Life Sciences 218 (2019) 292–299

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Sclareol-loaded lipid nanoparticles improved metabolic profile in obese mice

Gabriela Cavazza Cerri[a], Leandro Ceotto Freitas Lima[a], Deborah de Farias Lelis[c], Lucíola da Silva Barcelos[a], John David Feltenberger[d], Samuel Vidal Mussi[b], Renato Sobral Monteiro-Junior[c], Robson Augusto Souza dos Santos[a], Lucas Antônio Miranda Ferreira[b], Sérgio Henrique Sousa Santos[c,e,*]

[a] Department of Physiology and Pharmacology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[b] Department of Pharmaceutics, Faculty of Pharmacy, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[c] Laboratory of Health Science, Postgraduate Program in Health Sciences, Universidade Estadual de Montes Claros, Montes Claros, Minas Gerais, Brazil
[d] Health Sciences Center, School of Medicine, Texas Tech University, Lubbock, TX, United States
[e] Institute of Agricultural Sciences, Food Engineering College, Universidade Federal de Minas Gerais, Montes Claros, Minas Gerais, Brazil

ARTICLE INFO

Keywords:
High-fat diet
Metabolism
Obesity
Sclareol and solid lipid nanoparticles

ABSTRACT

Sclareol is a bioactive hydrophobic diterpene in the essential oil isolated from *Salvia sclarea* (Fam. Lamiaceae). Sclareol has been widely studied due to its anti-inflammatory and antioxidant effects.
Aims: The present study aimed to evaluate the effects of Sclareol in different formulations (solid lipid nanoparticle and free) on the metabolic profile of obese mice.
Main methods: Swiss male mice were randomly divided into two groups: standard diet (STD) and high-fat diet (HFD). After obesity induction, each group was divided into three treatment groups: free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). Treatments were performed every day during 30 days.
Key findings: L-Sc improves obese mice metabolic profile by decreasing adiposity, ameliorating insulin sensitivity, glucose tolerance and increasing the HDL plasma levels. In addition, L-Sc decreased the expression of NF-KB, MCP-1 and SERBP-1.
Significance: The use of sclareol together with lipid nanocarriers may be promising for the treatment of metabolic disorders by reducing adipose tissue.

1. Introduction

Obesity is a public health problem that affects many countries. Worldwide the prevalence of obesity has increased over the years, since 1975, obesity has almost tripled [1,2]. If the same pattern remains in 2030, 58% of the adult population worldwide will be overweight or obese [3].

White adipose tissue is responsible for secretion of essentials factors that regulate inflammation, food intake and energy expenditure [4,5]. Obesity generates several metabolic disturbances that cause adipokines altered secretion [6–8]. Therefore, drugs that aim to reduce excess adipose tissue may be effective in the treatment of obesity and metabolic disorders [9].

Several studies show the effects of phytochemicals in metabolism improvement [10–14]. Popularly known the *salvia* teas are usually used as homemade recipes for the renowned effects: anti-inflammatory and antioxidants properties and promote a waist circumference decrease. Interestingly, some studies have shown the scientific significance of the beneficial metabolic effects of some species of *Salvia*. *Salvia plebeia*, for example, decreases the expression of genes related to adipogenesis and lipogenesis in epididymal white adipose tissue [15]. *Salvia hispanica* seeds were effective in promoting weight loss and improving glycemic profile in obese adult patients [16].

Sclareol is a bioactive diterpene in the essential oil isolated from *Salvia sclarea* (Fam. Lamiaceae). This diterpene, has low solubility in water and has a tendency to accumulate in adipose tissue [17]. Furthermore, it has been widely studied due to its antioxidant and anti-inflammatory effects. Sclareol attenuates LPS-induced pulmonary inflammation interfering in different pathways including NF-κB and MAPKs [18]. Besides that, exhibits chondro protective effect by

* Corresponding author at: Laboratory of Health Science, Universidade Estadual de Montes Claros, Av. Cula Mangabeira 562, 31401-001 Montes Claros, MG, Brazil.
E-mail address: sergiosousas@ufmg.bra (S.H.S. Santos).

https://doi.org/10.1016/j.lfs.2018.12.063
Received 15 October 2018; Received in revised form 28 December 2018; Accepted 31 December 2018
Available online 02 January 2019
0024-3205/

inhibiting the expression of iNOS and COX-2 *in vitro* and *in vivo* [19]. Sclareol also reduces the expression of oncogenes, modulates the immune response by affecting cytokine patterns and reduces the growth of certain cells [20–22]. In addition, in view of the metabolic benefits of various species of *Salvia*, it is extremely important to investigate the effects of its components, such as Sclareol.

Despite the beneficial effects, Sclareol has high lipophilicity; therefore, the use of Sclareol *in vivo* is hampered [20,23,24]. The lipid nanocarriers such as liposomes, nanoemulsions and solid lipid nanoparticles (SLN) appear as alternatives to overcome this drawback, increasing the bioavailability of lipophilic substances [25]. However, there is a deficiency in studies regarding the form of performance of the diterpene sclareol, as well as on its possible use for treatment of metabolic syndrome.

Considering all of the above information, this study's goal was to evaluate the effect of Sclareol in different formulations (lipid nanoparticle and free) on the metabolic profile of mice with HFD induced obesity.

2. Materials and methods

2.1. Animals and in vivo experiments

All animal experiments were performed according to procedures approved by the Ethics Committee on Animal Experimentation of Universidade Estadual de Montes Claros, Minas Gerais, Brazil. The experiment was conducted with 48 male *swiss* mice (4 weeks old) obtained from the animal care center at the Universidade Federal de Minas Gerais. In the first month, the animals were randomly divided into two groups (n = 24) according to their experimental diets: standard mice (ST) were fed a normal diet and induced-obese mice (HF) were fed a high-fat diet (HFD). Mice were kept in an environmentally controlled room with free access to tap water and food, according to the experimental diets, throughout the study [29,30].

2.2. Diets

HFD was prepared according to the protocols described previously [31], composed by 24.55% carbohydrate, 14.47% protein and 60.98% fat, presenting a total of 5.28 kcal per 1 g of diet. The HFD induces obesity (60%) and is consisted by: BHT (0.014 g), Bitartaro choline (2.50 g), methionine (3 g), vitamins AIN93M (10 g), minerals (35 g), cellulose (50 g), maize starch (62 g), casein (200 g), soybean oil (20 g), gooseberry syrup (310 g) and fat (355 g) [29,30]. Standard diet (STD) (Purina - Labina®), which was used for the regular maintenance of our mice, is composed of 50.30% carbohydrate, 31.90% protein and 17.80% fat, presenting a total of 2.18 kcal per 1 g of diet [30]. All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, Brazil).

2.3. Measurements of body composition and food intake

The animals were weighed on the first experimental day and twice a week during the whole experimental period to record their body mass gain. Food intake was measured twice a week during the study. After euthanasia, the interscapular brown adipose tissue, epididymal, retroperitoneal and mesenteric white adipose tissues were collected and weighed [32].

2.4. Sclareol

Sclareol is a natural component in the essential oil isolated from *Salvia sclarea* (Fam. Lamiaceae). In this work (for free formulation and for the nanoparticle preparation), the purified sclareol was purchased from Sigma company - ID: 49944 Sigma-Aldrich; PubChem Substance ID:329757414; Beilstein Registry Number: 2054148; Empirical Formula (Hill Notation): $C_{20}H_{36}O_2$ and Molecular Weight: 308.50.

