

## Green Synthesis of Silver Nanoparticles Using *Persea Americana* Fruit Extract Prevents Adipogenesis of 3T3-L1 Cells Via Down Regulating Ppar- $\gamma$

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Cite this paper as: Dr. R. Roghini, Dr. M. Muthulakshmi, K. Selvi, Mrs. M. Krishnaveni, Dr. Muni Kumar Dokka, (2025) Green Synthesis of Silver Nanoparticles Using *Persea Americana* Fruit Extract Prevents Adipogenesis of 3T3-L1 Cells Via Down Regulating Ppar- $\gamma$ . *Journal of Neonatal Surgery*, 14 (4), 188-197.

### ABSTRACT

Obesity is one of the major public health concerns. In the last two decades, Obesity and its comorbidities were witnessed among the people worldwide which making fifth main cause of human mortality. Excessive weight gain occurs due to sedentary life style, imbalance in utilization and consumption of calories, and intake of high energy dense food rich in high sugar and fat content, that leads to onset of several health issues such as Type II diabetes, cancer, cardio vascular diseases, respiratory diseases, and Musculo skeletal disorders. Various synthetic drugs are available in the market for the treatment of obesity, but on the other side these are accompanied with inimical effects. To overcome this problem, and in the view of current scenario, emphasizing more on the development of natural bioactive products for the obesity management. However, experimental evidence is required to support obesity management mechanisms or unveil new ones. Hence silver nanoparticles were prepared from *Persea americana* and its anti-obesity mechanisms were tested with nuclear receptors called PPAR $\gamma$  which plays a significant role on adipogenesis and fat metabolism.

The silver nanoparticles were prepared from the extract of *Persea americana* and it is encapsulated with Orlistat which is a standard drug used as positive control. The nanoparticles were characterized physically and spectroscopically. The viability of nanoparticles was assessed with 3T3L1 cell line by MTT assay. The lipid content was estimated in 3T3L1 cell line by oil-O-red staining method. The level of triglycerides was estimated on 3T3L1 cell line. The impact of the *Persea americana* silver nanoparticle and Orlistat encapsulated silver nanoparticles on anti-adipogenic effect by using epigenetic markers with 3T3L1 cell lines.

**Keywords:** Orlistat, obesity, *Persea americana*, 3T3-L1 cell line

### 1. INTRODUCTION

Obesity is a multifactorial global epidemic disease affecting all age groups of both sexes. Now one third of population is obese and obesity becomes world-wide prevalence in recent era. BMI is an indirect measure of adiposity in epidemiological studies and it is defined as their weight in kilograms divided by the square of height in meters. 'Normal weight' is classified as Body Mass Index between 18.5 and 25 Kg/m<sup>2</sup>, 'overweight' is over 25 Kg/m<sup>2</sup>, and 'obesity' is greater than 30 Kg/m<sup>2</sup><sup>(1)</sup>. The worsen condition in the population ages is 40.9% obese prevalence among adults in Asia-Pacific region will cause a financial influence up to 12% of over-all healthcare spending on corpulence and its associated diseases <sup>(2)</sup>. The global epidemic obesity is unfortunate for more reasons than the poor physical appearance. Sedentary life style, new dietary habitats, and westernization that associated increased risk of obesity related diseases like hypertension, type II diabetes, raised circulating cholesterol, cancer, fateful hormonal imbalances in women that can leads to infertility, heart diseases, stroke, arthritis, chronic renal disease, worse pregnancy outcome, and even Alzheimer's disease which is commonly experienced in adults <sup>(3)</sup>.

Some strategies adopted to drop body fat is to limits caloric intake, Drug therapy, behavior reshaping, increased physical exertion and, in risky cases, surgery. In recent years, the availability and popularity of natural dietary supplements has been

increased in weight loss journey [4]. The prospective of natural products for the treatment of obesity is still mostly unmapped and it might be noticeable alternative strategy for the development of defended and effective drugs to reduce obesity and overweight [5].

Plant based medications and its therapeutic use provides valuable approaches in the treatment and prevention of numerous diseases. This may afford to sufficient, upturned metabolism, and accelerated weight loss [6]. Plant products have long been a source for invention of new drugs will be a complementary and alternative medicine system. In recent decades the plant - based products are investigated and reported in the treatment of obesity. There are several herbs, and plant- based products were reported for weight management. But so far, a systematic, well designed and proper screening of compound from the herbal source for anti-obesity treatment is not attempted.

