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Anti-Obesity Effects of *Cissus Quadrangularis* (Cissuslean®) in C57bl/6j Mice with High-Fat-Diet Induced Obesity and 3t3-L1 Adipocytes

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Abstract Obesity is gradually becoming a widespread health problem, and treatment using natural compounds has seen an increasing trend. The aim of this study investigated the effects and underlying mechanisms of *Cissus quadrangularis* (Cissuslean®) on adipogenesis and high-fat diet (HFD)-induced obesity. The inhibitory effects of Cissuslean® on adipocyte differentiation were evaluated *in vitro* using 3T3-L1 preadipocyte cells, focusing on AMPK signaling and lipid metabolism. Subsequently, an *in vivo* trial with male C57BL/6J mice fed HFD was conducted to examine the effects of Cissuslean® at 25, 50, and 100 mg/kg on adipose tissue characteristics, body weight, fat mass, and obesity-related gene expression. Cissuslean® significantly inhibited preadipocyte differentiation by reducing intracellular lipid accumulation and suppressing the expression of key adipogenic regulators. It also enhanced AMPK phosphorylation during early adipocyte differentiation. In HFD-induced obese mice, Cissuslean® administration resulted in marked reductions in body weight gain, adipose tissue mass, and hepatic lipid accumulation. Furthermore, decreased serum ALT and AST levels indicated attenuation of HFD-induced hepatic injury. According to these results, Cissuslean® reduces body fat mass by inhibiting lipase activity and regulating genes and proteins involved in adipogenesis and lipogenesis in epididymal adipose tissue. Our results suggest that Cissuslean® may be used as an anti-obesity agent.

Keywords: *Anti-obesity,Cissus quadrangularis,Cissuslean®, DEXA,high-fat diet*

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1. Introduction

Obesity is a complex health and global epidemic that affects different age groups, with more than 10% of the adult population being affected. The disease poses a serious threat to public health and is defined by a body mass index (BMI) of 30 kg/m^2 or higher [1]. Obesity increases morbidity and mortality and is associated with type 2 diabetes, cancer, stroke, and coronary heart disease [2]. According to WHO estimates, being overweight or obese would cause 167 million people, including children, to lose their health by 2025 [3]. The link between obesity and metabolic issues, as well as the relationship between obesity and adipose tissue—an endocrine organ thought to be metabolically active—has been the focus of years of research [4].

As an alternative to conventional treatments for obesity and its associated problems, naturopathic treatments have been extensively explored since ancient times and are gaining momentum in the present scenario. Natural products are appealing sources of anti-obesity agents because many of them can modify host metabolic processes and preserve glucose homeostasis by stimulating metabolism and thermogenesis, regulating appetite, inhibiting pancreatic lipase and amylase, improving insulin sensitivity, inhibiting adipogenesis, and inducing adipocyte apoptosis [5].

Cissus quadrangularis L. (Vitaceae) is an ancient medicinal plant that is endemic to Sri Lanka and India. In India, the plant has been used to enhance the fracture healing process [6]. It has been used in Ayurvedic medicine as an anthelmintic, dyspeptic, digestive tonic, and analgesic for eye and ear ailments and for treating irregular menstruation and asthma [7]. In India, stems and leaves are used for food preparation and raw drug treatment for various ailments [8]. The plant's main ingredients include ascorbic acid, carotene A, ketosteroids, calcium, and triterpenoids. It contains three unsymmetrical tetracyclic triterpenoids and β-sitosterol, β-amyrin, and β-amyrone. Flavonoids, phytosterols, δ-amyrin, δ-amyron,

resveratrol, piceatannol, pallidol, parthenocissine, quadragularins, and water-soluble glycosides in it [9,10,11]. Several studies have indicated that *Cissus quadrangularis* has antiobesity properties in animals and humans by inhibiting amylase, lipase, and α-glucosidase [12,13,14].

The hallmark of obesity is an increase in adipose tissue mass, which is related to an increase in fat cell size and number [15]. Adipocytes are vital biological constituents of fatty tissues. Excess lipid (triglycerides) accumulation in adipose tissues is associated with augmented adipogenesis/lipogenesis expression levels and subsequent body weight gain [16]. Obesity and its associated disorders are associated with adipocyte differentiation and fat storage [17]. The prevalence of obesity is increased by a high-fat diet (HFD) and is related to an increase in adipose tissue mass induced by adipocyte hyperplasia and hypertrophy [18]. Adipocytes play an important role in energy balance by producing triglycerides and breaking down fatty acids [19]. In the present study, we aimed to investigate the anti-obesity and anti-adipogenic effects of Cissuslean® in HFD-induced male C57BL/6J mice and in 3T3-L1 preadipocyte models.

2. Materials and Methods

2.1. Preparation and Extraction of *Cissus quadrangularis* (CISSUSLEAN®)

Cissus quadrangularis (CISSUSLEAN®) was provided by Star Hi Herbs Pvt. Ltd. (Bengaluru, INDIA). In a brief overview of the extraction process, *Cissus quadrangularis* dried stems and leaves were extracted with water at 100°C for 3h. The extract was filtered and concentrated by vacuum evaporation. The filtrate was dried using a spray dryer so that the moisture content was less than 5%. The powder was analyzed using high-performance liquid chromatography (HPLC) for standardization using 12.2 mg/g quercetin and 0.37 mg/g isorhamnetin.