2.5. Solid lipid nanoparticles

The Solid Lipid Nanoparticles (SLN) were prepared by the hot melting homogenization method. The oil phase (OP) was composed of 150 mg of compritol, 100 mg of tween 80 and 0.2% w/v of sclareol. For blank SLN the OP was composed of 150 mg of compritol and 100 mg of tween 80. The aqueous phase (AP) was composed of purified water. The SLN were prepared using the mixer Ultra Turrax T-25 (IkaLabortechnik, Germany), an ultrasound and a homogenization device with high power probe (Ultra-cell 750 W; Sonics Materials Inc., EUA). The OP was heated to 80 °C. Simultaneously, the AP was heated to the same temperature. The AP was gently dropped onto the OP under constant agitation (7656 G) with an Ultra Turrax T-25 mixer. The formed emulsion was homogenized for 10 min on ultrasound with high power probe (21% amplitude). The pH was adjusted to 7.0 to 7.4 with HCl or 1 M NaOH. Then, the suspension of the nanoparticles was stored in penicillin bottles, protected from light and kept cool at 4 °C [33,34].

2.6. Particle size analysis and zeta potential

The average diameter of the SLN was determined by photon correlation spectroscopy using a Zetasizer 3000HSA (Malvern Instruments, England) at a fixed angle of 90° and temperature 25 °C. The SLN dispersions were diluted with distilled water previously filtered through a membrane cellulose ester with a 0.45-μM pore size (HAWPO4700, Millipore, USA). All measurements were performed in triplicate, each triplicate corresponding to the mean of ten measurements.

The zeta potential was determined by dynamic light scattering technique and analysis of the electrophoretic mobility of the nanoparticles. The zeta potential measurements were performed using a Zetasizer 3000HSA (Malvern Instruments, England) at a temperature of 25 °C. The dispersions of SLN were diluted in sodium chloride 1 mM solution previously filtered through a cellulose ester membrane with a 0.45 μM pore diameter (HAWPO4700, Millipore, USA), to a count of 100 to 1000 Kcps. All measurements were performed in triplicate [34,35].

2.7. Treatment

After a month of obesity induction with the high-fat diet, both groups (ST and HF) were randomly distributed into three subgroups as follows: free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). The treatment was performed every day during one month. The dose of Sc, Sc-L and L was 1 mg·kg $^{-1}$. All administrations were intraperitoneal with a volume of 300 μL and dilutied with saline.

2.8. Glucose tolerance and insulin sensitivity tests

A glucose tolerance test was performed on overnight fasted mice after intraperitoneal injection of glucose (2 g·kg $^{-1}$ body weight; Sigma–Aldrich®, St. Louis, USA). Tail blood samples were taken at 0, 15, 30, 60 and 90 min after the injection for measurement of blood glucose levels using an Accu-Check glucometer (Roche Diagnostics®, Indianapolis, USA).

An insulin sensitivity test was performed on overnight-fed mice, after an intraperitoneal injection of insulin (0.75 units·kg^{-1} body weight; Sigma–Aldrich®, St. Louis, USA). Tail blood samples were taken at 0, 30, 60, 90 and 120 min after the injection for measurement of blood glucose levels using an Accu-Check glucometer (Roche Diagnostics®, Indianapolis, USA) [29].

2.9. Euthanasia and tissue collection

Mice were euthanized by guillotine without anesthesia. Samples of blood were immediately collected for serum analysis. Interscapular brown adipose tissue, epididymal, retroperitoneal and mesenteric white adipose tissues were collected, weighed and immediately frozen in liquid nitrogen and stored at −80 °C for subsequent analysis [32].

2.10. Plasma measurements

Dosages of serum were obtained after centrifugation (3200 rpm for 10 min at 4 °C). Total cholesterol, triglycerides, and HDL were assayed using the enzymatic method through a commercial kit from the company Doles, Brazil [32].

2.11. Histology

Epididymal adipose tissue were stored at −80 °C, rehydrated with serial washes of ethanol (70%, 50%, 30% in PBS) for 1 h each time to avoid breaking the adipocytes. The samples were stored in 10% formaldehyde solution for 24 h. Soon after, the samples were processed and embedded in paraffin. Transverse sections of 8 μm were made and mounted on slides. Tissues were stained with hematoxylin and eosin (Sigma–Aldrich®, St. Louis, USA). The samples were analyzed by microscopy, adipocyte numbers and diameters were measured from each animal using ImageJ software and a 20 objective lens [36].

2.12. Reverse transcription and qRT-PCR

Total RNA from epididymal adipose tissue was prepared using TRIzol reagent (Invitrogen Corp.®, San Diego, CA, USA), treated with DNAse and reverse transcribed with M-MLV (Invitrogen Corp.®) using random hexamer primers. The NF-kB, MCP-1 and SREBP-1 cDNA were amplified using specific primers and SYBR green reagent (Appllied Biosystems®, USA) in a PlusOne platform (Applied Biosystems®) [29].

2.13. Statistical analysis

All data were transferred to GraphPad Prism software (Version 5.0, GraphPad Software Inc., San Diego, CA, USA) and analyzed with a confidence of 95% ($P < 0.05$). Data are expressed as mean ± SEM. The statistical significance of differences in mean values among mice groups was assessed by two-way ANOVA and one-way ANOVA followed by Bonferroni post-test [29].

3. Results

3.1. Solid-lipid nanoparticles

The final SLN components (% mass/volume percentage) were: Compritol (1.50%), sclareol (0.20%) and tween 80 (1.00%). Further, the blank SLN was composed of compritol and tween 80. Table 1 presents the evaluations of size, zeta potential and the polydispersity index (PI) of SLN.

Table 1
Particle size analysis, zeta potential and polydispersity index (PI) of solid lipid nanoparticle (SLN).

Parameters			
Formulation	Diameter (nm)	Zeta potential (mV)	PI
SLN blank	120 ± 4	−31 ± 2	0.23 ± 0.08
SLN-sclareol	128 ± 10	−29.3 ± 0.9	0.21 ± 0.05

3.2. Body composition and food intake

The first month of the experiment, body weight gain was greater in mice fed with HFD as compared to the ST group (Fig. 1A). The HFD was effective inducing obesity. Food intake and energy intake were calculated for each group and no significant differences were found in ST or HF controls groups (Fig. 1D and E). The body weight of HF L-Sc mice decreased significantly compared with HF L mice during the treatment period (Fig. 1B and C).

In order to verify if the weight reduction in the HF L-Sc group is connected to white adipose tissue loss, adiposity was measured for each animal after the euthanasia. These results were used to form the adiposity graph per group (Fig. 1F). HF L-Sc animals showed a significant decrease in adiposity compared with HF L mice. Adiposity was measured by adding the epididymal, retroperitoneal and mesenteric white adipose tissues and by correction with the whole body weight (Fig. 2). In addition, HF L-Sc animals had an increase in interscapular brown adipose tissue weight compared to the other HF groups (Fig. 2).

3.3. Glucose tolerance and insulin sensitivity tests

A day before euthanasia, the glucose tolerance and the insulin sensitivity tests were performed (Fig. 3). A significant improvement of glycemic profile was observed in HF L-Sc mice compared with the HF L group (Fig. 3). Basal glycemia of the HF L-Sc animals was similar than that of the ST L group. In addition, the return time of glycemia to near-basal values after insulin or glucose injection, was lower in the HF L-Sc group than in the HF L group.

