Molecular Biology Reports (2020) 47:8679–8686
https://doi.org/10.1007/s11033-020-05912-7

ORIGINAL ARTICLE

Hypericum perforatum L.: a medicinal plant with potential as a curative agent against obesity-associated complications

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Received: 24 July 2020 / Accepted: 10 October 2020 / Published online: 22 October 2020
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Abstract

Obesity is a low-grade inflammatory disease that is getting increasingly common among adults and children and causes different complications. Insulin resistance, Type II diabetes, atherosclerosis, metabolic syndrome and hypertension are among the major health problems, that are associated with obesity. Some medications are used to treat obese individuals and metabolic surgery is recommended, if appropriate, for individuals with a BMI ≥ 40. Due to the fact that medications and metabolic surgery are not tolerated by all, researchers focus on alternative therapies. Medicinal plants comprise the most important group of these alternative treatments. *Hypericum perforatum* L. is the medicinal plant, which we focused on in this study. *Hypericum perforatum* L. has been recognized as a medicinally valuable plant for over 2000 years. It has been used for generations to treat anxiety, depression, insomnia, gastritis, hemorrhoids, wounds, and burns. Recent studies have indeed shown promising effects for the treatment of obesity. In this study, 3T3-L1 adipocytes were used to mimic the adipocyte differentiation associated with obesity in cellular terms. Lipoprotein lipase (Lpl), Diacylglycerol-*O*-acyltransferase 1 (Dgat1), Fatty acid synthase (Fasn) markers were used to study the lipid accumulation, and Collagen V (ColV) was used to study cell elasticity to investigate the relationship of the effects of the administration of *Hypericum perforatum* L. with obesity.

Keywords *Hypericum perforatum* L. · Insulin resistance · Inflammation · Obesity · Medicinal plants

Abbreviations

BMI	Body mass index
Dgat1	Diacylglycerol-*O*-acyltransferase
ColV	Collagen V
H. perforatum	Hypericum perforatum
qPCR	Quantitative PCR
HDL	High-density lipoprotein
CPT 1	Carnitine palmitoyltransferase 1
IL-6	Interleukin-6
Lpl	Lipoprotein lipase
Fasn	Fatty acid synthase
VLDL	Very low-density lipoprotein
CRP	C-reactive protein
IBMX	Isobuthylmethilxsantin
FATP1	Fatty acid transport protein 1
TNF-α	Tumor necrosis factor-α
FABP	Fatty acid binding protein

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Introduction

Obesity is a well characterized mild chronic inflammatory disease, which plays an important role in the pathogenesis of several chronic diseases, such as Type II diabetes, hypertension, atherosclerosis, fatty liver, cancer, asthma and sleep apnea [1]. Obesity is positively correlated with an increase or expansion of adipocyte cells [2, 3]. In most living organisms, triglycerides are the major molecules, where the metabolic energy and fatty acids are deposited. However, the over-accumulation of triglycerides correlates with diseases such as obesity and diabetes mellitus. The enzymes that are mainly involved in the catalytic reactions related to the synthesis of the triglycerides within a cell are the diacylglycerol-*O*-acyltransferase 1 (Dgat1) enzymes.

Harris et al. [4] showed that the presence of Dgat1 contributes significantly to the formation of lipid droplets and triglycerides, and that the triglyceride formation can only occur in the activity of these two Dgat enzymes (Dgat1 and Dgat2) [4]. One of the main characteristics of obesity is the deposition of over-accumulated triglycerides in white adipose tissues and it is often associated with an abnormal storage of triglycerides in tissues such as skeletal muscle and

liver. Since the over-expression of Dgat1 will increase the accumulation of triglycerides, it is expected that the elevated levels of Dgat1 directly correlates with obesity [5]. The lipoprotein lipase (Lpl) has an important effect on adipocyte metabolism by hydrolyzing the circulating triglycerides to fatty acids. Thus, a decrease in Lpl activity contributes to the decrease in total body fat stores [6]. The Lpl levels in adipose tissue increases with the insulin release and after meals, but decreases in the fasting state. Studies have shown that the Lpl protein levels per adipocyte are elevated in obese humans and rodents. Intrestingly however, in obesity, the response of Lpl to insulin and nutrition is reduced [7–10]. The key lipogenic enzyme responsible from the biosynthesis of the long chain fatty acids from the acetyl-CoA precursors is known as the Fatty acid synthase (Fasn) [11].

