells, **B**; Differentiated - untreated cells, **C**; Differentiated - 10 µg/mL *A. vera* extract treated cells, **D**: Differentiated - 20 µg/mL *A. vera* extract treated cells, **E**: Differentiated - 30 µg/mL *A. vera* extract treated cells, **F**: Differentiated - 40 µg/mL *A. vera* extract treated cells, **G**: Differentiated - 50 µg/mL *A. vera* extract treated cells)

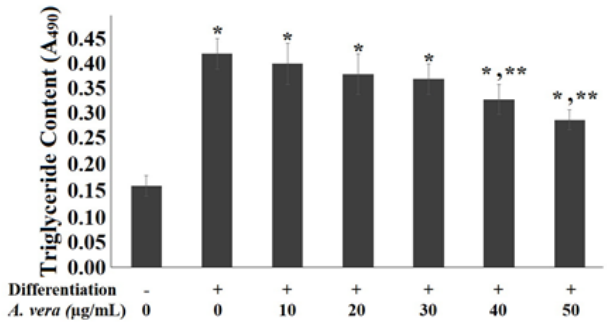

Fig. 4. Effect of *A. vera* extract administration at different concentrations on relative intracellular triglyceride content in 3T3-L1 cells.
* p<0.05 in comparison to undifferentiated - untreated cells
** p<0.05 in comparison to differentiated - untreated cells

obesity are being performed with many natural products-derived medicines or ingredients (11, 12). There are several studies in the literature trying to reveal the anti-obesity activity of *Aloe vera* (22-25). These studies conducted so far are generally *in vivo* studies, and there are only limited studies investigating the anti-obesity effect of *Aloe vera* at the cellular and molecular level. The aim of this study was not only to investigate the effect of *Aloe vera* on adipogenesis inhibition, but also to reveal which mechanism is effective on adipogenesis. The 3T3-L1 cell line has been selected for this study because this cell line is an adipocyte progenitor cell widely used in obesity studies, and is known as a cell line that can best reproduce the obesity state by inducing adipogenesis by treatment with a differentiation inducing agent (45). To mimic a successful mature adipocyte model, differentiation media was used as a differentiation inducer to stimulate confluent 3T3-L1 cells, furthermore altered cells appearance

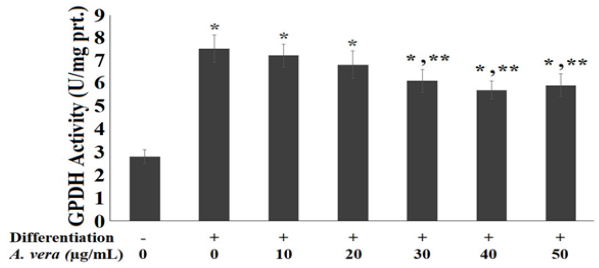

Fig. 5. Effects of *A. vera* extract administration at different concentrations on GPDH activity in 3T3-L1 cells.
* $p<0.05$ in comparison to undifferentiated - untreated cells
** $p<0.05$ in comparison to differentiated - untreated cells

from fertilized egg to fibroblast, preadipocytes and spherical shape of adipocytes in sequential order (45, 46).

Non-cytotoxic concentrations of *Aloe vera* extract have been determined by using MTT test (47). While *Aloe vera* at concentrations of 10-50 µg/mL did not show any cytotoxic effects, the application of higher concentrations led to an excessive increase in cell death. In our study, IC_{10} value of *Aloe vera* extract on 3T3-L1 cells was also calculated as 43.83 µg/mL. Wong-a-nan et al. (48) determined the IC_{10} value of *Zingiber cassumunar* Roxb. rhizome extracts as 11.8 µg/mL. Oon et al. (49) determined the IC_{10} value of xanthorrhizol as 35 µg/mL. It can be understood that the cytotoxic effect of *Aloe vera* extract is lower than *Zingiber cassumunar* rhizome extract. This shows that *Aloe vera* can be safely used without causing too much cell death.