3.4. Serum measurements

The peripheral blood was collected at the time of euthanasia for serum measurements. Circulating levels of triglycerides were increased in ST group (Fig. 4B and C). Serum levels of HDL increased expressively in the HF L-Sc group as compared to HF L group (Fig. 4A).

3.5. Histological analysis

According to the histological analysis, HF L-Sc mice decreased their adipocyte area as compared to HF L, resembling the ST mice (Fig. 5). These results demonstrate the possibility that the significant weight loss of the HF L-Sc mice could come from decreased fat accumulation in the adipose tissue.

3.6. Reverse transcription and qRT-PCR

The mRNA expression of pro-inflammatory cytokines and adipogenesis-related markers, shown by qRT PCR in the EAT, indicated a significant decrease in NF-kB, MCP-1, and SREBP-1 in the HF L-Sc group (Fig. 6). L-Sc treatment was effective in decreasing the expression of inflammatory and lipogenic factors in the white adipose tissue of obese mice.

4. Discussion

We evaluated the intraperitoneal administration of L-Sc and Sc in obesity-induced mice. The main findings were the following metabolic alterations in HF L-Sc mice: (i) body weight reduction (ii) significant body adiposity reduction, (iii) improved glycemic profile, (iv) increased HDL plasma levels and (v) decreased expression of pro-inflammatory cytokines and adipogenesis-related markers. The administration of L-Sc was effective at improving the metabolism of obese mice.

The efficacy of some natural products in obesity improvement are evidenced in several studies [10,37–39]. Despite the beneficial effects, most studies with natural products have limitations when tested *in vivo*, this is because several phytochemicals have low bioavailability,

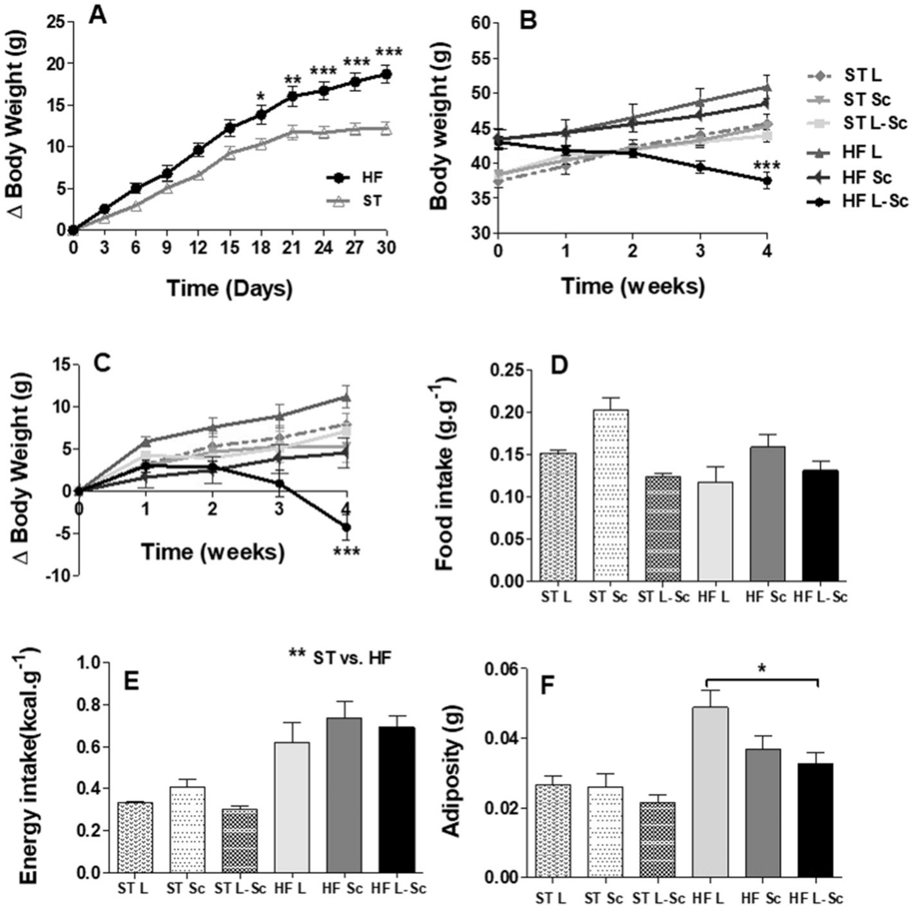

Fig. 1. Body composition and food intake. (A) Relative body weight before the treatment. (B) Absolute body weight during the sclareol treatment. (C) Relative body weight during the sclareol treatment. (D) Food intake (E) Energy intake ** represents the difference between HF groups and the respective ST groups. (F) Adiposity. Differences between groups were analyzed by Two-way ANOVA (A, B and C) and One-way ANOVA (D, E and F) followed by Bonferroni's post test. The data represent the mean ± SEM, *P < 0.05; **P < 0.01; ***P < 0.001. Free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). High-fat diet (HF) or standard diet (ST).

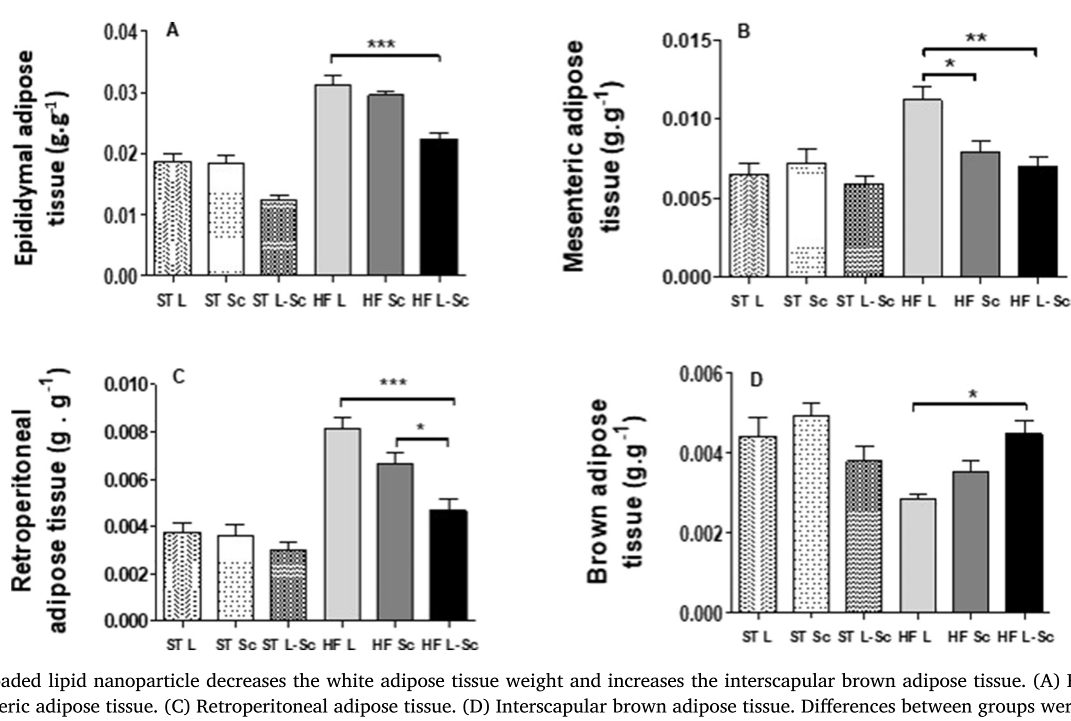

Fig. 2. Sclareol-loaded lipid nanoparticle decreases the white adipose tissue weight and increases the interscapular brown adipose tissue. (A) Epididymal adipose tissue. (B) Mesenteric adipose tissue. (C) Retroperitoneal adipose tissue. (D) Interscapular brown adipose tissue. Differences between groups were analyzed by One-way ANOVA followed by Bonferroni's post test. The data represent the mean ± SEM, *P < 0.05; **P < 0.01; ***P < 0.001. Free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). High-fat diet (HF) or standard diet (ST).