By keeping these conditions in our knowledge and urge to design a system (*Persea americana* silver nanoparticles) in effective treatment plans with minimal or no risk/complications.

Consequently, by using the knowledge from existing evidence- based science on natural products, we are endeavoring qualitative research to provide the permanent solution for weight loss without surgery and side effects. In this research work, we are endeavors to separate the specific compound which is responsible for effective anti-obesity agent. In this study, *Persea americana* are used to develop active formulation (Silver nanoparticle) for the management of obesity. The primary and secondary metabolites in *Perseana americana* exert their activity in inhibition of PPAR $\gamma$  (Peroxisome Proliferator Activated Receptor  $\gamma$  expression). The mechanism at genetic level is explained in order to generate conviction amongst users as dynamic agent for weight management.

*Persea americana* is significantly valued for its nutritional value and its bioactive compounds at global level. *Persea americana* belongs to the family Lauraceae [7]. It has various pharmacological properties includes anti-hypertensive, anti-fungicidal, anti-larvicidal, antioxidant, amoebicidal and giardicidal activities. *Persea americana* contains high protein and fat-soluble vitamins. 3T3-L1 cells mimic the developing fat cell appearance and formation. Avacado is an oil-rich fruit and its intake leads to protection of cellular metabolic activities from oxidative damage and enhance the immune system [8]. Avocado is a subtropical fruit tree plant which is commercially cultivated in Mediterranean climates of many countries in which Mexico is the chief producer of avocado at global level. Avocado is a nutrient rich fruit and it have a higher content of potassium than bananas. It is rich in Monosaturated fatty acids and water-soluble fibers. Avocado has lot of medicinal properties like cure for high cholesterol, Angina pectoris and Alzheimer's disease. It aids in digestion, and blood sugar balance, skin care. It is having anti- inflammatory, Anti-microbial, anti-ulcer [9].

## 2. MATERIAL AND METHODS

All materials were purchased in Sigma Aldrich.

### 2.1 Extraction:

The sample (*Perseana Americana*) was allowed to dry for a week and grinded well into powder. The powder is dissolved in 100ml distilled water mixed well with magnetic stirrer at 37°C for 20minutes and it was filtered with Whatman filter paper No.4. The filtrate is centrifuged for 10 minutes at 2000rpm. The supernatant was taken for the further analysis.

### 2.2 Preparation, encapsulation and characterization of nanoparticles:

#### 2.2.1 Preparation:

16.98 mg of silver nitrate is dissolved in 100 ml of distilled water. 10 ml of *Persea americana* fruit extract and 25 mg of sodium citrate a capping agent was added to above silver nitrate solution and kept overnight at room temperature. The solution containing nanoparticles was centrifuged for 30 mins at 12000 rpm. Then the nanoparticles were dried in oven at 80°C. The clumped nanoparticles were separated by ultra sonicator. The dried nanoparticles were used for further analysis.

#### 2.2.2 Encapsulation:

16.98 mg of silver nitrate is dissolved in 100 ml of distilled water. 10 ml fruit extract of *Persea Americana*, 2mg of orlistat, 25 mg of capping agent sodium citrate was added to above silver nitrate solution and incubated for overnight at room temperature. The obtained nanoparticle solution was centrifuged for 30 mins at 12000 rpm. The nanoparticles were dried in oven at 80°C. The dried nanoparticles were used for further analysis.

#### 2.2.3. In vitro study:

##### 2.2.3.1. Characterization of *Perseana americana* silver nanoparticles:

##### 2.2.3.2 SEM analysis:

The morphology of *Perseana americana* silver nanoparticles and Orlistat loaded silver nanoparticles were analyzed by Scanning Electron Microscope (SEM: JSM-52- TOKYO, JAPAN.) and samples were sputtered with gold.

### 2.2.3.3 UltraViolet-visible spectroscopy analysis

UV-vis absorption spectrum was measured using a UV-vis double-beam spectrophotometer (UV-SHIMADZU, JAPAN) in the range of 200-800nm.

### 2.2.3.4 X-Ray Diffraction analysis

It is one of the microstructural analysis method of identification of *Perseana americana* silver nanoparticles and Orlistat loaded silver nanoparticles and analysis of crystalline phases (polymorphism). XRD pattern of sample was analysed with a CAD4-X-Ray Diffractometer (M/S Nonius BV, The Netherlands) with Cu(K $\alpha$ ) radiation at 30 mA and 40kV.