2.2. *In Vivo* Antiobesity Activity

Male C57BL/6J mice, aged four weeks, were procured from Invivo Biosciences, Bangalore, allowed to adjust to a controlled environment with specific conditions (temperature, 24 ± 5 ◦C; relative humidity, 55 ± 5%), and provided with ad libitum access to water and food within a 12/12 h light/dark cycle for a period of 1 week. The experimental procedures were approved by the Ethics Committee of the Invivo Biosciences, Bangalore, Karnataka (Invivo/017/2023), Via a random selection process, mice were allocated into six groups: group 1, the control group that consumedan 18% protein diet; group 2, customized high-fatrodent feed (VRK high-fat pellets amounting to 45 kcalper gram of feed); group 3, HFD + Cissuslean® 25 mg/kg/day; group 4, HFD + Cissuslean® 50 mg/kg/day; group 5, HFD + Cissuslean®100mg/kg/day; and group 6, HFD + 50 mg/kg/day Simvastatin (positive control). The body weight of each mouse was measured once weekly for 9 weeks. Weekly food intake measurements were made in each cage throughout the study. At the end of the 9-week treatment period, the mice were anesthetized and blood samples were collected for biochemical analysis. The liver and WAT were excised immediately, and each tissue was rinsed, weighed, frozen in liquid nitrogen, and kept at −80^{o} C until analysis.

2.3. Blood plasma Biochemical Analysis

Mice were starved for 4 h before being anesthetized with ether at the end of the experiment, and blood was collected through the retro-orbital plexus. Plasma was obtained from the collected blood via centrifugation at 2,500×g for 15 min at 4°C for biochemical analyses of plasma parameters. The separated plasma was stored at −80°C until analysis. The levels of serum glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, alanine transaminase (ALT), and aspartate transaminase (AST) were measured using commercial assay kits (Sigma Aldrich, USA) according to the manufacturer's instructions.

2.4. Histological Analysis

Liver and adipose tissues were dissected, fixed immediately in 10% neutral buffered formalin phosphate solution, and embedded in paraffin. Tissue sections (4 µm) were cut and stained with hematoxylin and eosin (H&E). The sections were viewed using a light microscope (Olympus, Tokyo, Japan) and photographed under ×200 magnifications.

2.5. Dual-energy X-ray (DXA)

Body fat was determined in each rat by dual - energy X - ray absorptiometry after 9 weeks of administration using an INALYZER scanner (MEDIKORS Inc., Seongnam, Gyeonggi, Korea). Rats were anesthetized by inhalation of isoflurane during scanning.

2.6. Cell Viability Assay

The MTT assay was performed as described previously; briefly, cells were seeded in 96-well plates and allowed to attach overnight. The cells were then treated with increasing concentrations of compound. The cells were incubated for 48 h and subjected to the MTT assay. The absorbance of solubilized formazan was measured at 570 nm using a microplate reader. A minimum of three independent experiments were conducted, and error bars were calculated.

2.7. Cell Culture and Adipocyte Differentiation

Mouse 3T3-L1 pre-adipocytes (American Type Culture Collection, Manassas, VA, USA) were cultured at 37 °C in a 5% CO_2 atmosphere in Dulbecco's modified Eagle's medium (Gibco BRL, NY, USA) supplemented with 10% FBS and penicillin/streptomycin (Gibco BRL). The 3T3-L1 pre-adipocytes were incubated for 2 days until confluence. Here the cells were exposed to an adipogenic differentiation medium (DMI; DMEM

containing 5% FBS, 0.5 mM 3-isobutyl-1-methylxanthine, 1 mM dexamethasone, and 10 µg/mL insulin) for 4 days. After 2 days, the DMI was changed with DMEM containing 5% FBS and 10 µg/mL insulin, and then changed with DMEM containing 5% FBS every other day. The Cissuslean® was dissolved in phosphate buffered saline (PBS) at 100 mM, and then reconstituted in each medium at the working concentration. Additionally, cells were pre-treated with compound C (20 µM) for one hour and then treated with Cissuslean® (5 µM).

2.8. Western Blot Analysis

Western blotting was performed as previously described. Tissues were washed with PBS, lysed using Qiagen tissue laser II, lysate was centrifuged, and proteins were quantified. Proteins were separated on different gel percentages and subjected to Western blotting to identify the expressed proteins. Blots were blocked and treated with primary and secondary antibodies to detect altered proteins and loading control proteins. HRP-conjugated secondary antibodies were developed using chemiluminescent solution and scanned using ChemiDoc(UViTech, UK).

2.9. Statistical Analysis

The results were processed using one-way ANOVA followed by Turkey's test (GraphPadPrism10.3.1 (509)) was applied to all the parameters, namely glucose, AST, ALT, TG, TC, LDL, HDL, and body weights, and Duncan's multiple range test was applied to lipolytic activity to determine the overall significance. The Student t-test was applied to the food intake results to determine the overall significance. Values of $P < 0.05$ were considered statistically significant.