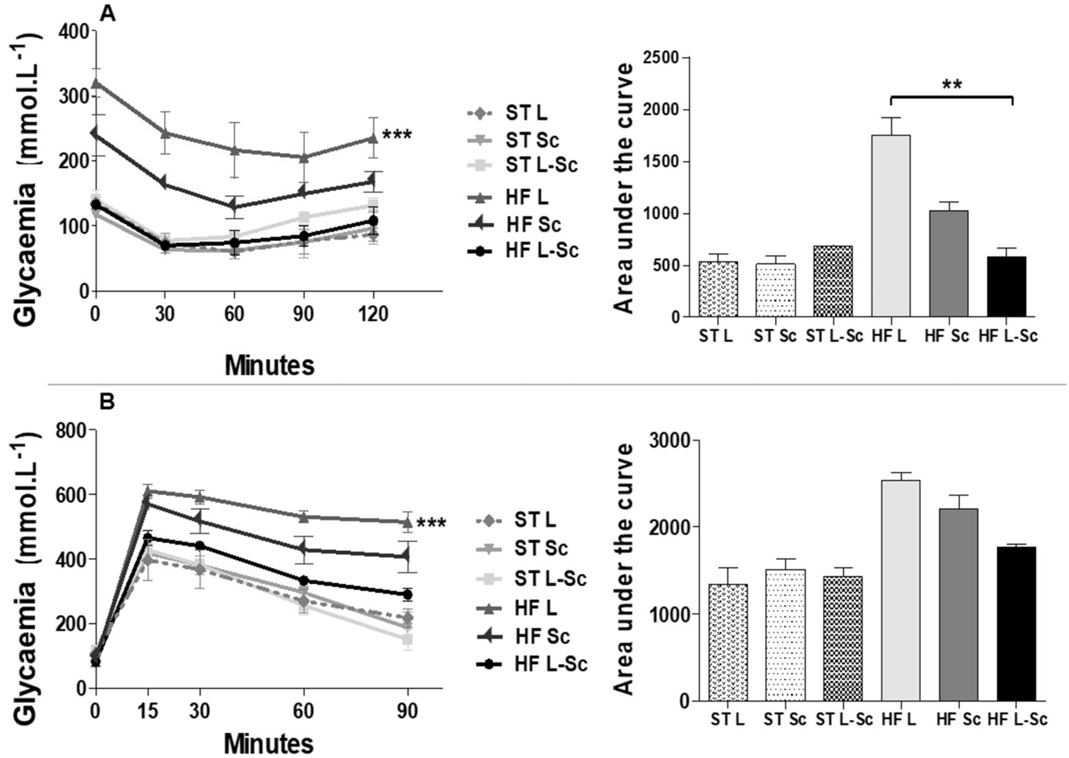

Fig. 3. The use of Sclareol-loaded lipid nanoparticle improves glycemic profile of obese mice. (A) Insulin sensitivity test (IST) and Area under curve. (B) Glucose tolerance test (GTT) and Area under curve. Differences between groups were analyzed by Two-way and One-way ANOVA followed by Bonferroni's post test. The data represent the mean ± SEM, *P < 0.05; **P < 0.01; ***P < 0.001. Free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). High-fat diet (HF) or standard diet (ST).

difficult distribution and absorption. Also because of that, high local concentrations of the drug may be necessary for the demonstrated effect. These characteristics hinder the *in vivo* study of various natural compounds [27]. Various efforts in natural compounds delivery have been made to prolong the context of sensitive half-lives of drugs and improve overall pharmacokinetics. Several studies have shown the improved efficiency of drugs associated with lipid carriers [10,26,27,33,40]. Doxorubicin and docosahexaenoic acid associated with SLN led to improvement of *in vitro* antitumor activity [33]. Resveratrol has beneficial metabolic effects and despite its low bioavailability, association of resveratrol with a lipid nanoparticle has increased its therapeutic efficacy [10,28].

Sclareol is highly lipophilic, which led to difficult transport in plasma and administration for *in vivo* study [24]. Sclareol in mitochondria targeted liposomes improving apoptotic and cytotoxic action over non-targeted liposomes [41]. Interestingly, the SLN with Doxorubicin and sclareol is more effective against 4T1 cells and show potential as an alternative for cancer treatment [34]. Bearing in mind that the treatment with sclareol in free formulation did not demonstrate the same beneficial effects as sclareol in nanoformulation, we readily conclude that in our work the SLN positively modulated Sclareol. Since the mice treated with sclareol in nano formulation showed perceptible effects compared to the mice treated with free Sclareol.

The adipose tissue acts as an important endocrine organ, secreting factors that regulate the metabolism of lipids and glucose in the whole body [4,5,42]. Obese mice treated with L-Sc decreased adiposity significantly compared to obese mice treated with L alone (Fig. 1F). This finding may indicate that the nanoformulation does not augment the Sclareol effect on adiposity, thus evidencing this compound independent effect on this parameter. Georgieva, 1989 showed that the Sclareol glycol has inhibitory effects on mice aggressive behavior, through activation of the catalytic subunit of adenylate cyclase, increasing the level of cAMP in brain cells, and decreasing the activity of alpha1 adrenergic receptors [44]. There are several metabolic processes linked to cAMP, such as lipolysis. The increased intracellular level of cAMP activates hormone sensitive lipase in adipocytes and, therefore, increases lipolysis [45]. HF L-Sc mice significantly decrease adiposity. Further investigation of the possible metabolic pathways involved is

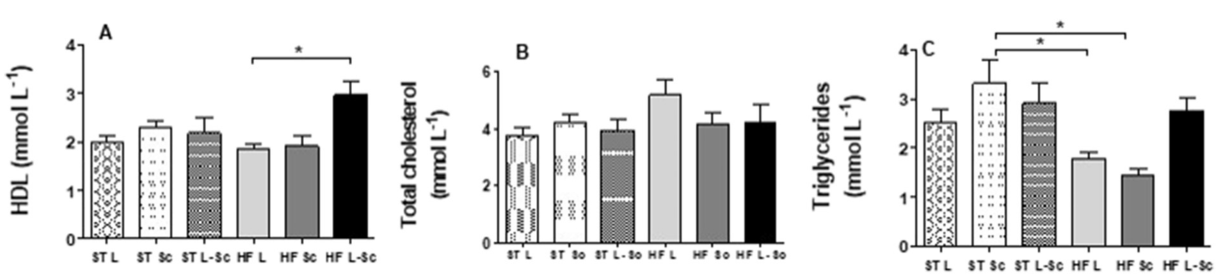

Fig. 4. Sclareol-loaded lipid nanoparticle increases HDL levels. (A) HDL. (B) Total cholesterol. (C) Triglycerides. Differences between groups were analyzed by One-way ANOVA followed by Bonferroni's post test. The data represent the mean ± SEM. *P < 0.05; **P < 0.01; ***P < 0.001. Free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). High-fat diet (HF) or standard diet (ST).