### 2.2.3.5 Cell viability:

The cytotoxicity was carried out using Methyl thiazolotetrazolium (MTT) assay with proliferation of 3T3-L1 preadipocyte. 3T3-L1 preadipocytes were seeded at a density of  $3 \times 10^6$  cells per well into 96-well plates and maintained with Dubelcco's modified eagle's medium (DMEM) containing 10% bovine serum. After 24 hours of incubation at 37°C, the medium was changed and the sample contains different concentrations (10  $\mu$ g - 100  $\mu$ g/ml) of silver nanoparticles from the extract of *Perseana americana*. After 48 hours of incubation, the medium containing 10  $\mu$ l of MTT solution (5  $\mu$ g/ml) was added. The plate was wrapped using foil and incubated for 48 hours at 37°C. The supernatant was discarded, and 100  $\mu$ l of Dimethyl sulfoxide (DMSO) was added to each well. The amount of formazan product was determined using a micro plate reader (BioRad, USA) and absorbance was read under the wavelength of 570nm in a microplate spectrophotometer. The optical density was converted into cell viability percentage which is compared with control group. Results were expressed as the percentage of MTT reduction.

### 2.2.3.6 Oil-O-red staining:

The cells washed with phosphate buffered saline after removing the supernatant from the culture plate. The culture plate is fixed with 4% formaldehyde in PBS for 15 minutes at room temperature. Lipid accumulation in the mature adipocytes were determined using the Oil Red O staining method. The effects of silver nanoparticle from the extract *Persea americana* may decrease the lipid accumulation. The 10% formalin was used to fix the cells for one hour, then washed with 60% isopropanol. It is allowed to dry. Then, the cells were stained with Oil Red O (Sigma-Aldrich) solution in isopropanol for 10 min. The solution was washed four times with distilled water. The stained red fat droplets were removed from cells using isopropanol. The absorbance was measured at a wavelength of 500 nm (Tecan M200 Infinite).

### 2.2.3.7 Estimation of Triglycerides:

The level of triglycerides of silver nanoparticles from the extract of *Perseana americana* will be noted. Cellular triglyceride contents were measured using triglyceride quantification assay kit (Asan Pharm. Co., Seoul, Republic of Korea). Cells were treated with samples at concentrations of 100  $\mu$ g/mL in 6-well plates for 6 days. The cells were washed with phosphate-buffered saline (PBS). The cells were scraped in 75  $\mu$ L of a homogenizing solution (154 mM KCl, 1 mM EDTA and 50 mM Tris, pH 7.4), and cell suspension was sonicated. To remove fat layer, the residual cell lysate was centrifuged at 3000rpm for 5 min at 25 °C. The supernatants were analyzed for triglycerides. Lipolysis was quantified by the measurement of glycerol released (Free glycerol reagent, Sigma, St. Louis, MO, USA) into the medium. Differentiated 3T3-L1 adipocytes were treated with samples for 24 h. After incubation, 50  $\mu$ L of the medium was incubated with 200  $\mu$ L of a free glycerol reagent for 15 minutes at room temperature. The glycerol was quantified by measuring absorbance at 540 nm <sup>(10)</sup>.

### 2.2.3.8 Epigenetic Markers for Examining the Effects of Antilipidemic Agents on PPAR $\gamma$ Gene Expression:

The protein expression (epigenetic markers) will be carried out by western blotting technique.

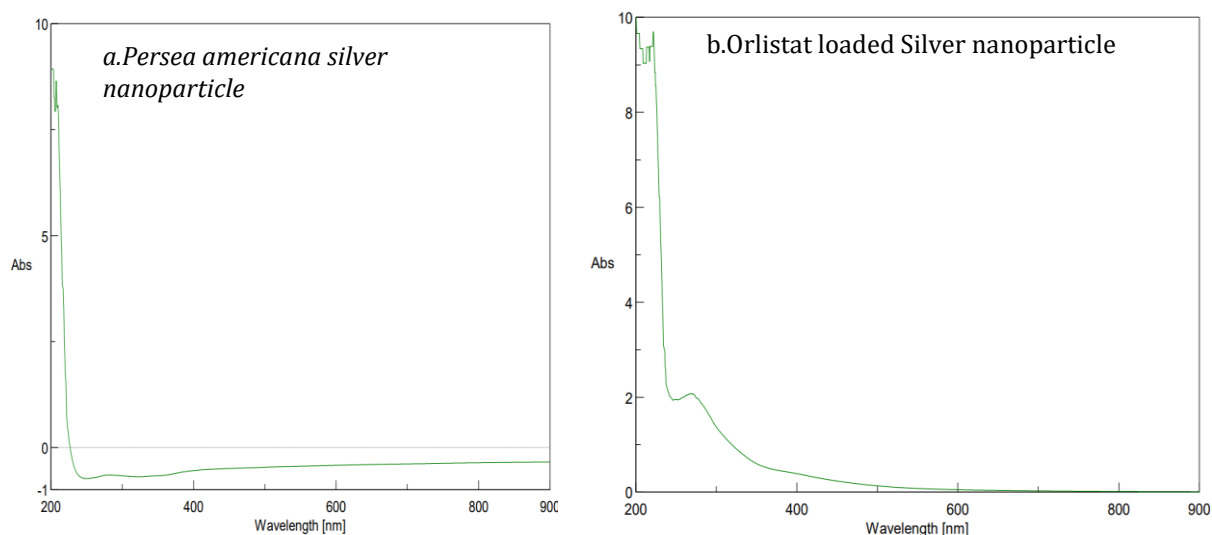
## 3. RESULT AND DISCUSSION:

### 3.1 Characterization of AgNPs:

Upon adding the fruit extract to silver nitrate solution, a faint brown color was observed after 24 hours of reaction in dark. The coloration of solution is due to the excitation of surface plasmon vibrations reveals spectroscopic signature of AgNPs formation <sup>(11)</sup>. (Li et al., 2007). Characterization of biosynthesized AgNPs was accomplished using UV-Visible spectroscopy, X-Ray Diffraction analysis and Scanning Electron Microscope.

### 3.2 Ultra violet Visible Spectroscopy analysis:

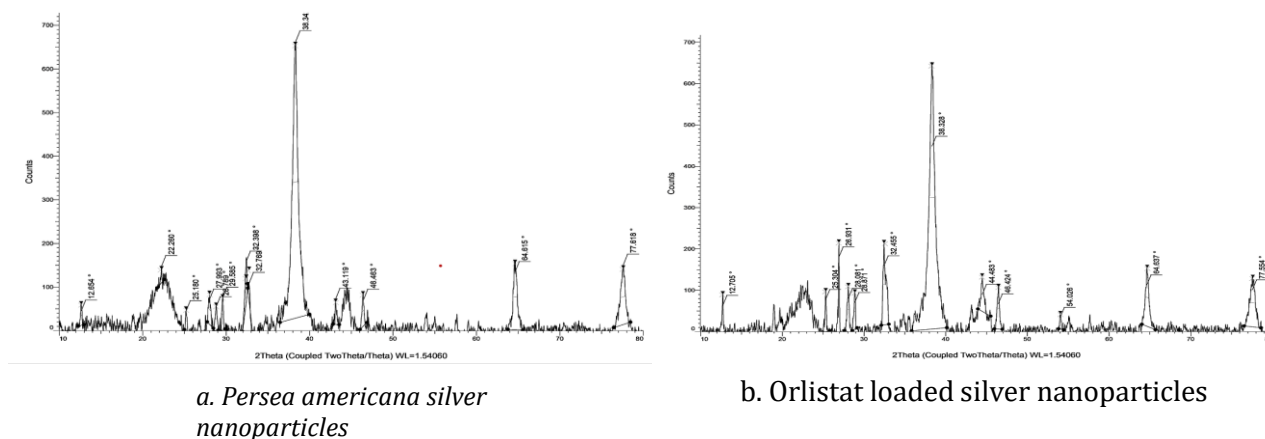
The UV visible spectrum of silver nanoparticles was noted from the reaction medium with a function of reaction time. *Persea americana* silver nanoparticle produce the absorption peak ranges from 250-350nm (Figure 1a) and orlistat loaded silver nanoparticle produces 200-250nm. The maximum absorption peak ( $\lambda_{max}$ ) of *Persea americana* silver nanoparticles and orlistat loaded silver nanoparticle were 290nm and 240 nm respectively (Figure 1b). The same absorption spectra observed by <sup>(12)</sup>. In their fungal mediated silver nanoparticle synthesis. In this study, the absorption spectrum observed at 250nm to 350nm. The deconvolution of the spectrum results due to characteristic physique of the silver nanoparticle <sup>(13)</sup>. The broadening of peak in silver nanoparticles designated that the particles in dispersed phase.



**Figure.1** UV-Visible spectroscopic analysis of (a) *Persea americana* silver nanoparticle (b) Orlistat loaded silver nanoparticle.