It was observed that this enzyme increased in mice fed with a high fat diet. There is a positive correlation between the adipocyte differentiation and the elevated Fatty acid synthase expression in obesity [12]. It is known that the levels of Collagen V, an extracellular matrix component known to have an elasticity-reducing effect on adipose tissue, increase upon adipocyte differentiation [13]. The 3T3-L1 cell line is the in vitro model system of choice to understand the underlying mechanisms of diseases such as obesity, metabolic syndrome, and diabetes, due to its ability to differentiate into mature adipocytes.

Hypericum perforatum L. (*H. perforatum* L.), also known as St. John's wort, is used by the public for many purposes. The pharmacological studies on showed the anti-inflammatory, antidepressant, antimicrobial and antiviral activities of the *H. perforatum* L. extracts [14–18]. We, therefore, wanted to determine whether this plant can be used as a natural alternative for the treatment of obesity and insulin resistance related to obesity. *H. perforatum* contains a wide variety of secondary metabolites, including alkaloids, terpenes and phenolics [19]. Hernández-Saavedra et al. investigated the effects of these secondary metabolites on obesity. It was suggested that the hypolipidemic effect of this plant may be related to the presence of some of the secondary metabolites [20]. Accordingly, he epigallocatechine gallate may stimulate thermogenesis [21] and reduce fat accumulation, which may be associated with its ability to inhibit pancreatic lipase [22] function. In addition, the epigallocatechine gallate was shown to decrease the C-reactive protein (CRP) secretion by reducing the production of the reactive oxygen species from vascular smooth muscle cells [23]. The anti-obesity effect of rutin was associated with the ability to reduce the accumulation of triglycerides in the adipose tissue thereby improving the lipid profile [24]. In addition, hypericin, hyperforin and adperforin, which are considered to be the main bioactive compounds in *H. perforatum*, were identified [25]. Hyperforin, a major bioactive component of *H. perforatum*, was reported to protect against the cytokine-induced cell damage, thereby decreasing the loss of β-cell function observed in diabetes and increasing survival, which could potentially be valuable for preventing or limiting β-cell damage [26]. Husain et al. [27] observed that *H. perforatum* extracts with standardized amounts of hypericin and hyperforin showed promising healing effects with respect to obesity related complications in rats fed with a high-fat diet [27].

In our study, to investigate the putative curative role of *H. perforatum* on molecular level, we focused on the changes of some genetic markers that are regulated by obesity and other molecular events associated with it, such as alterations in the extracellular matrix composition or the lipid metabolism. Therefore, we analyzed the expression of the genes such as ColV, Fasn, Lpl and Dgat1 in 3T3-L1 cells treated without and with the plant for different time courses and doses by qPCR.

Materials and methods

Plant material

H. perforatum L. was collected in Muğla and all collected samples were taxonomically identified by Prof. Dr. Güven Görk and Dr. Olcay Ceylan. The plants were dried for two weeks in a cool and moisture-free environment. The above-ground parts of the dried plants were ground to powder. The dried whole plant of *H. perforatum* L. was suspended in absolute ethanol. After the extract was filtered, the solvent was removed by evaporation [28]. The stock solutions were dissolved in ethanol to a final concentration of 20 mg/mL and stored at − 20 °C until use.

Cell culture and differentiation

3T3-L1 cells (ATCC) were cultured in Dulbecco's Modified Eagles Medium (DMEM) (L0102-500) containing 10% Newborn Calf Serum (Capricorn, NCS-1B) and 1% Penicillin–Streptomycin (Multicell, 450-201-EL) in 5% CO_2 and 95% air at 37 °C. 10% Fetal Bovine Serum (Capricorn, FBS-12B) and 1% Penicillin–Streptomycin containing DMEM was used to differentiate cells to adipocytes. Predifferentiation medium containing 0.5 mM IBMX (Sigma, STBF6061V), 1 µM dexamethasone (Sigma, BCBV5460) and 1 µg/mL insulin (Sigma, SLBV1793) was applied for 48 h, followed by the application of the differentiation medium containing 1 µg/mL insulin to induce 3T3-L1 cells to adipocytes.