3. Results

3.1. Effect of Cissuslean® on Body Weight, Food Intake, and Food Efficiency Ratio (FER) in Obese Mice

The final body weight, weight gain, food intake, and food efficiency ratio are summarized in Table 1 and Figure 1. From week 2 onwards, a notable weight gain was observed in the control group receiving the HFD compared with the group on a regular diet, indicating successful induction of obesity. Starting from week 3, daily administration of Cissuslean® at doses of 25, 50, and 100 mg/kg significantly prevented body weight gain in the 50 and 100 mg/kg Cissuslean®-treated groups. The final body weight was higher in the HFD groups than in the control group ($p < 0.05$), whereas the final body weight of all Cissuslean®-treated groups was lower than that in the HFD group ($p < 0.05$). The food intake was higher in the HFD group than the Cissuslean®-treated group. The FER was significantly higher in the HFD groups than in the control group; however, the FER of the HFD + Cissuslean® 100mg group was lower than those of both the HFD and HFD + Cissuslean® 25mg groups.

Table 1. Effect of Cissuslean® treatment on Body Weight (Grams) and food intake with High Fat Diet (HFD)-Fed on Male C57BL/6Jmice

Treatment Groups	Control	HFD	Cissuslean® 25mg+HFD	Cissuslean® 50mg+ HFD	Cissuslean® 100mg+HFD	Simvastatin+ HFD
Initial body weight(g)	14.6±0.2	14.5±0.3	14.8±0.3	14.4±0.3	14.6±0.3	14.4±0.3
Final body weight(g)	24.0±0.4	33.9±0.7	26.5±0.8**$^{\#}$	24.1±0.5**$^{\#}$	24.3±0.3**$^{\#}$	24.1±0.3**$^{\#}$
Body weight gain(g)	9.4±0.44	19.4±0.76**	11.7±0.85*$^{\#}$	9.7±0.58$^{\#}$	9.7±0.42$^{\#}$	9.7±0.42$^{\#}$
Lean mass percentage (%)	17.25±0.25	18.75±0.25	17.90±1.5	17.30±1.1	17.50±0.7	17.80±1.1
Fat mass percentage (%)	26.88±0.77	44.65±0.45****	31.80±0.8$^{**\#\#\#\#}$	28.75±0.45$^{\#\#\#\#}$	27.95±0.25$^{\#\#\#\#}$	27.60±0.1$^{\#\#\#\#}$
Food Intake(g/day)	2.84±0.02	2.52±0.03	2.20±0.01$^{\#}$	2.13±0.01$^{\#}$	2.10±0.02$^{\#}$	2.32±0.007$^{\#}$
Food efficiency ratio	0.052±0.04	0.119 ±0.03	0.081±0.07	0.07±0.04	0.071±0.03	0.064±0.03

****P<0.0001, **P<0.05, where in obese group values were significantly higher as compared against control group.$^{\#\#\#\#}$P<0.0001, $^{\#\#}$P<0.005, $^{\#}$P<0.05, where in obese group showed significant activity as compared against HFD control

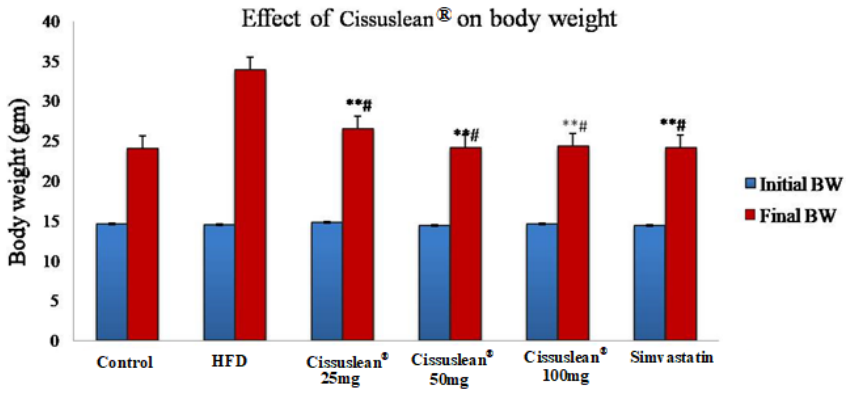


Figure 1. Effects of Cissuslean® on body weight in male C57BL/6J mice fed with HFD. The data were shown relative mean ± SEM. Statistical significance was calculated using one-way ANOVA followed by Turkey's test.**P<0.05 Control vs All other groups., $^{\#}$P<0.05 HFD vs all other treatment groups

3.2. Effects of Cissuslean® on Whole-Body Fat Mass

To determine whether the observed reduction with Cissuslean® treatment was due to reduced fat accumulation, whole-body fat mass was estimated using dual energy X-ray absorptiometry (DEXA) with a small animal apparatus. At the end of the ninth week of intervention, mice in the high-fat diet group exhibited significantly higher whole-body fat mass than those on a regular diet. In contrast, daily oral administration of Cissuslean® at doses of 25, 50, and 100 mg/kg significantly reduced whole-body fat mass compared with the HFD group (HFD: 15.15 vs. Cissuslean® 25mg/kg: 8.4, Cissuslean® 50mg/kg: 7.0, and Cissuslean® 100mg/kg: 6.8). In parallel with whole-body fat mass, significant decreases in fat mass percentage were observed in mice treated with 50 and 100 mg/kg Cissuslean® compared with the HFD group (HFD: 44.65 vs. Cissuslean® 50mg/kg: 28.75 and Cissuslean® 100mg/kg: 27.95) (Table 2).