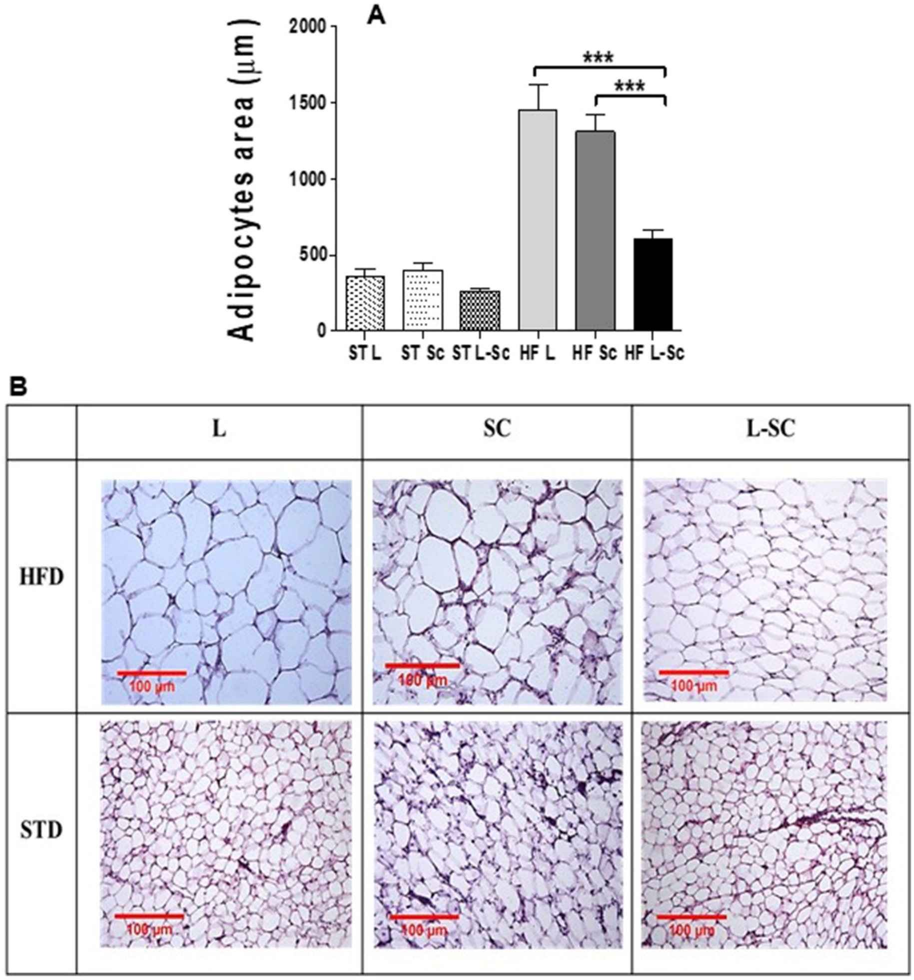

Fig. 5. Histological analysis of the epididymal adipose tissue. (A) Adipocytes area. (B) EAT histology. Differences between groups were analyzed by One-way ANOVA followed by Bonferroni's post test. The data represent the mean ± SEM, *P < 0.05; **P < 0.01; ***P < 0.001. Free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). High-fat diet (HF) or standard diet (ST).

necessary to assert a hypothesis. However, this result is very important, since the decrease in body fat can restore metabolic homeostasis through the normalization of important adipokines secretion. It is important to mention that other parameters may be contributing to the Sclareol body weight-lowering effects, such as by the modulation of muscle mass, which should be investigated in future studies.

Besides that, the treatment with L-Sc in obese mice increased the brown adipose tissue weight, had no anorectic effect (confirmed by the food intake assessment) and decreased adiposity (Figs. 1 and 2D). It was noticed a slight increase, although not significant, in the ST-free sclareol group food intake, however the energy intake remained similar among the control groups, thus justifying the absence of statistical differences. These results are very interesting, considering that the brown adipose tissue has a great participation for the increase of thermogenesis and consequently for the decrease of adiposity [43]. However, it is necessary to evaluate markers related to thermogenesis to conclude if weight loss was related to increased energy expenditure.

The intensity of lipogenesis depends of circulating glucose and insulin levels. This process occurs through the activity of specific transcription factors. One example is the sterol regulatory element binding protein 1c (SREBP-1c), activated by insulin. SREBP-1 activates glycolytic gene expression and increases glucose metabolism and lipogenic genes [46]. Several studies show increased SREBP-1 expression in obese mice [10,31]. The effects of SREBP-1 contribute to the understanding of how L-Sc decreases the adiposity of obese mice. With this information, we conclude that L-Sc decreases the expression of SREBP-1 (Fig. 6D) and consequently contribute by inhibiting lipogenesis (evidenced by decrease in white adipose tissue weight).

One of the well-known consequences of obesity and increased adiposity is insulin resistance [47]. Intraperitoneal administration of L-Sc was effective improving glycemic profile of obese mice with insulin resistance (Fig. 3). Interventions that decrease abdominal obesity and insulin resistance have important implications for type 2 diabetes prevention. Histological analysis of the epididymal adipose tissue indicated that HF L-Sc mice decreased adipocyte area as compared to HFL (Fig. 5). Thus, lipid nanoparticles of Sclareol improved glycemic profiles of the HF mice, perhaps by decreasing the white adipose tissue weight. This study suggests that the use of L-Sc can prevent occurrence of diabetes type 2.

Obesity and insulin resistance are both conditions associated with oxidative stress and increased inflammation, mainly in adipose tissue. In patients with obesity, the white adipose tissue is characterized by high-intensity inflammation, increased secretion and expression of pro-inflammatory cytokines, including, NF-kB and MCP-1 [42,48,49]. Nuclear Factor kappa B (NF-kB) is involved in various cellular responses, mainly in vascular inflammation, oxidative stress and endothelial dysfunction. HFD leads to increased vascular inflammation *via* different signaling pathways, one of which is linked to increased oxidative stress, reduction of endogenous antioxidant mechanisms and activation of NF-kB [50]. Increased NF-kB is involved in several metabolic complications including the development of insulin resistance [51]. MCP-1 is a chemotactic factor for monocytes and is upregulated in obesity. Increased