LDH release assay is the most widely used enzyme release cytotoxicity test in *in vitro* models (50). The cytotoxic effect of *Aloe vera* extract in differentiated cells was found higher than the cytotoxicity in undifferentiated cells, due to the fact that increased triglyceride and fat vacuoles

in differentiated adipocytes made the cells more fragile. Treatment of *Aloe vera* extract at different concentrations to differentiated cells increased the cytotoxicity. It is predicted that this situation is due to the decrease in the amount of triglycerides and fat vacuoles in adipocytes and the decrease in cell fragility. A slight increase in cell death was observed after *Aloe vera* applications at concentrations of 30 µg/mL and above, and it was thought that *Aloe vera* applications at high concentrations could adversely affect cell viability.

Oil Red O staining is a valuable method for the determination of cellular morphology and intracellular triglyceride storage (51). After the transformation of pre-adipocytes into mature adipocytes, a significant increase in the triglyceride content of adipocytes was observed. In addition, when pre-adipocytes were small, hollow and numerous, the volumes and triglyceride content of mature adipocytes increased, while their number decreased. It is thought that the higher number of undifferentiated cells compared to differentiated cells may be due to the high proliferation of these cells and the relative decrease in the number of differentiated cells due to the fact that cells lose their dividing capacity and become more fragile and easily damaged due to the increase in lipid content (52, 53). Dietary triglyceride accumulation and endogenous lipogenic signal increase the size of adipocytes, while increasing proliferation and differentiation increase the number of adipocytes. Therefore, pharmacological solutions that limit both the number and size of adipocytes form the basis of anti-obesity drugs. Differentiated cells treated with *Aloe vera* extract at different concentrations decreased the triglyceride content, and also resulted in decreased cell size and intracellular triglyceride storage. This situation shows that *Aloe vera* reduced anabolic activity in differentiated cells. Application of *Aloe vera* extract at concentrations of 40 and 50 µg/mL to mature adipocytes significantly decreased the cell size and intracellular triglyceride content, thus it is estimated that these doses may be the most effective ones.

Since GPDH activity is a marker of terminal differentiation, it is frequently used in adipogenesis studies (54). In the current study, differentiated adipocytes have higher GPDH activity compared to undifferentiated adipocytes. Treatment of differentiated cells with *Aloe vera* extract has been shown to reduce GPDH activity, and this result proves the reducing effect of *Aloe vera* on adipogenesis. In addition, no significant reducing effect of *Aloe vera* extract treatment on the viability of 3T3-L1 cells was observed, indicating that the suppressive effect of *Aloe vera* on adipogenesis occurred independent of cytotoxicity.

PPARγ, *C/EBPa*, and *SREBP-1c* play important roles during adipocyte differentiation, because they mediate the transcription of numerous genes (55). *PPARγ* is the key regulator of adipogenesis, whereas *C/EBPa* may play an accessory role for *PPARγ* by inducing and maintaining *PPARγ* expression (56). To determine whether *Aloe vera* extract affects the expression of transcription factors, mRNA levels of *PPARγ*, *C/EBPa*, *and SREBP-1c* were analyzed. In the current study, *PPARγ*, *C/EBPa*, *and SREBP-1c* gene expressions of differentiated adipocytes was found higher than the undifferentiated cells. This