Evaluation of changes in morphology and viability of cells

The cells were placed onto petri dishes and incubated within a media containing 50 µg/mL, 100 µg/mL and 150 µg/mL *H. perforatum* extracts for 24-, 48- and 72 h

[29]. The adherence and the morphological characteristics of the cells on petri dishes were evaluated using the light microscopy.

Staining of 3T3-L1 cells with Oil Red O Dye

The differentiation of 3T3-L1 adipocytes was performed as described previously. Oil Red O staining was done according to the manufacturer's instructions, using the Biovision Oil Red O Staining kit (Biovision, K580-24).

Total RNA isolation and Real Time Reverse Transcription-Polymerase Chain Reaction (qPCR)

Total RNA from adipocyte cells was extracted using the RiboEx™ (Cat. No. 301-001) total RNA isolation solution from GeneAll (Cat. No. 301-902). Ultraviolet light spectrophotometry followed by formaldehyde- agarose gel electrophoresis was used to determine the quantity and the quality of the isolated RNA, respectively. 500 nanograms of total RNA was reverse transcribed using oligo- dT primers with EasyScript Plus cDNA synthesis kit from ABM Alfagen (Cat. No. G236). The amplification of the reverse-transcribed RNA was achived using Ampliqon RealQ Plus 2 × Master Mix Green in the presence of 0.3 mM gene-specific forward and reverse primers by a thermocycling on a Roche Light Cycler 96 Real Time PCR machine for 45 cycles. The temperatures and durations for denaturation, annealing and extension were 95 °C for 30 s; 55 °C to 58 °C for 30 s and 72 °C for 30 s, respectively. The normalization of the differences in individual samples was done by the use of the amplified 18S expression as a standard control. The list of the mouse Dgat1, Lpl, Fasn, Col V and 18S RNA primer sequences are given in Table 1. The information about the primers and their accession numbers were obtained from The National Center for Biotechnology Information https://www.ncbi.nlm.nih.gov/genbank/ [30].

Statistical analysis

The comparison of the groups with respect to the continious variables and the comparison of baseline and posttreatment measurements were done by using one-way ANOVA, followed by the Tukey's multiple comparison test. The significance level was set at $p < 0.05$.

Results

In this study, 3T3-L1 cells with fibroblastic morphology and preadipocyte nature were used. When 3T3-L1 cells are stimulated with chemicals such as insulin, dexametasone and IBMX, they become fully differentiated adipocytes. Oil Red O staining was used to evaluate lipid droplet formation at the end of a time course of two weeks Fig. 1. In our experiments with 3T3-L1 preadipocytes, the 24- and 48 h application of 50-, 100- and 150 µg/mL extracts of *H. perforatum* (HypE) dissolved in ethanol was determined to be suitable for the cell viability. Therefore, these doses were used for the experiments shown in Fig. 2.

RNA was extracted from the 3T3-L1 cells with RiboEx™, followed by cDNA synthesis. Electrophoretic and spectrophotometric analyses were performed to determine the quality and the quantity of extracted RNA samples. The mRNA expression experiments of Fasn, ColV, Dgat1 and Lpl molecular markers, which were designed on the Primer3, were performed by quantitative Real Time Reverse Transcription-Polymerase Chain Reaction (qPCR) using samples of 3T3-L1 preadipocytes treated with *H. perforatum* and nontreated control cells. In addition, the expression levels of ColV, Fasn, Lpl and Dgat1 in adipocytes were analyzed by qPCR. The administration of *H.perforatum* extracts to adipocytes led to the downregulation of the ColV, Fasn, Dgat1 and Lpl genes Figs. 3, 4. The time courses and doses of

Table 1 Sequences and NCBI accession number for primers designed for Real Time PCR reactions