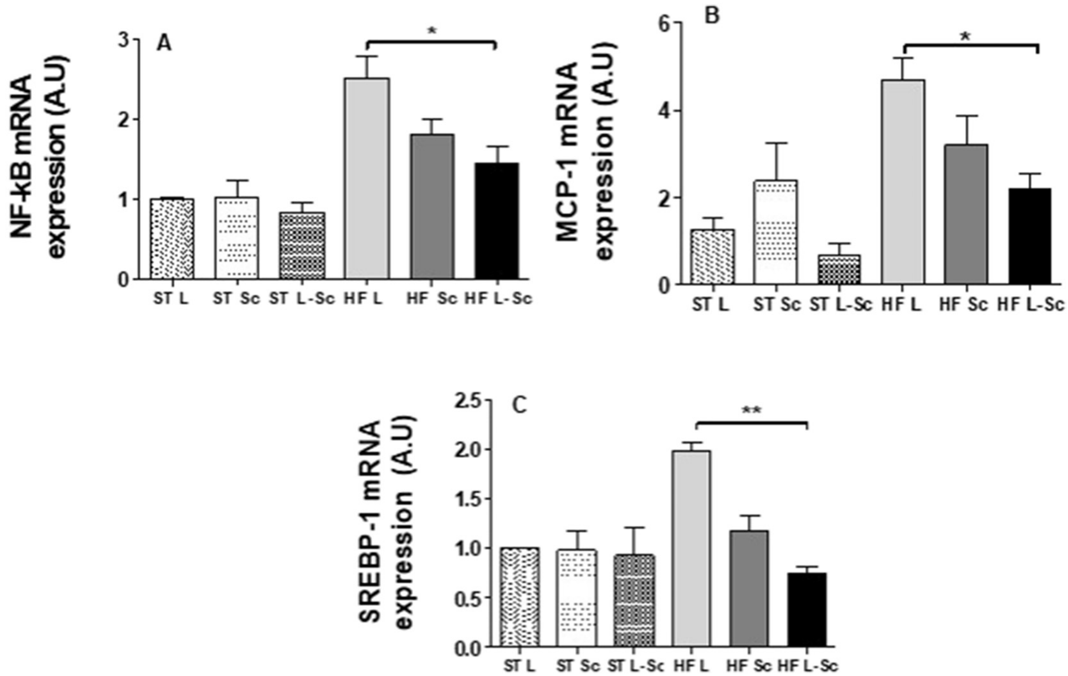

Fig. 6. Reverse transcription and qRT-PCR. (A) NF-KB (B) MCP-1 (C) SREBP-1 gene expression in EAT. Differences between groups were analyzed by One-way ANOVA followed by Bonferroni's post test. The data represent the mean ± SEM, *P < 0.05; **P < 0.01; ***P < 0.001. Free Sclareol (Sc), Sclareol-loaded lipid nanoparticle (L-Sc) and blank lipid nanoparticle (L). High-fat diet (HF) or standard diet (ST).

expression of *MCP-1* in white adipose tissue improves the macrophage infiltration into this tissue favoring hepatic steatosis and insulin resistance [24,49,51,53]. L-Sc prevents the increase of inflammation and oxidative stress contributing to the prevention of diabetes type 2, since HF L-Sc decreased MCP-1 and NF-kB expression in white adipose tissue (Fig. 6). This represents a step closer to normality compared to the altered balance of adipokines in obesity.

Our evaluation also showed that L-Sc intraperitoneal administration significantly increased HDL levels in HF mice. Interestingly, we observed increased triglycerides levels in ST-fed animals, which may be explained by the high-carbohydrate content of this diet that when in excess are converted into triglycerides, as stated in the literature [54,55]. Dyslipidemia is a metabolic disorder characterized by high plasma triglycerides levels, total cholesterol and low HDL [52]. Dyslipidemia is often associated with complications of obesity and metabolic syndrome and is a risk factor for cardiovascular disease [56]. The HDL level has an inverse ratio with the risk for coronary artery disease [57]. HDL is also an anti-atherogenic lipoprotein and displays anti-oxidants properties, removes excess cholesterol from cells, helps the maintenance of endothelial cell function and provides protection against thrombosis, by inhibiting pro-coagulant activity induced by calcium and maintaining the viscosity of normal blood [57]. Since the L-Sc administration increased HDL levels in HF mice (Fig. 4A), it is possible that lipid nanoparticles of Sclareol have a protective role for dyslipidemia and its consequences.

5. Conclusion

The present study indicated that the SLN modulated Sclareol, increasing its effectiveness. Intraperitoneal administration of L-Sc in obesity-induced mice was effective in metabolic improvement, decreasing adiposity, increasing brown adipose tissue weight, increasing HDL levels and improving glycemic profile. The L-Sc effect is due to decreased pro-inflammatory cytokines (NF-KB and MCP-1) and adipogenesis-related marker SREBP-1 mRNA expressions. Further studies are needed to evaluate the possible metabolic pathways involved. However, this work was very important to demonstrate that with intraperitoneal administration of Sclareol in SLN in obese mice, in fact, drastically decreases adiposity. And this is the main finding. Thus, the use of L-Sc may be promising for treatment of metabolic disorders caused by an increase in adipose tissue.

Acknowledgments

This work was supported by grants from Coordenadoria de Aperfeiçoamento do Pessoal de Nível Superior (CAPES), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Fundação de Amparo à Pesquisa de Minas Gerais (FAPEMIG).

Conflict of interest statement

The authors declare no conflicts of interest.

References

[1] World Health Organization http://www.who.int/mediacentre/factsheets/fs311/en/ (Last access: April), (2018).

[2] World Health Organization, World Health Statistics, WHO, Geneva, 2012, pp. 36–37.

[3] T. Kelly, W. Yang, C.S. Chen, K. Reynolds, J. He, Global burden of obesity in 2005 and projections to 2030, Int. J. Obes. 32 (2008) 1431–1437.

[4] F. Zhang, G. Hao, M. Shao, K. Nham, Y. An, Q. Wang, Y. Zhu, C.M. Kusminski, G. Hassan, R.K. Gupta, Q. Zhai, X. Sun, P.E. Scherer, O.K. Oz, An adipose tissue atlas: an image-guided identification of human-like BAT and beige depots in rodents, Cell Metab. 27 (2007) 252–262.

[5] V. Peirce, S. Carobbio, A. Vidal-Puig, The different shades of fat, Nature 510 (2014) 76–83.

[6] M. Iwabu, T. Yamauchi, M. Okada-Iwabu, K. Sato, T. Nakagawa, M. Funata, M. Yamaguchi, S. Namiki, R. Nakayama, M. Tabata, H. Ogata, N. Kubota, I. Takamoto, Y.K. Hayashi, N. Yamauchi, H. Waki, M. Fukayama, I. Nishino, K. Tokuyama, K. Ueki, Y. Oike, S. Ishii, K. Hirose, T. Shimizu, K. Touhara, T. Kadowaki, Adiponectin and AdipoR1 regulate PGC-1alpha and mitochondria by Ca(2+) and AMPK/SIRT1, Nature 464 (2010) 1313–1319.

[7] R.H. Eckel, S.M. Grundy, P.Z. Zimmet, The metabolic syndrome, Lancet 365 (2005) 1415–1428.

[8] S.M. Grundy, H.B. Brewer, J.I. Cleeman, S.C. Smith, C. Lenfant, Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition, Arterioscler. Thromb. Vasc. Biol. 24 (2004) e13–e18.