3.3. Blood Biochemical Changes Induced by Cissuslean®

Following blood collection from each experimental animal group, serum was separated, and biochemical alterations in the blood were noted (Figure 2 & 3). All groups treated with Cissuslean® had higher ALT and AST levels than the control group, and all groups treated with Cissuslean® had lower levels of both variables than the HFD group (Table 3). Cissuslean® significantly reduced the amount of liver damage brought on by fatty liver. The effects of Cissuslean® on blood glucose regulation, which is generally impeded by obesity, were assessed by observing changes in serum glucose. While glucose levels were considerably lower than those in the HFD group, they were higher in all Cissuslean®-treated groups than in the control group (Table 3). Changes in blood caused by Cissuslean® were confirmed by measuring triglyceride, total cholesterol, HDL cholesterol, and LDL cholesterol levels. Blood triglyceride levels in all Cissuslean®-treated groups were higher than those in the control group and lower than those in the HFD group; this difference was significant for the 25, 50, and 100 mg/kg groups (Table 3). All groups treated with Cissuslean® had higher total cholesterol levels than the control group, and they were significantly lower than those in the HFD group (Table 3). There were no noticeable changes in HDL cholesterol in the Cissuslean®-treated groups when compared to the HFD group (Table 3). While LDL cholesterol was considerably lower in all Cissuslean®-treated groups than in the HFD group, it was higher in all groups when compared to the control group (Table 3). As a result, Cissuslean® was discovered to regulate aberrant hepatotoxicity, blood sugar, triglyceride, and cholesterol levels caused by obesity by assessing the biochemical changes in the blood, and this effect was concentration dependent.

Table 2. Body composition measured by DEXA after 9 weeks of Cissuslean® administration

	Control	HFD	Cissuslean® 25mg+HFD	Cissuslean® 50mg+ HFD	Cissuslean® 100mg+HFD	Simvastatin+ HFD
Area(cm^2)	13.80±0.1	14.61±0.04	14.38±0.16	14.14±0.01	13.92±0.01	13.71±0.15
BMC(g)	0.98±0.008	1.29±0.03***	1.25±0.02***	1.18±0.01$^{**\#}$	1.01±0.01$^{\#\#\#}$	1.01±0.01$^{\#\#\#}$
BMD(g/cm^2)	0.08±0.0005	0.08±0.0005	0.08±0.001	0.08±0.0005	0.08±0.0005	0.08±0.002
Fat Mass(g)	6.35±0.15	15.15±0.05***	8.4±1.0$^{\#\#\#}$	7.0±0.6$^{\#\#\#}$	6.8±0.2$^{\#\#\#}$	6.8±0.4$^{\#\#\#}$
Lean+BMC (g)	17.25±0.25	18.75±0.25	17.90±1.5	17.30±1.1	17.50±0.7	17.80±1.1
Total Mass(g)	23.60±0.1	33.90±0.2*	26.30±2.5	24.30±1.7$^{\#}$	24.23±0.9$^{\#}$	24.60±1.5$^{\#}$
Percentage Fat	26.88±0.77	44.65±0.45****	31.80±0.8$^{**\#\#\#\#}$	28.75±0.45$^{\#\#\#\#}$	27.95±0.25$^{\#\#\#\#}$	27.60±0.1$^{\#\#\#\#}$

Effects of Cissuslean® on fat mass and body mass in male C57BL/6J mice fed with HFD. The data were shown relative mean± SEM. Statistical significance was calculated using one-way ANOVA followed by Turkey's multiple test.****P<0.0001, ***P<0.0005, **P<0.005, *P<0.05Control vs All other groups. $^{\#\#\#\#}$P<0.0001, $^{\#\#\#}$P<0.0005, $^{\#\#}$P<0.005, $^{\#}$P<0.05 HFD vs all other treatment groups

Table 3. Effects of Cissuslean® on the Biochemical parameters with High Fat Diet (HFD)-Fed on Male C57BL/6J mice

Sl No	Control	HFD	Cissuslean® 25mg+HFD	Cissuslean® 50mg+ HFD	Cissuslean® 100mg+HFD	Simvastatin+ HFD
ALT	46.6±1.7	196.9±3.1**	85.3±2.1**#	82.1±2.1**#	63.8±2.8**#	65.1±1.7**#
TG	123.0±1.1	240.5±11.5**	210.1±4.1**#	199.9±4.1**#	178.1±2.5**#	138.1±5.8**#
TC	123.3±2.4	216.6±4.4**	199.6±3.4**#	184.5±4.1**#	170.6±4.2**#	134.5±1.9**#
LDL	15.7±0.4	37.2±1.9**	33.1±1.4**	29.0±2.0**#	22.5±1.6**#	18.8±0.5**#
HDL	93.6±1.4	148.1±2.8	146.8±2.2	141.9±2.9	145.8±1.7	128.4±7.5*
AST	54.9±1.8	154.6±5.4**	94.1±5.1**#	80.3±3.1**#	67.4±2.4#	74.9±3.3**#
BLOOD GLUCOSE	91.5±2.9	162.9±4.5**	154.4±3.6**	140.75±2.6*#	135.0±3.4**#	126.6±2.80*#