GeneBank accesion number	Gene	Primer sequence
NM_010046.3	*Mus musculus* Dgat1; (Diacylglycerol-O-acyltransferase)	(F): 5′-CCTCAGCCTTCTTCCATGAG-3′ (R): 5′-ACTGGGGCATCGTAGTTGAG-3′
NM_008509.2	*Mus musculus* Lpl; (Lipoprotein lipase)	(F): 5′-ACTCGCTCTCAGATGCCCTA-3′ (R): 5′-TTGTGTTGCTTGCCATTCTC-3′
NM_007988.3	*Mus musculus* Fasn; (Fatty acid synthase)	(F): 5′-CTGAGATCCCAGCACTTCTTGA-3′ (R): 5′-GCCTCCGAAGCCAAATGAG-3′
NM_015734.2	*Mus musculus* ColV (Collagen V)	(F): 5′-CTCAGGGGTAACGAAAACCA-3′ (R): 5′-GGAGAAGTCCTCGGGAAAAC-3′
NR_003278.3	*Mus musculus* 18S ribosomal RNA (Rn18s)	(F): 5′-TTCGAACGTCTGCCCTATCAA-3′ (R): 5′-ATGGTAGGCACGGCGACTA-3′

H. perforatum extracts used in these studies were 24 h and 48 h application of 50 µg/mL, 100 µg/mL and 150 µg/mL respectively (Table 2).

Discussion

Obesity is the most common nutritional disease and a growing public health problem worldwide [31]. Obesity is a disease with increasing prevalence dramatically in adult and pediatric populations and that causes different complications [2, 3].

The prevalence of diseases, such as obesity and related insulin resistance, and diabetes is increasing all over the world. Treatment options such as the use of oral hypoglycemic agents and insulin are currently available for diabetes. Glycemic drugs, which are used to treat obesity have serious side effects [32]. The bariatric surgery can also be performed in obese individuals with severe comorbidity. Drugs are not tolerated well by every individual and the bariatric surgery may cause diarrhea, nausea and vomiting in patients [33]. The increasing prevalence of obesity even has adverse effects in countries, that have to deal with this health issue. Therefore, these lead researchers to seek for alternative treatment options. The anti-obesity and anti-diabetic effects of *H. perforatum* was demonstrated in vivo and in vitro [27, 34, 35]. In in vivo studies, the parameters of sugar and fat intake were investigated in rats, fed with high fat diet to reveal the effects of the plant on lipid metabolic pathways. It was observed that the application of this plant reduced the levels of elevated cholesterol, triglyceride and blood glucose [20, 27].

Arokiyaraj et al. showed the effect of *H. perforatum* on blood sugar and blood lipid parameters in diabetic rats induced with streptozotocin [36]. Streptozotocin causes pancreatic ß-cell damage, which results in insufficient insulin secretion and thus hyperglycemia. The excessive glucose production and decreased glucose utilization in tissues is known as hyperglycemia and is a hallmark of diabetes mellitus. In their study, the researchers made use of the diabetic and the non-diabetic Wistar rats. They administered diabetic rats *H. perforatum* in increasing doses and showed that the administration of *H. perforatum* extract reduced the blood glucose levels, which were elevated due to diabetes, back to an equal (or comparable) level with the healthy group. They reported that *H. perforatum* extract normalized this hyperglycemic condition. The elevated plasma insulin levels in streptozotocin-treated rats, compared to the normal rats, decreased by 2.5 fold upon the administration of *H. perforatum*. Moreover, the total cholesterol levels increased approximately fourfold in streptozotocin-treated diabetic rats, compared to normal control group. The HDL-cholesterol levels were threefold lower than normal in diabetic control, and the administration of 200 mg/kg *H. perforatum* was reported to normalize the HDL levels. It was shown that the triglyceride level increased fourfold in diabetic control and *H. perforatum* significantly decreased this level. These in vivo findings demonstrated the anti-obesity and the anti-diabetic properties of *H. perforatum,* and encouraged researchers to work at a molecular level to better understand the mechanism of action of this plant in treatment of obesity and obesity-related diseases. In our study, the genes that are associated with lipid accumulation and cell matrix components were used as markers in order to understand the relationship between *H. perforatum* and obesity. The elevated expression levels of ColV, Dgat1, Lpl and Fasn in adipocytes were downregulated upon the administration of the plant extract, which is in line with the work by Arokiyaraj et al. [36].