### 3.3 XRD STUDIES:

The XRD results supporting that the presence of silver colloids in sample. The Bragg's reflections were observed in the XRD pattern of *Persea americana* silver nanoparticle at  $2\theta = 12.6^\circ, 22.2^\circ, 25.18^\circ, 27.9^\circ, 28.7^\circ, 29.5^\circ, 32.3^\circ, 32.7^\circ, 38.34^\circ, 43.19^\circ, 46.46^\circ, 64.6^\circ, 77.6^\circ$  (Figure 2a). The orlistat loaded silver nanoparticle produces  $2\theta = 12.7^\circ, 25.3^\circ, 26.9^\circ, 28.08^\circ, 32.45^\circ, 38.328^\circ, 38.32^\circ, 44.4^\circ, 46.4^\circ, 54.02^\circ, 64.63^\circ, 77.5^\circ$  (Figure 2b). From  $2\theta = 12^\circ$  to  $2\theta = 32^\circ$  reveals the peak reflection due to the drug Orlistat. The *Persea americana* silver nanoparticles show the reflection pattern at  $2\theta = 38.3^\circ, 46.18^\circ, 64.6^\circ$  and  $77.5^\circ$ . Comparable results were observed in the green synthesis of silver nanoparticles using *Taxacum officinale* <sup>(14)</sup>. On the account of reflections, the silver nanoparticles sorted as cubic close-packed structure or face centered cubic structure. The broaden peak of XRD specified that the existence of smaller sized silver particles. Hence XRD pattern thus evidently exemplified that silver nanoparticles formed in the present synthesis are crystalline in nature. The unassigned peaks were also noticed that suggests the crystallization of bioorganic phase occurred on the surface of the nanoparticles.



**Figure.2** X-ray Diffraction analysis of (a) *Persea americana* silver nanoparticle (b) Orlistat loaded silver nanoparticle.

### 3.4 SEM STUDIES:

SEM techniques visualized the size and shape of orlistat loaded silver nanoparticle and SEM images were obtained from *Persea americana* silver nanoparticles which is encapsulated with orlistat. The morphological dimension of the orlistat encapsulated silver nanoparticles were 74-83nm with interparticle distance (Figure 3). The shape of the orlistat loaded silver

nanoparticles evidenced to be spherical.

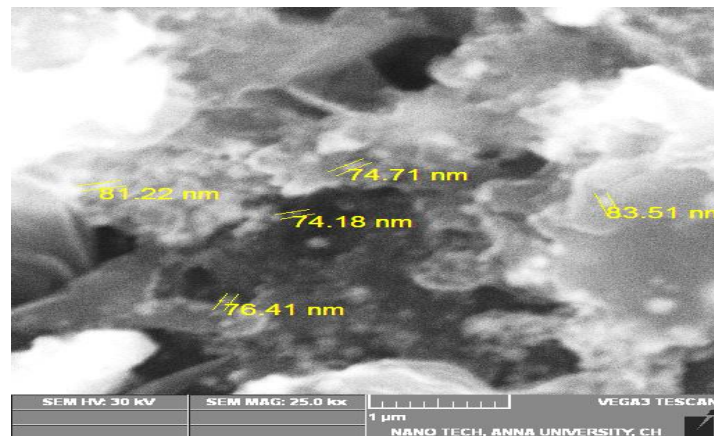


Figure 3. SEM image of Orlistat loaded silver nanoparticles.

### 3.5 Cell viability by MTT assay:

The excess energy is stored in adipose tissue from intake food. In carried invitro studies mice 3T3-L1 preadipocytes were used which elaborate all of the features of adipocytes. Adipocytes are differentiated by the cell division, inhibition and formation of lipid droplets <sup>(15)</sup>. This process initially observed after 7 days of differentiation. We aimed to assess the *Persea americana* silver nanoparticles as adipogenesis process inhibitors but without divulging any toxic effects on cellular viability after the time of differentiation process. The results of cytotoxic activity were presented in the Table 1.