Effects of Cissuslean® on biochemical parameters in male C57BL/6J mice fed with HFD. The data were shown relative mean ± SEM. Statistical significance was calculated using one-way ANOVA followed by Turkey's test.**P<0.05Control vs All other groups., $^{\#}$P<0.05HFD vs all other treatment groups

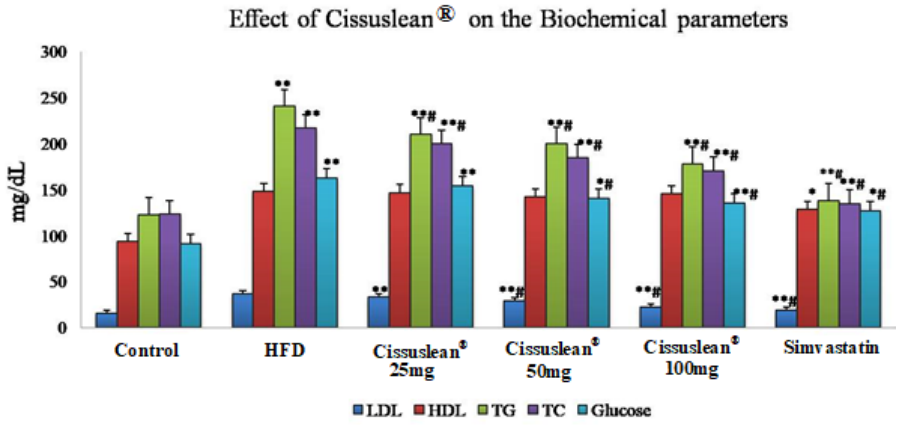


Figure 2. Effects of Cissuslean® on biochemical parameters in C57BL/6J mice fed a HFD. The data were shown relative mean ± SEM. Statistical significance was calculated using one-way ANOVA followed by Turkey's test. ** P>0.05 vs. Control and # P>0.05vs.HFD. TG, triglycerides; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein

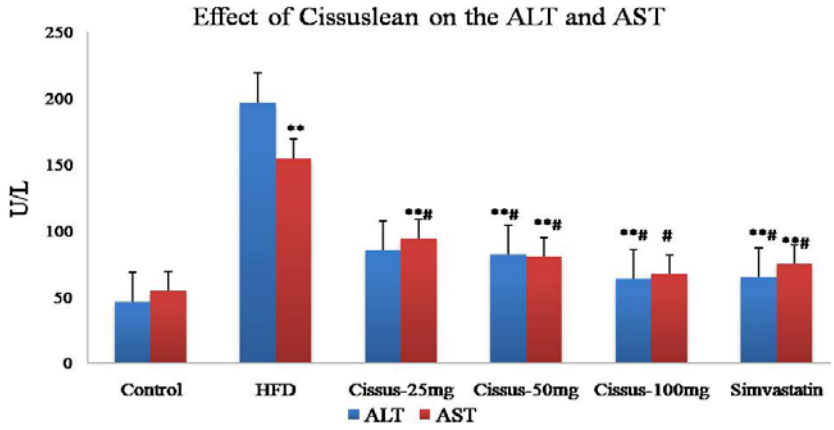


Figure 3. Effects of Cissuslean® on biochemical parameters in C57BL/6J mice fed a HFD. The data were shown relative mean ± SEM. Statistical significance was calculated using one-way ANOVA followed by Turkey's test. ** P>0.05 vs. Control and # P>0.05vs.HFD. AST, aspartate amino transferase; ALT, alanine transaminase

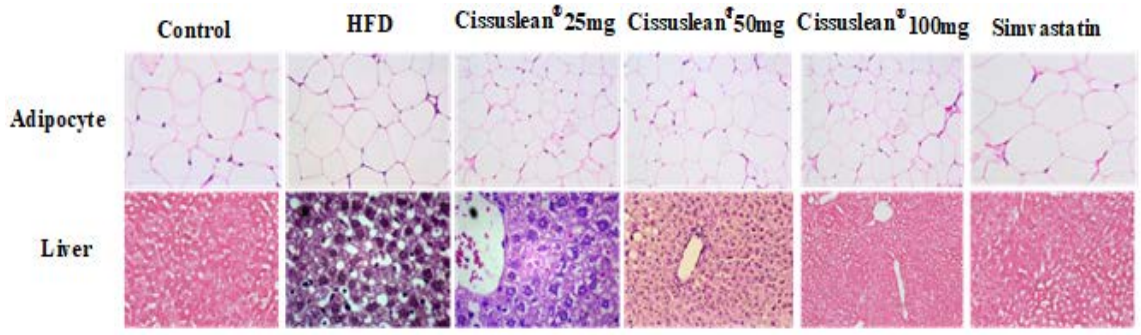


Figure 4. H&E-stained images of liver and subcutaneous adipose tissue samples from the normal diet (control), vehicle-treated, Cissuslean®-treated groups

3.4. Histopathological Evaluation

Hematoxylin and eosin were used to stain the experimental animal liver and WAT (white adipose tissue) to evaluate the morphological alterations caused by fatty liver. A marked increase in adipocyte size and number was observed in the HFD group compared with the treatment groups, thereby indicating the presence of activity in all Cissuslean®-treated groups. Fatty liver was observed in the HFD, low-dose, and mid-dose Cissuslean® groups, whereas in the high-dose Cissuslean®-treated groups, normal architecture was restored (Figure 4).