In the study conducted by Husain et al. [27], the hypolipidemic and the anti-obesity activity of *H. perforatum* was investigated. A high-fat and fructose diet was initially applied to rats followed by the administration of the increasing doses of *H. perforatum*. *H. perforatum* extract significantly reduced the daily food intake after 30 days. *H. perforatum* extract also inhibited the accumulation of adipose tissue [27]. This study demonstrating the anti-obesity effect of *H. perforatum* is consistent with our study, in which we

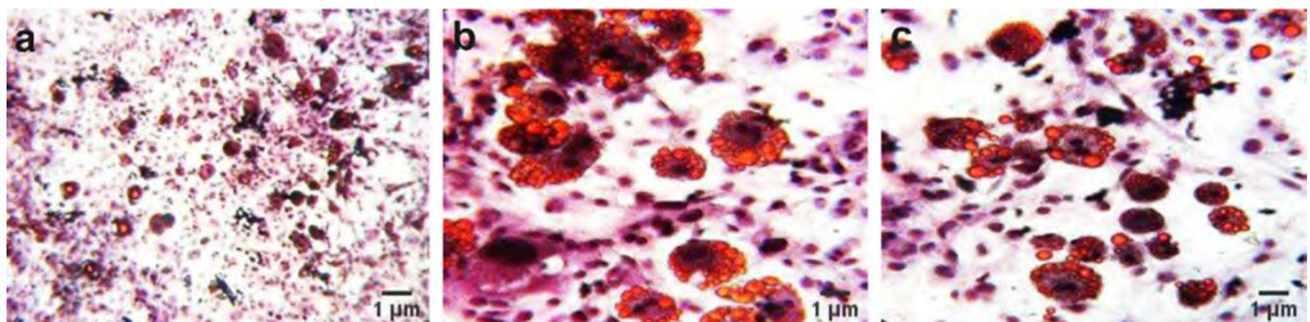

Fig. 1 Oil Red O Staining to determine the lipid accumulation in fully differentiated 3T3-L1 cells. Cells were visualized before and after staining (**a**, × 10), and only after staining (**b**, **c**, × 40), respectively. Scale bars in each micrograph represent 1 µm