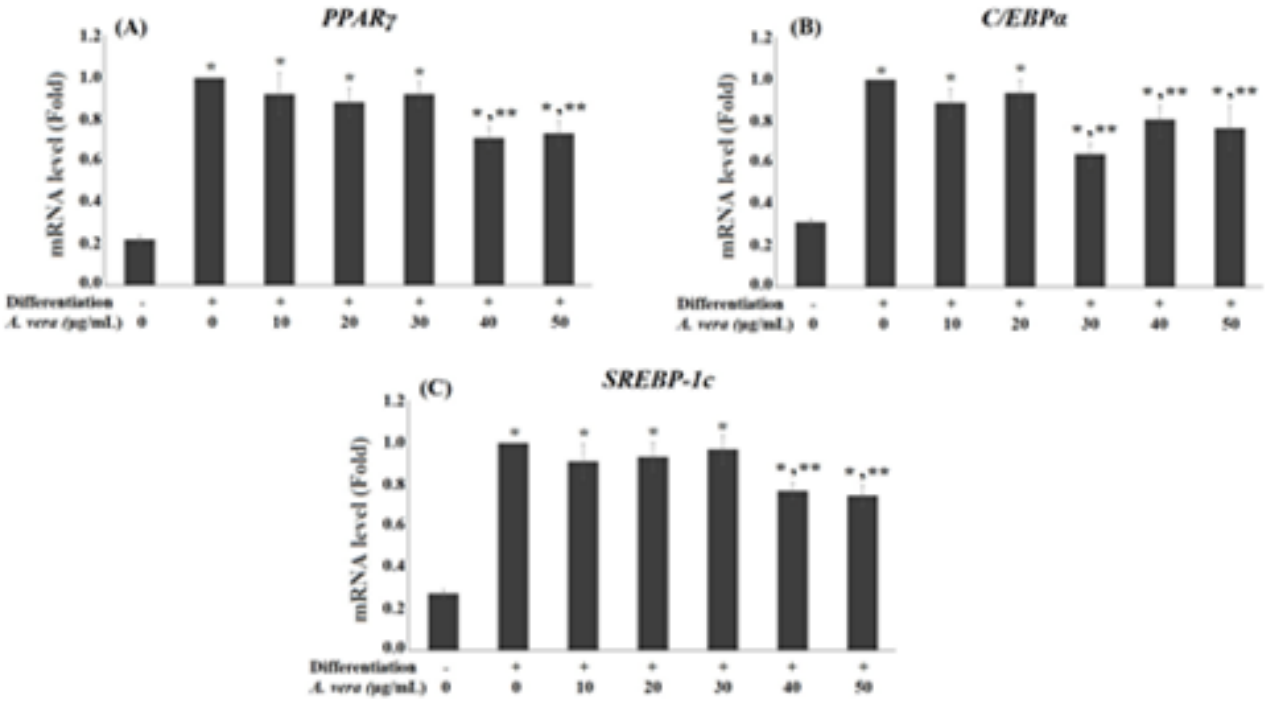

Fig. 6. Effect of *A. vera* extract administration at different concentrations on relative **(A)** *PPARγ*, **(B)** *C/EBPa*, and **(C)** SREBP-1c gene expressions in 3T3-L1 cells.
* $p<0.05$ in comparison to undifferentiated - untreated cells
** $p<0.05$ in comparison to differentiated - untreated cells