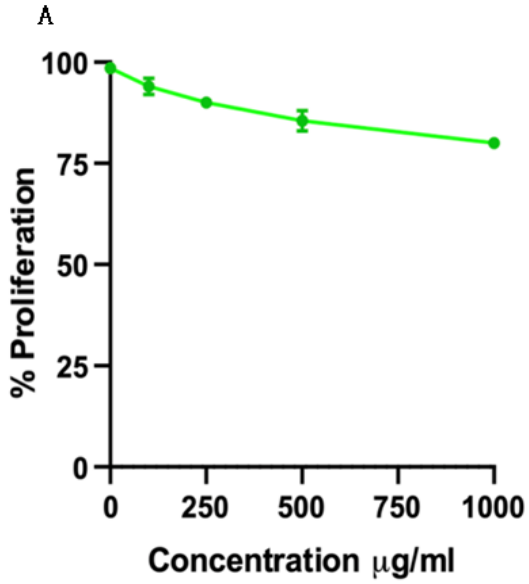
3.5. Cissuslean® Exhibit Nontoxic Effects on Normal Cells

To assess the cytotoxicity profile of Cissuslean®, HFF1 cells were treated with various concentrations of Cissuslean®. The viability of the cells was then measured using the MTT assay. MTT assesses the metabolic activity of cells as a measure of cell viability. The results demonstrated that across all tested concentrations, cell viability remained high, comparable to that of the untreated control group (Figure 5A). This indicates that

the extract had no negative effects on the ability of cells to metabolize. Furthermore, microscopic evaluation of HFF1 treated with Cissuslean® revealed normal cell morphology, with no visible signs of cell membrane disruption, cell shrinkage, or detachment from the cell culture substrate. These observations are consistent with a lack of cytotoxicity, as damaged or dying cells typically exhibit such morphological changes. This study demonstrated that Cissuslean® did not induce any significant cell death or exhibit toxic effects on normal fibroblasts, highlighting its potential for use in various applications, including dietary supplements and therapeutic formulations. Further studies should explore the effects of this agent on other cell types to fully establish its safety profile.

3.6. Cissuslean® Depletes Triglycerides Formation in NIH/3T3 Cells

To evaluate the effect of Cissuslean® on triglyceride accumulation, differentiated NIH/3T3 cells were exposed to various concentrations of Cissuslean®, and the results demonstrated that Cissuslean® treatment inhibited triglyceride accumulation in a concentration-dependent manner (Figure 5B). The intracellular triglyceride level significantly decreased with increasing Cissuslean® concentration. This suggests that the extract effectively suppresses fat storage in these cells. The inhibition of triglyceride accumulation indicates that Cissuslean® interferes with the adipogenic process, potentially through the modulation of key signaling pathways involved in lipid metabolism. By reducing the formation of lipid droplets within differentiated adipocytes, Cissuslean® can limit fat storage and promote lipid lysis. These results demonstrate the potential of Cissuslean® as a therapeutic agent for the management of obesity and related metabolic disorders by inhibiting excessive fat accumulation in adipose tissue.



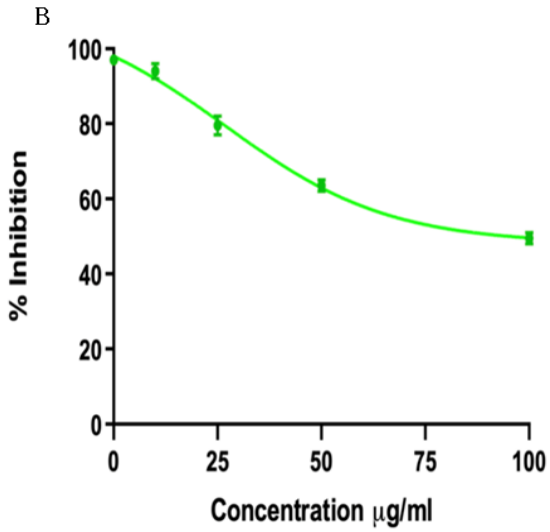


Figure 5. (A). Effect of Cissuslean® on normal cells: NIH3T3 cells were exposed to Cissuslean® at various concentrations and subjected to MTT assay to evaluate toxicity. **(B).** Effect of Cissuslean® on lipid accumulation: Differentiated NIH3T3 cells were treated with various concentrations of Cissuslean® and subjected to triglyceride glow assay to estimate triglyceride levels.