[9] J.M. Andrade, F.O. Lemos, P.S. Fonseca, R.D. Millán, F.B. de Sousa, A.L. Guimarães, M. Qureshi, J.D. Feltenberger, A.M. de Paula, J.T. Neto, M.T. Lopes, H.M. Andrade, R.A. Santos, S.H. Santos, Proteomic white adipose tissue analysis of obese mice fed with a high-fat diet and treated with oral angiotensin-(1–7), Peptides 60 (2014) 56–62.
[10] J.M. Andrade, A.F. Paraíso, M.V. de Oliveira, A.M. Martins, J.F. Neto, A.L. Guimarães, A.M. de Paula, M. Qureshi, S.H. Santos, Resveratrol attenuates hepatic steatosis in high-fat fed mice by decreasing lipogenesis and inflammation, Nutrition 30 (2014) 915–919.
[11] R. Chaiittianan, K. Sutthanut, A. Rattanathongkom, Purple corn silk: a potential anti-obesity agent with inhibition on adipogenesis and induction on lipolysis and apoptosis in adipocytes, J. Ethnopharmacol. 201 (2017) 9–16.
[12] H.S. Choi, H.J. Jeon, O.H. Lee, B.Y. Lee, Indole-3-carbinol, a vegetable phytochemical, inhibits adipogenesis by regulating cell cycle and AMPK signaling, Biochimie 104 (2014) 127–136.
[13] S.H. Kim, H.S. Park, M.J. Hong, J.Y. Yoo, H. Lee, J.A. Lee, J. Hur, D.Y. Kwon, M.S. Kim, Tongqiaohuoxue decoction ameliorates obesity-induced inflammation and the prothrombotic state by regulating adiponectin and plasminogen activator inhibitor-1, J. Ethnopharmacol. 92 (2016) 201–209.
[14] L.A. Monteros, E. Ramón-Gallegos, N.T. Torres, R. Mora-Escobedo, Effect of germinated soybean protein hydrolysates on adipogenesis and adipolysis in 3T3-L1 cells, Plant Foods Hum. Nutr. 66 (2011) 355–362.
[15] S.I. Choi, I.H. Cho, S.H. Han, Y.J. Jeon, J.G. Choi, J.S. Kim, J.H. Lee, Antiobesity effects of *Salvia plebeia* R. Br. extract in high-fat diet-induced obese mice, J. Med. Food 19 (2016) 1048–1056.
[16] V. Vuksan, A.L. Jenkins, C. Brissette, L. Choleva, E. Jovanovski, A.L. Gibbs, R.P. Bazinet, F. Au-Yeung, A. Zurbau, H.V. Ho, L. Duvnjak, J.L. Sievenpiper, R.G. Josse, A. Hanna, Salba-chia (*Salvia hispanica* L.) in the treatment of overweight and obese patients with type 2 diabetes: a double-blind randomized controlled trial, Nutr. Metab. Cardiovasc. Dis. 27 (2017) 138–146.
[17] Environment Canada, Health Canada, Draft Screening Assessment for the Challenge 1-Naphthalenepropanol, α-ethenyldecahydro-2-hydroxy-α,2,5,5,8α-pentamethyl-, [1R-[1α(R*),2β,4αβ,8αα]]-(Sclareol), Draft Screening Assessment, 2013 (CAS RN 515-03-7).
[18] Y. Hsieh, J. Deng, H. Pan, J. Liao, S. Huang, G. Huang, Sclareol ameliorate lipopolysaccharide-induced acute lung injury through inhibition of MAPK and induction of HO-1 signaling, Int. Immunopharmacol. 44 (2017) 16–25.
[19] Y. Zhong, Y. Huang, M.B. Santos, L. Wu, Sclareol exerts anti-osteoarthritic activities in interleukin-1β-induced rabbit chondrocytes and a rabbit osteoarthritis model, Int. J. Clin. Exp. Pathol. 8 (2015) 2365–2374.
[20] K. Dimas, S. Hatziantoniou, S. Tseleni, H. Khan, A. Georgopoulos, K. Alevizopoulos, J.H. Wyche, P. Pantazis, C. Demetzos, Sclareol induces apoptosis in human HCT116 colon cancer cells in vitro and suppression of HCT116 tumor growth in immune deficient mice, Apoptosis 12 (2007) 685–694.
[21] G.J. Huang, C.H. Pan, C.H. Wu, Sclareol exhibits anti-inflammatory activity in both lipopolysaccharide-stimulated macrophages and the λ-carrageenan-induced paw edema model, J. Nat. Prod. 75 (2012) 54–59.
[22] L.G. Mahaira, C. Tsimplouli, N. Sakellaridis, K. Alevizopoulos, C. Demetzos, Z. Han, P. Pantazis, K. Dimas, The labdanediterpenesclareol (labd-14-ene-8, 13-diol) induces apoptosis in human tumor cell lines and suppression of tumor growth in vivo via a p53-independent mechanism of action, Eur. J. Pharmacol. 666 (2011) 173–182.
[23] S.P. Bhatia, D. Mc Ginty, C.S. Letizia, A.M. Api, Fragrance material review on sclareol, Food Chem. Toxicol. 46 (2008) S270–S274.
[24] S. Hatziantoniou, K. Dimas, A. Georgopoulos, N. Sotiriadou, C. Demetzos, Cytotoxic and antitumor activity of liposome-incorporated sclareol against cancer cell lines and human colon cancer xenografts, Pharmacol. Res. 53 (2006) 80–87.
[25] N.P. Aditya, A.S. Macedo, S. Doktorovova, E.B. Souto, S.H. Kim, P.S. Chang, K. Ko, Development and evaluation of lipid nanocarriers for quercetin delivery: a comparative study of solid lipid nanoparticles (SLN), nanostructured lipid carriers (NLC), and lipid nanoemulsions (LNE), Food Sci. Technol. 59 (2014) 115–121.
[26] R. Liu, Z. Liu, C. Zhang, B. Zhang, Nanostructured lipid carriers as novel ophthalmic delivery system for mangiferin: improving in vivo ocular bioavailability, J. Pharm. Sci. 101 (2012) 3833–3844.
[27] M. Coimbra, B. Isacchi, L. Van Bloois, J.S. Torano, A. Ket, X. Wu, F. Broere, J.M. Metselaar, C.J. Rijcken, G. Storm, R. Bilia, R.M. Schiffelers, Improving solubility and chemical stability of natural compounds for medicinal use by incorporation into liposomes, Int. J. Pharm. 416 (2011) 433–442.
[28] S. Pund, R. Thakur, U. More, A. Joshi, Lipid based nanoemulsifying resveratrol for improved physicochemical characteristics, in vitro cytotoxicity and in vivo antiangiogenic efficacy, Colloids Surf. B: Biointerfaces 120 (2004) 110–117.
[29] S.H. Santos, L.R. Fernandes, E.G. Mario, A.V. Ferreira, L.C. Pôrto, J.I. Alvarez-Leite, L.M. Botion, M. Bader, N. Alenina, R.A. Santos, Mas deficiency in FVB/N mice produces marked changes in lipid and glycemic metabolism, Diabetes 57 (2008) 340–347.
[30] J.M. Andrade, A.C. Frade, J.B. Guimarães, K.M. Freitas, M.T. Lopes, A.L. Guimarães, A.M. de Paula, C.C. Coimbra, S.H. Santos, Resveratrol increases brown adipose tissue thermogenesis markers by increasing SIRT1 and energy expenditure and decreasing fat accumulation in adipose tissue of mice fed a standard diet, Eur. J. Nutr. 53 (2014) 1503–1510.