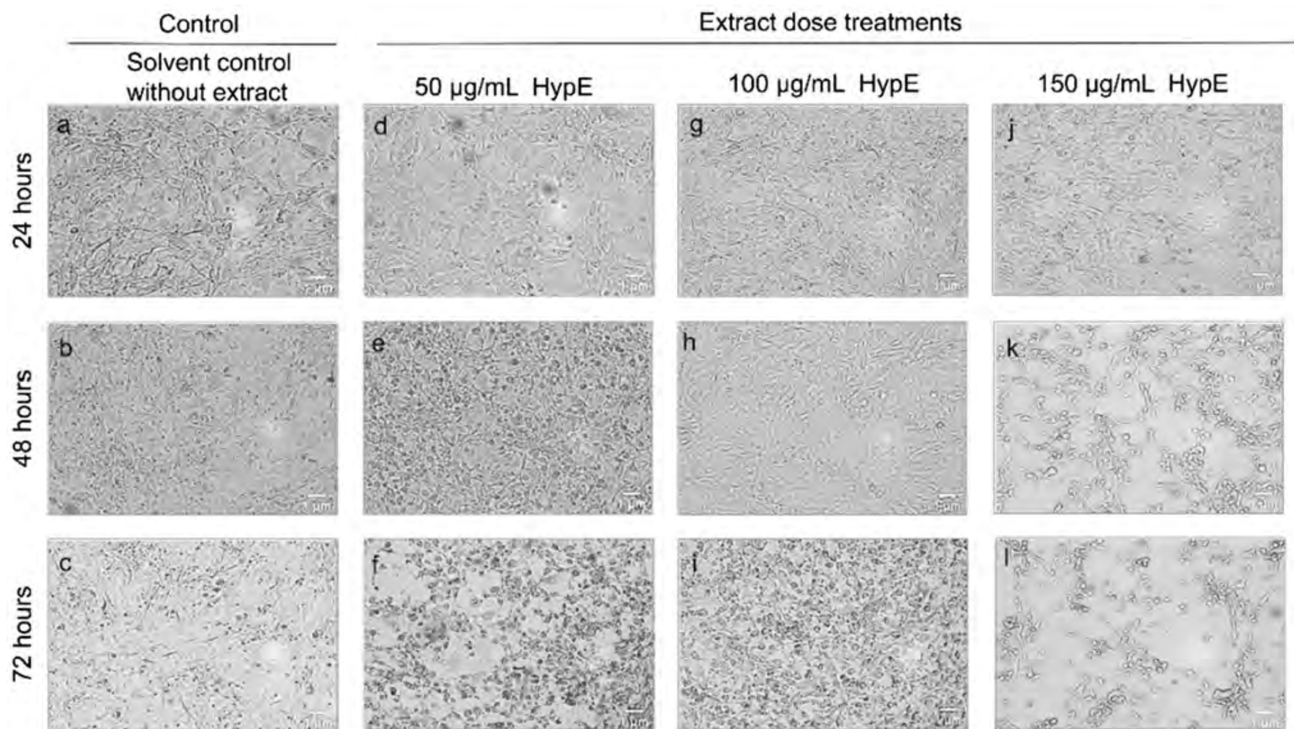

Fig. 2 Microscopic visualization and the evaluation of morphological changes and cell viability in 3T3-L1 cells, treated with plain solvent or *H.perforatum* extracts (HypE) with a dose of 50 μg/mL, 100 μg/mL and 150 μg/mL for 24 h (**a**, **d**, **g**, **j**), 48 h (**b**, **e**, **h**, **k**), and 72 h (**c**, **f**, **i**, **l**). Scale bars in each micrograph represent 1 μm

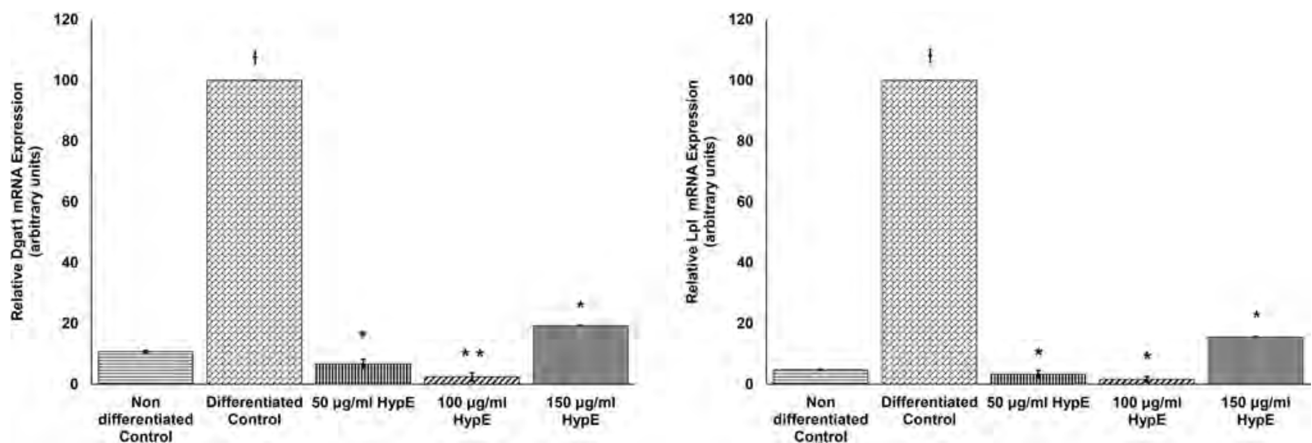

Fig. 3 Quantitative measurements of Dgat1 and Lpl mRNA in 3T3-L1 cells. qPCR analyses of Dgat1 (left) and Lpl (right) using 3T3-L1 adipocytes, differentiated with IBMX, Dexamethasone and insulin for 24 h. The results represent duplicate measurements in three separate experiments (*$p < 0.05$, comparison with differentiation control; **$p < 0.001$, comparison with differentiation control. $^{+}p < 0.05$, comparison with nondifferentiation control)

mimiced obesity in vitro and investigated the effects of *H. perforatum*. We observed that the expressions of Dgat1, Lpl and Fasn, which are the markers related to adipocyte differentiation, were downregulated as a result of the administration of *H. perforatum.*

Tian et al. [35] investigated the effect of *H. perforatum* on lipid metabolic pathways [35]. In their study, the mice were fed on a high-fat diet for 14 days, after which high dose and low dose of *H. perforatum* were administered to these mice. The development of hypercholesterolaemia was observed in mice receiving the high-fat diet. When the levels