3.7. Cissuslean® Declines the Lipid Accumulation by Down Modulating Adipogenesis Proteins

To evaluate the effect of Cissuslean® on lipid accumulation, the tissues were lysed using Qiagen tissue lyser II, defatted the fat, extracted the proteins, quantified, and subjected to SDS PAGE to resolve the proteins (Figure 6). The results demonstrate the Cissuslean® treatment induces PPARα expression in a concentration-dependent manner. PPARα is a nuclear receptor that plays a crucial role in lipid metabolism, particularly in the regulation of fatty acid oxidation. The study observed an increase in PPARα expression upon Cissuslean® treatment. The elevated expression of PPARα is associated with enhanced fatty acid catabolism, promoting the oxidation of fatty acids and thus reducing the lipid accumulation within cells. The Cissuslean® treatment displayed reduced PPARγ expression, another nuclear receptor involved in adipogenesis and lipid storage. PPARγ promotes expression of genes involved in lipid uptake and storage and contributes to fat accumulation. The results suggest Cissuslean® down regulates lipogenic processes leading to decreased lipid synthesis and storage. The AKT pathway plays a crucial role in cell growth and metabolism. AKT phosphorylation activates anabolic processes, including lipogenesis. The results demonstrate concentration-dependent inhibition of AKT phosphorylation, indicating suppression of AKT activity and decreased lipogenesis activity. AKPK is a key energy sensor that promotes catabolic processes, including fatty acid oxidation, while inhibiting lipogenesis. The Cissuslean® treatment exhibited increased AMPK phosphorylation, suggesting enhanced metabolic activity

by increasing fatty acid oxidation and inhibiting the synthesis of fatty acids and triglycerides. Cissuslean® treatment also displayed enhanced expression of phosphorylated ACC protein in a concentration-dependent manner. ACC (acetyl-CoA carboxylase) is a pivotal enzyme involved in fatty acid biosynthesis, which converts acetyl-CoA to malonyl-CoA, a fatty acid precursor. Phosphorylation of ACC inactivates ACC and reduces lipogenesis. The results further substantiate the inhibition of lipogenesis and promotion of lipolysis activity by Cissuslean® treatment. P53, a master regulator, was slightly unregulated at higher concentrations or unaltered by Cissuslean® treatment, and it down modulates the phosphorylated NFkB, a transcription factor that plays a crucial role in inflammation and in metabolism. The results unveiled that decreased phosphorylation of NFkB leads to reduced NFkB activity, which leads to decreased inflammatory response and has been associated with improved metabolic profiles. The results collectively suggest a potent lipolytic ability of Cissuslean® by inhibiting lipogenic proteins to reduce the lipid accumulation and favoring lipolysis to reduce the lipid accumulation and highlight the possible benefits in managing lipid-related disorders or promoting a healthier metabolic state.

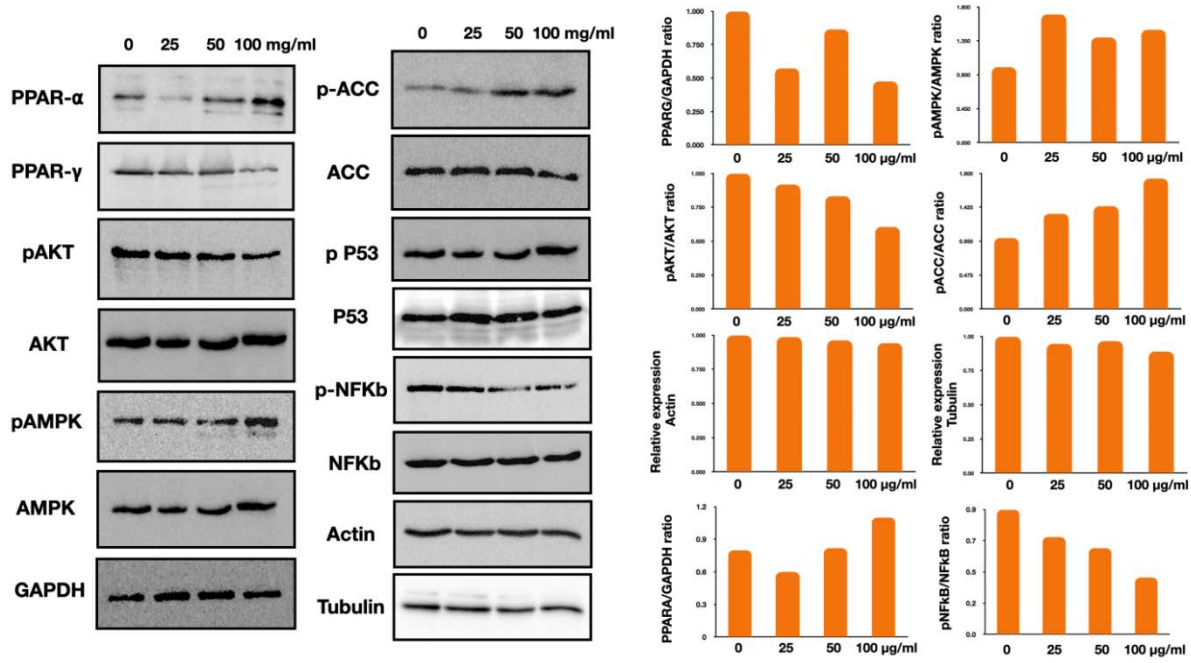


Figure 6. Effect of Cissuslean® on the expression of proteins related to lipogenic and lipolytic activity in 3T3-L1. PPAR- α, PPAR-γ, pAKT/AKT, pAMPK/AMPK, p-ACC/ACC, p-NFkB/NFkB, Actin and Tubulin. All results are presented as the mean ± SD of three independent in triplicate. Bars with different letters indicate significant differences at $p < 0.05$ using Duncan's multiple range test