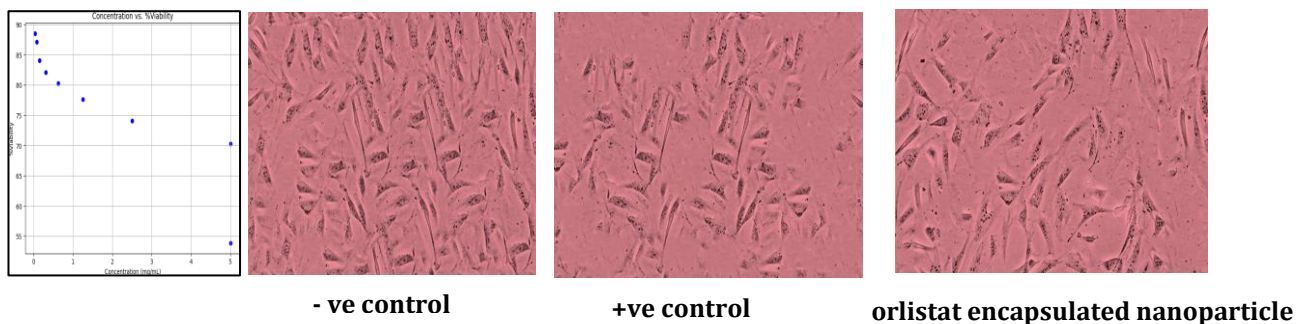


Figure 4. Cytotoxicity evaluation of *Persea americana* silver nanoparticle

. The recorded IC<sub>50</sub> values of *Persea americana* silver nanoparticle and Orlistat loaded silver nanoparticles are 5mg/ml (Figure 4). It reveals that upto 5mg/ml of *Persea americana* Ag-NPs is enough to administer for in vivo studies. This report is with data in the literature survey, reported that dose-dependent toxicity of silver nanoparticles <sup>(16), (17)</sup>. In addition to the dose concentration of sample, configuration, distribution and surface chemistry of silver nanoparticles also decided the cytotoxicity. Hence biosynthesized silver nanoparticles are recommended for biomedical applications <sup>(18)</sup>. In the present study, results of cell viability show that, cell death occurs at high concentration. The live cells ratio was augmented when the concentration of *Persea americana*-AgNPs were lowered.

ID	Conc. (mg/mL)	OD I	OD II	Mean OD	SD	%CV	%Viability
Blank	NA	0.0651	0.0662	0.06565	0.000777817	1.1848	NA
NC	NA	0.122	0.1238	0.1229	0.001272792	1.0356	100
PC	5	0.0589	0.0585	0.0587	0.000282843	0.4818	47.76



TC1	5	0.0873	0.0874	0.08735	7.07107E-05	0.081	71.07
TC2	2.5	0.0904	0.0915	0.09095	0.000777817	0.8552	74.00
TC3	1.25	0.0941	0.0945	0.0943	0.000282843	0.2999	76.73
TC4	0.625	0.097	0.0981	0.09755	0.000777817	0.7974	79.37
TC5	0.312	0.0991	0.1005	0.0998	0.000989949	0.9919	81.20
TC6	0.156	0.1014	0.1031	0.10225	0.001202082	1.1756	83.20
TC7	0.078	0.1054	0.1068	0.1061	0.000989949	0.933	86.33
TC8	0.039	0.107	0.1084	0.1077	0.000989949	0.9192	87.63

**Table 1 Cytotoxicity evaluation of *Persea americana* silver nanoparticle**

### 3.6 Oil O staining method and assay for lipid quantification:

**Chloesterol is the major component of body cells which acquired from food that we eat.** This is occurred as phospholipid bilayer, insoluble in nature and it moves to all area of body through lipoproteins <sup>(19) (20)</sup>. The cholesterol existed as High-Density lipoproteins (HDL) Low-Density Lipoproteins (LDL) and Very Low-Density Lipoproteins (VLDL). The state of low level of HDL and high level of triglycerides are the hallmark of development of obesity <sup>(21)</sup>. In the current study supplementation of biosynthesized *Persea americana* silver nanoparticles decreases the risky cholesterol level. The *Persea americana* silver nanoparticle exhibits at the lowest concentration (0.5 mg/mL), there is a 15% inhibition compared to the control group and at a medium concentration (1 mg/mL), the inhibition rises to 35% and at the highest concentration tested (2.5 mg/mL), the inhibition reaches 80%, representing a significant dose-dependent adipogenesis inhibition on 3T3 cell lines. In Sample 2 (Orlistat loaded silver nanoparticle), the concentration-dependent inhibitory effect with 20% inhibition at 0.5 mg/mL, 40% at 1 mg/mL, and 70% at 2.5 mg/mL (Figure5). The dose-dependent response suggests that higher concentrations of antilipidemic lead to more significant inhibition of the differentiation process of adipocytes. This indicates a promising potential application in controlling or reducing adipogenesis, which can be beneficial in treating obesity and metabolic disorders. It demonstrates the ability of antilipidemic to inhibit lipid synthesis or storage, in addition to its anti-adipogenic effects.