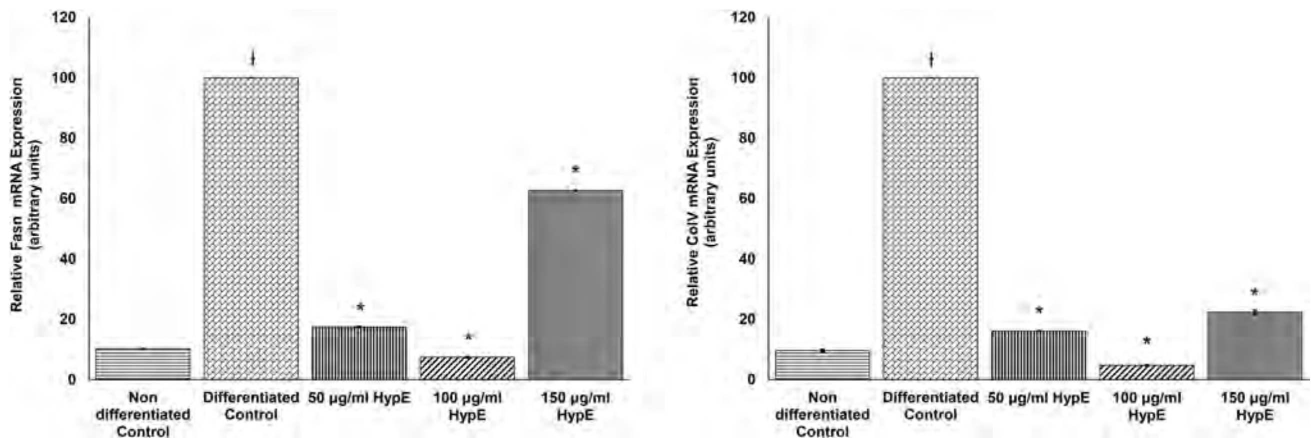

Fig. 4 Quantitative measurements of Fasn and ColV mRNA in 3T3-L1 cells. qPCR analyses of Fasn (left) and ColV (right) using 3T3-L1 adipocytes, differentiated with IBMX, Dexamethasone and insulin for 48 h.The results represent duplicate measurements in three separate experiments (*$p < 0.05$, comparison with differentiation control. ⁺$p < 0.05$, comparison with nondifferentiation control)

Table 2 The change in the expression levels of Dgat1, Lpl, Fasn, ColV in 3T3-L1 preadipocytes (nondifferentiated control), adipocytes (differentiated control) and *Hypericum perforatum* extract (HypE) treatment to 3T3-L1 adipocyte groups (50-, 100-, 150 µg/mL HypE treatment to 3T3-L1 adipocytes; *$p < 0.05$, comparison with differentiation control; **$p < 0.001$, comparison with differentiation control; ⁺$p < 0.05$, comparison with nondifferentiation control)

Gene of interest	Relative mRNA expression (arbitrary units)				
	Samples				
	Non-differentiation control	Differentiation control	50 µg/mL HypE treatment to 3T3-L1 adipocytes	100 µg/mL HypE treatment to 3T3-L1 adipocytes	150 µg/mL HypE treatment to 3T3-L1 adipocytes
Dgat1	10.71 ± 0.35	100⁺	6.70* ± 1.48	2.44** ± 1.35	19.24* ± 0.18
Lpl	4.73 + 0.21	100⁺	3.31* ± 1.20	1.64* ± 0.87	15.50* ± 0.31
Fasn	10.21 ± 0.12	100⁺	17.46* ± 0.20	7.43* ± 0.24	62.63* + 0.24
ColV	9.64 ± 0.40	100⁺	16.09* ± 0.14	4.73* ± 0.25	22.34* ± 0.68

of chosen metabolic parameters in mice were compared, it was revealed that in the groups, treated with low and high doses of *H. perforatum*, the total cholesterol levels decreased significantly by 16.2% and 22.2%, and the serum triglyceride levels decreased by 13.1% and 22.6% in a dose-dependent manner, respectively. The intramyocellular lipid accumulation in skeletal muscle was associated with insulin resistance and dyslipidemia. They reported that the triglyceride content in skeletal muscles increased significantly in mice fed with a high-fat diet and this increase was reversed with low and high doses of *H. perforatum* extracts (100 mg/kg, 200 mg/kg). Gene expression studies on lipid metabolism were performed to understand the mechanisms of *H. perforatum* on dyslipidemia. Fatty acid transport protein 1 (FATP1), the main carrier of fatty acid, was reported to increase significantly in skeletal muscle in obese mice fed with a high-fat diet, and the administration of *H. perforatum* reduced the gene expression of FATP1. Carnitine palmitoyltransferase 1 (CPT1), an important regulator of fatty acid oxidation, was reported to increase slightly in the skeletal muscle of obese mice fed with a high-fat diet, while *H. perforatum* elevated the expression of CPT1 dramatically. Lpl is an enzyme that plays a role in the conversion of triglycerides to fatty acid and glycerol, and in our study, *H. perforatum* inhibited the elevation of adipocyte Lpl, associated with fully differentiated adipocytes, to the level of Lpl, characteristic for preadipocyte cells. Our results on adipocyte associated gene expressions are consistent with the study by Tian et al. [35].