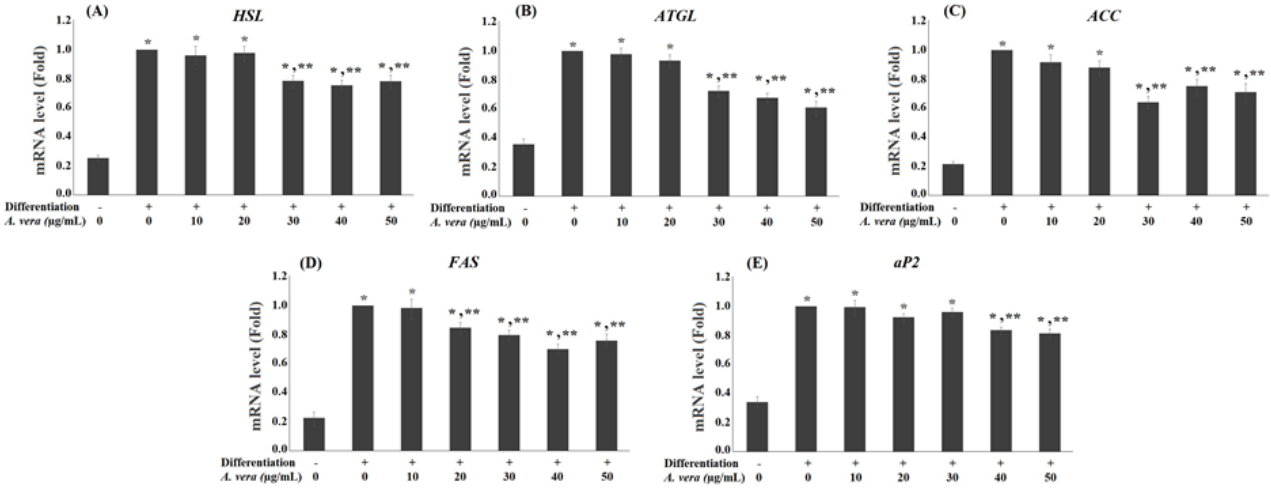

Fig. 7. Effect of *A. vera* extract administration at different concentrations on relative **(A)** *HSL*, **(B)** *ATGL*, and **(C)** *ACC*, **(D)** *FAS*, and **(E)** *aP2* gene expressions in 3T3-L1 cells.
* $p<0.05$ in comparison to undifferentiated - untreated cells
** $p<0.05$ in comparison to differentiated - untreated cells

demonstrates that 3T3-L1 cells have undergone complete terminal differentiation. *Aloe vera* treatment downregulated the mRNA levels of *PPARγ*, *C/EBPa*, *and SREBP-1c* on final stage of differentiation. Several studies suggest that *PPARγ*, *C/EBPa*, *and SREBP-1c* mutually co-regulate their expression (55, 57).

Adipocyte differentiation process requires a complex algorithm of various adipogenic gene expression. The downregulation of *SREBP-1c* and *C/EBPa* following *Aloe vera* treatment may reduce the fatty acid synthesis, as well as the synthesis and activity of *PPARγ*, thereby inhibiting adipogenesis and lipid accumulation (56 57). *PPARγ* and *C/EBPa* are master transcription

factors through the differentiation course, thus they activate *SREBP-1c* to up-regulate the expression of adipocyte-specific proteins: ACC, FAS, aP2 and so on (58). ACC expression is indirectly inhibited via the suppression of SREBP-1c. The downregulation of aP2 and FAS considerably decreased fatty acid utilization and fatty acid transport in 3T3-L1 cells. aP2 is a terminal differentiation maker of adipocytes, and facilitates the cellular uptake of long-chain fatty acids in a pathway linked to fatty acid metabolism and obesity. FAS is associated with differentiation and lipid accumulation in 3T3-L1 pre-adipocytes (59). Therefore, the *Aloe vera* induced downregulation of PPARγ and C/EBPα may suppress other adipogenic mRNA expressions.

ATGL and HSL mediate the hydrolysis of TG into FFAs and glycerol during lipolysis (39, 60, 61). Gene expression of *ATGL* and *HSL* were determined for determination of the lipolytic responses during adipocyte differentiation. Treatment with *Aloe vera* significantly downregulated *ATGL* and *HSL* gene expressions during adipocyte differentiation. According to Kong et al (62), treatment with sulfated glucosamine decreased *HSL* gene expression level during adipocyte differentiation compared to fully differentiated adipocytes, resulting in a decrease in lipid accumulation. In addition, after the administration of *Aloe vera* extract to adipocytes, the reduction in the intracellular triglyceride content probably occurred due to the decrease in the expression of the *ATGL* and *HSL* genes which are involved in lipolysis.

In conclusion, this study suggests that *Aloe vera* extract inhibits adipocyte differentiation and prevents obesity through the transcriptional factors and adipogenic genes. These results suggest that *Aloe vera* may have a therapeutic effect in prevention and/or treatment of adipogenesis-related obesity.

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