[31] J.D. Feltenberger, J.M. Andrade, A.F. Paraíso, L.O. Barros, A.B. Filho, R.D. Sinisterra, F.B. Sousa, A.L. Guimarães, A.M. de Paula, M.J. Campagnole-Santos, M. Qureshi, R.A. dos Santos, S.H. Santos, Oral formulation of angiotensin-(1–7) improves lipid metabolism and prevents high-fat diet–induced hepatic steatosis and inflammation in mice, Hypertension 62 (2013) 324–330.
[32] S.H. Santos, J.M. Andrade, L.R. Fernandes, R.D. Sinisterra, F.B. Sousa, J.D. Feltenberger, J.I. Alvarez-Leite, R.A. Santos, Oral angiotensin-(1–7) prevented obesity and hepatic inflammation by inhibition of resistin/TLR4/MAPK/NF-kappaB in rats fed with high-fat diet, Peptides 46 (2013) 47–52.
[33] S.V. Mussi, V.P. Torchilin, Recent trends in the use of lipidic nanoparticles as pharmaceutical carriers for cancer therapy and diagnostics, J. Mater. Chem. B 1 (2013) 5201–5209.
[34] M.S. Oliveira, B.H.S. Lima, G.A.C. Goulart, S.V. Mussi, G.S.M. Borges, R.L. Oréfice, L.A.M. Ferreira, Improved cytotoxic effect of doxorubicin by Its combination with sclareol in solid lipid nanoparticle suspension, Nanotechnology 18 (2018) 5609–5616.
[35] S.V. Mussi, R.C. Silva, M.C. Oliveira, C.M. Lucci, R.B. Azevedo, L.A. Ferreira, New approach to improve encapsulation and antitumor activity of doxorubicin loaded in solid lipid nanoparticles, Eur. J. Pharm. Sci. 48 (2013) 282–290.
[36] S.H. Santos, J.F. Braga, E.G. Mario, L.C. Pôrto, M.G. Rodrigues-Machado, A. Murari, L.M. Botion, N. Alenina, M. Bader, R.A. Santos, Improved lipid and glucose metabolism in transgenic rats with increased circulating angiotensin-(1–7), Arterioscler. Thromb. Vasc. Biol. 30 (2010) 953–961.
[37] D.P. Arçari, W. Bartchewsky, T.W. dos Santos, K.A. Oliveira, A. Funck, J. Pedrazzoli, M.F. de Souza, M.J. Saad, D.H. Bastos, A. Gambero, P.O. Carvalho, M.L. Ribeiro, Antiobesity effects of yerba maté extract (*Ilex araguariensis*) in high-fat diet–induced obese mice, Obesity 17 (2009) 2127–2133.
[38] H. Cao, I. Hininger-Favier, M.A. Kelly, R. Benaraba, H.D. Dawson, S. Coves, A.M. Roussel, R.A. Anderson, Green tea polyphenol extract regulates the expression of genes involved in glucose uptake and insulin signaling in rats fed a high fructose diet, J. Agric. Food Chem. 55 (2007) 6372–6378.
[39] O. Tabatabaei-Malazy, B. Larijani, M. Abdollahi, Targeting metabolic disorders by natural products, J. Diabetes Metab. Disord. 14 (2015) 57.
[40] S.V. Mussi, R. Sawant, F. Perche, M.C. Oliveira, R.B. Azevedo, L.A. Ferreira, V.P. Torchilin, Novel nanostructured lipid carrier co-loaded with doxorubicin and docosahexaenoic acid demonstrates enhanced in vitro activity and overcomes drug resistance in MCF-7/Adr cells, Pharm. Res. 31 (2014) 1882–1892.
[41] N.R. Patel, S. Hatziantoniou, A. Georgopoulos, C. Demetzos, V.P. Torchilin, V. Weissig, G.G. D'Souza, Mitochondria-targeted liposomes improve the apoptotic and cytotoxic action of sclareol, J. Liposome Res. 20 (2010) 244–249.
[42] S.H. Santos, A.C. Silva, The therapeutic role of renin-angiotensin system blockers in obesity-related renal disorders, Curr. Clin. Pharmacol. 9 (2014) 2–9.
[43] M. Saito, Brown adipose tissue as a regulator of energy expenditure and body fat in humans, Diabetes Metab. J. 37 (2013) 22–29.
[44] J.V. Georgieva, Diterpene sclareol glycol inhibits clonidine-induced aggressive responses in mice, Pharmacol. Biochem. Behav. 34 (1989) 503–505.
[45] Y. He, H. Zhang, J. Teng, L. Huang, Y. Li, C. Sun, Involvement of calcium-sensing receptor in inhibition of lipolysis through intracellular cAMP and calcium pathways in human adipocytes, Biochem. Biophys. Res. Commun. 404 (2011) 393–399.
[46] P. Ferré, F. Foufelle, Hepatic steatosis: a role for de novo lipogenesis and the transcription factor SREBP-1c, Diabetes Obes. Metab. 2 (2010) 83–92.
[47] M.Y. Donath, Targeting inflammation in the treatment of type 2 diabetes: time to start, Nat. Rev. Drug Discov. 13 (2014) 465–476.
[48] R.A. Heijden, F. Sheedfar, M.C. Morrison, P.P. Hommelberg, D. Kor, N.J. Kloosterhuis, N. Gruben, S.A. Youssef, A. de Bruin, M.H. Hofker, R. Kleemann, D.P. Koonen, P. Heeringa, High-fat diet induced obesity primes inflammation in adipose tissue prior to liver in C57BL/6j mice (MCP-1), Aging 7 (2015) 256–268.
[49] B.C. Lee, J. Lee, Cellular and molecular players in adipose tissue inflammation in the development of obesity-induced insulin resistance, Biochim. Biophys. Acta 1842 (2014) 446–462.
[50] J. Quilley, Oxidative stress and inflammation in the endothelial dysfunction of obesity: a role for NF-KB? J. Hypertens. 28 (2010) 2010–2011.
[51] H. Carlsen, F. Haugen, S. Zadelaar, R. Kleemann, T. Kooistra, C.A. Drevon, R. Blomhoff, Diet-induced obesity increases NF-kB signaling in reporter mice, Genes Nutr. 4 (2009) 215–222.
[52] S.H. Santos, J.M. Andrade, Angiotensin 1–7: a peptide for preventing and treating metabolic syndrome, Peptides 59 (2014) 34–41.
[53] H. Kanda, S. Tateya, Y. Tamori, K. Kotani, K. Hiasa, R. Kitazawa, S. Kitazawa, H. Miyachi, S. Maeda, K. Egashira, M. Kasuga, MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity, J. Clin. Invest. 16 (2006) 1494–1505.
[54] Elizabeth J. Parks, Effect of dietary carbohydrate on triglyceride metabolism in humans, J. Nutr. 131 (10) (1 October 2001) 2772S–2774S.
[55] Frank M. Sacks, Martijn Katan, Randomized clinical trials on the effects of dietary fat and carbohydrate on plasma lipoproteins and cardiovascular disease, Am. J. Med. 113 (9) (2002) 13–24 (Supplement 2).
[56] C.F. Santos, S.H. Santos, A.V. Ferreira, L.M. Botion, R.A. Santos, M.J. Campagnole-Santos, Association of an oral formulation of angiotensin-(1–7) with atenolol improves lipid metabolism in hypertensive rats, Peptides 43 (2013) 155–159.
[57] A.M. Cohen, D.J. Rader, Dyslipidemia, Curr. Treat. Options Cardiovasc. Med. 3 (2001) 347–357.