4. Discussion

A lot of research is being conducted on anti-obesity medications and functional diets to deal with the rising number of obesity cases recently [20,21]. Numerous studies are also being conducted to confirm the efficacy of natural-substance-based functional foods and anti-obesity medications, as well as to lessen the different adverse effects of some commercial anti-obesity medications [22,23]. An imbalance between energy intake and expenditure leads to obesity, and extra energy is built up in adipose tissue as TG [24]. *Cissus quadrangularis* is a succulent vine that is indigenous to West Asia and Africa and is a member of the Vitaceae family. For over a century, Ayurvedic medicine has utilized *Cissus quadrangularis* for its traditional medicinal properties to treat various ailments, such as bone fractures, digestive issues, eye and ear disorders, and asthma [25].In the present study, the biochemical and biological activity of Cissuslean® was examined in animal obesity models that mimic human metabolic disturbances exhibited as a result of high-fat diet consumption.

The epididymal, mesenteric, peritoneal, and cardiac depots comprise the fat masses identified by DEXA. Subcutaneous interscapular and inguinal depots were computed for fat masses in addition to the visceral adipose tissues [26]. When determining an animal's body composition, DEXA models assume that the animal's body composition consists of three components: fat, bone mineral, and residual lean soft tissue. Compared with the HFD-fed animal groups, the Cissuslean®-treated groups showed a significant reduction in fat mass, lean mass,and subsequently percentage fat.

It has been reported that HFD is suitable for inducing obesity and expresses an increase in body weight, adipose tissue weight, and hyperlipidemia in mice [27]. We confirmed that Cissuslean® treatment for 9 weeks decreased body weight gain in 25, 50, and 100 mg/kg. In addition, Cissuslean® treated mice significantly decreased the number and size of adipocyte tissue compared with the HFD-fed group, and 100 mg/kg of the Cissuslean®-treated group restored normal liver architecture. These results suggest that Cissuslean® treatment affects a decrease in body weight gain, which is likely related to reducing WAT weight.

In our study, the anti-obesity effect of Cissuslean® was confirmed. The Cissuslean®-treated groups showed a decrease in BW gain, body fat mass, number and size of adipocytes, and serum levels of glucose, ALT, AST, TG, cholesterol, and LDL. Thus, this study can be used as a foundation to investigate the safety and efficacy of Cissuslean® as a functional ingredient and anti-obesity medication. Additionally, by lowering blood fat levels, Cissuslean® may help prevent several disorders linked to obesity.

High levels of total cholesterol, glycerides, or LDL caused by an HFD are known to increase the risk of coronary vascular disorders, insulin resistance, and nonalcoholic fatty liver [28,29]. In addition to adipogenesis, high-fat diet consumption of free or saturated fatty acids causes metabolic disorders and long-term inflammation [30]. By lowering serum lipid concentrations, hypocholesterolemic and anti-obesity medications may lessen many clinical consequences of obesity. In the present study, lower serum concentrations of total cholesterol, triglycerides, free fatty acids, and LDL indicated that treatment with Cissuslean® considerably improved the lipid profiles of the rats. Because the liver is a crucial organ involved in both general metabolism and chemical detoxification, liver function tests are significant markers of liver function [31]. The current study found that elevated blood ALT and AST levels in the HFD group were indicative of changes in liver metabolic activity. Cissuslean® administration successfully reduced the increased levels of certain hepatic enzymes and lipid profiles associated with HFD consumption, indicating its protective effect. It can be concluded that Cissuslean® at low, mid, and high doses has anti-obesity activity, which was complemented by the lipid profile results.

The nontoxic effect of Cissuslean® was demonstrated using an MTT assay against NIH/3T3 cells, and the unaltered morphology and metabolic activity revealed its safety profile *in vitro*. The decrease in lipid accumulation upon Cissuslean® treatment revealed its antiadipogenic effect. The protein expression profile of HFD-and drug-treated mice revealed the lipolytic and anti-adipogenic effects of Cissuslean®. Elevated levels of PPAR gamma levels indicate fatty acid oxidation, indicating enhanced lipolytic activity. Decreased AKT levels reveal decreased lipogenesis activity and depleted NF-κB levels, indicating a decreased inflammatory response and have been associated with improved metabolic profiles.

5. Conclusion

This study used diet-induced obesity models in mice to show that Cissuslean® had antiobesity properties. Cissuslean® reduces lipid accumulation and suppresses adipogenic markers by blocking adipocyte development in 3T3-L1 cells. By decreasing mice's body weight, mostly through lowering food efficiency ratios and preventing fat accumulation in WAT and visceral organs. Our findings provide a basis for the future development of novel therapeutic strategies for obesity management.

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Statement of Competing Interests

The authors have no competing interest.

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List of Abbreviations

HFD - High fat diet, BMI - Body mass index, TC - Total cholesterol, TG – Triglycerides, LDL - Low-density lipoprotein cholesterol, HDL – High density lipoprotein cholesterol, ALT - Alanine transaminase, AST - Aspartate transaminase, DEXA - Dual energy X-ray absorptiometry,WAT - White adipose tissue,ACC - Acetyl-CoA carboxylase.

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