In the results of Oil Red'O staining assay, *Persea americana* silver nanoparticle shows, 25% reduction in lipid staining in 0.5 mg/mL concentration. With increasing the concentration to 1 mg/mL leads to a 45% reduction. At high concentration (2.5 mg/mL), an 80% reduction is observed. The pattern is similar, with 30% reduction at 0.5 mg/mL, 50% at 1 mg/mL, and 70% at the highest concentration in the case of orlistat encapsulated silver nanoparticle (Table 2a). This assay further solidifies the findings of the 3T3-L1 Cell Assay by showing a concentration-dependent reduction in lipid accumulation (Table 2b). It demonstrates the ability of antilipidemic to inhibit lipid synthesis or storage, in addition to its anti-adipogenic effects.

Both assays consistently demonstrate that antilipidemic state exerts a significant, concentration-dependent inhibition effect on adipocyte differentiation and lipid deposition in 3T3-L1 cells. Our findings coincide with other research studies. According to Mohammad et al., *Persea americana* pulp decrease the total cholesterol level with increase of beneficial cholesterol called high density lipoprotein. This facilitates the mitochondria to break the food into energy and it also breaks the cholesterol. This therapeutic intervention involves in treating congenital heart disease <sup>(22)</sup>.

Our findings reveal that antilipidemic as a potential therapeutic agent for diseases associated with abnormal lipid metabolism and adipose tissue accumulation, such as obesity and related metabolic syndromes. The high levels of inhibition at the concentrations tested emphasizes the robustness of these effects and open avenues for further mechanistic studies and potential medical development.

When compared both samples, *Persea americana* mediated silver nanoparticles shows significant antilipidemic effect than orlistat drug encapsulated silver nanoparticles. Moreover, the balance between adipogenesis and adipolysis determined by the size of fat cells <sup>(23)</sup>. Thus, *Persea americana* extracts and their constituents decreases lipid deposition in adipocytes along with the stimulation of triglyceride mobilization which is responsible for anti-obesogenic mechanisms <sup>(24)</sup>.

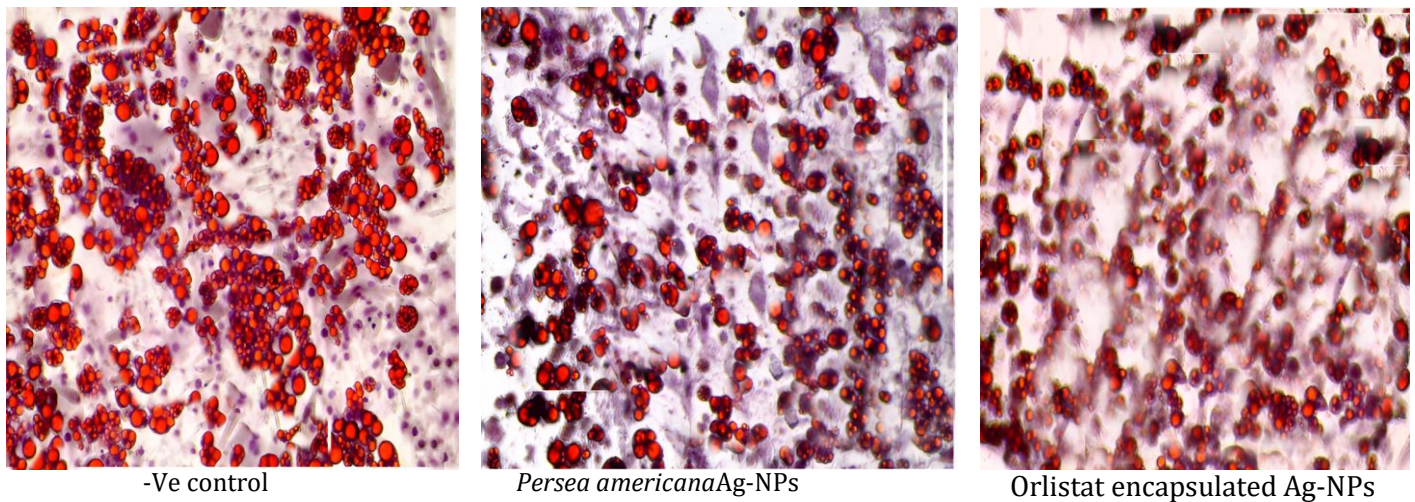


Figure.5 OilO' staining method for lipid quantification

Sample	Assay	Concentration (mg/mL)	Absorbance	% Inhibition (Compared to Control)
<i>Persea americana</i> AgNPs	Oil Red O Staining	0.5	0.75	25%
<i>Persea americana</i> AgNPs	Oil Red O Staining	1	0.55	45%
<i>Persea americana</i> AgNPs	Oil Red O Staining	2.5	0.4	80%
Orlistat AgNPs	Oil Red O Staining	0.5	0.7	30%
Orlistat AgNPs	Oil Red O Staining	1	0.5	50%
Orlistat AgNPs	Oil Red O Staining	2.5	0.35	70%