Perez-Ramirez et al. [37] used healthy and obese mice in order to observe the hepatoprotective feature of *H. perforatum*. Mice in the obese group were given *H. perforatum* extract prior to the administration of *H. perforatum*, in the obese group compared with the healthy group, an increase in body weight, lipid accumulation in the liver, increase in serum triglyceride levels and serum fatty acid levels were observed. *H. perforatum* extract was observed to cause a

significant decrease in the body weight of the mice in the obese group. It was reported that the application of *H. perforatum* extract decreased the serum fatty acid and serum triglyceride levels. In order to observe the effect of obesity on inflammation, the expression of inflammatory agents, such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) genes were investigated. It was observed that the elevated TNF-α and IL-6 expressions as a consequence of obesity were decreased with the administration of *H. perforatum*. In addition, researchers examined the level of expression of genes associated with the lipid metabolism, such as Fatty acid synthase, Fatty acid binding protein, Acetyl CoA carboxylase and Acetyl CoA dehydrogenase. The study reported that *H. perforatum* improved the effects in gene expression on the lipid metabolism [37]. In our study, we showed that the elevated Fatty acid synthase mRNA expression in adipocytes were decreased upon *H. perforatum* extract treatment.

Our results showed that the Dgat1 gene, which plays a role in the biosynthesis of triglycerides, increased with adipocyte differentiation and that the extracts of *H. perforatum* at different doses significantly reduced Dgat1 expression, in a statistically significant manner. We observed that Lpl, which is known as an enzyme converting triglycerides to fatty acids and glycerol, increased with adipocyte differentiation and this elevation was reduced back to the preadipocyte control level with *H. perforatum* extract application. *H. perforatum* extracts downregulated the expression of Fasn, which is correlated with fully differentiated adipocytes. In addition, ColV, which exhibits size reducing effect on fat tissue, increases with adipocyte differentiation and this increase is inhibited by the administration of the plant.

The data obtained support previous studies with *H. perforatum* and provide guidance to understand the molecular mechanisms of the effect of *H. perforatum* on obesity. In the present study, 3T3-L1 preadipocytes were transformed into fully differentiated adipocytes, followed by 50 µg/mL, 100 µg/mL, 150 µg/mL *H. perforatum* extract treatments for 24- and 48 h. We observed that the administration of *H. perforatum* extract reduced the expression of Dgat1, Lpl, Fasn and ColV on transcriptional level. Dgat1, Fasn, ColV and Lpl both were downregulated and downregulation of these markers was statistically significant. The fact that these markers show a significant decrease with the application of the plant extract is in accordance with the previous obesity related in vivo studies of the *H. perforatum*.

Conclusions

The application of *H. perforatum* L. at different doses downregulated the expression of Dgat1, Fasn, ColV and Lpl in fully differentiated 3T3-L1 cells. Our results underline the importance of the transcriptional regulation of *H. perforatum* L. to understand its effects on obesity.

Acknowledgements This work was supported by the Scientific Resarch Project (BAP) Grant (AR-GE 17/059) from the Muğla Sıtkı Koçman University granted to Dr. Filiz ALTAN. Authors would like to thank to Dr. Reşat ÜNAL for valuable scientific support and critical reading; to Dr. Zekiye Buket YILMAZ for critical reading and language editing; to Dr. Güven GÖRK and Dr. Olcay CEYLAN for providing the plant material and to Dr. Mehmet VAROL for his expertise and assistance in technical editing.

Compliance with ethical standards

Competing interests The authors declare that they have no competing interest.

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