Table 2(a). Oil Red O Staining method for lipid quantification

Sample	Assay	Concentration (mg/mL)	Absorbance	% Inhibition (Compared to Control)
<i>Persea americana</i> AgNPs	3T3-L1 Cell Assay	0.5	0.85	15%
<i>Persea americana</i> AgNPs	3T3-L1 Cell Assay	1	0.65	35%
<i>Persea americana</i>	3T3-L1 Cell Assay	2.5	0.5	80%

AgNPs				
Orlistat encapsulated AgNPs	3T3-L1 Cell Assay	0.5	0.8	20%
Orlistat encapsulated AgNPs	3T3-L1 Cell Assay	1	0.6	40%
Orlistat encapsulated AgNPs	3T3-L1 Cell Assay	2.5	0.45	70%

Table 2(b). 3T3-L1 cell assay method for lipid quantification

### 3.7 Epigenetic Markers for Investigating the Effects of Antilipidemic Agents on PPAR $\gamma$ Gene Expression:

PPAR $\gamma$  is a nuclear receptor which regulate the adipogenesis and controls glucose metabolism <sup>(25)</sup>. The natural and synthetic agonists activated PPAR $\gamma$  gene along with adipocyte genes including aP2, and SREBP-1 were enhanced and expressed <sup>(26)</sup>. The active SREBP-1c increased the lipogenic gene such as FAS and ACC-1 were expressed <sup>(27)</sup>. Hence our findings prove that *Persea americana* AgNPs and Orlistat encapsulated AgNPs downregulating the gene expressions for lipid synthesis and upregulating the fatty acid  $\beta$ oxidation metabolism.

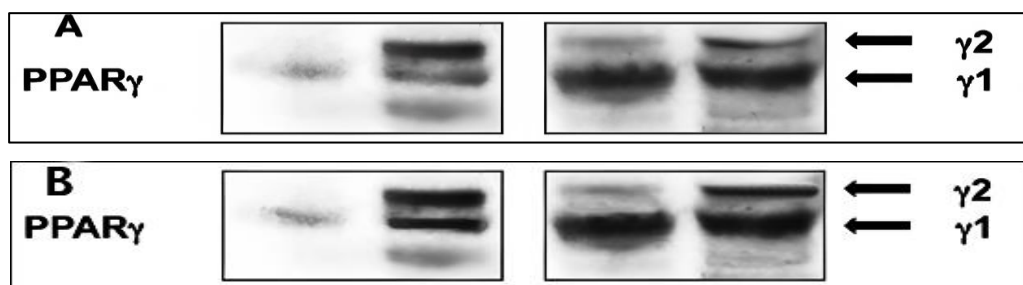


Figure 6. Effect of Antilipidemic Agents on PPAR $\gamma$  Gene Expression

Sample	Assay	Concentration (mg/mL)	PPAR $\gamma$ Expression (Fold Change)	% Reduction in Lipid Content
<i>Persea americana</i> AgNPs	PPAR $\gamma$ Assay	0.5	1.2	20%
<i>Persea americana</i> AgNPs	PPAR $\gamma$ Assay	1	1.5	40%
<i>Persea americana</i> AgNPs	PPAR $\gamma$ Assay	2.5	2.1	60%
Orlistat AgNPs	PPAR $\gamma$ Assay	0.5	1.1	30%
Orlistat AgNPs	PPAR $\gamma$ Assay	1	1.4	50%
Orlistat AgNPs	PPAR $\gamma$ Assay	2.5	1.8	70%

Table 3 Effects of Antilipidemic Agents on PPAR $\gamma$  Gene Expression:



The outcome of epigenetic studies put forward a strong correlation between the modulation of PPAR $\gamma$  gene expression by antilipidemic agents (*Persea americana* AgNPs and Orlistat encapsulated AgNPs) and the observed reduction in lipid content within 3T3-L1 cells (Table 3). When comparing both samples, Orlistat encapsulated silver nanoparticle shows significant percentage of reduction content in lipid (70%). Thus, the epigenetic studies shown to confer that, the antilipidemic agents that is *Persea americana* silver nanoparticles highly (70%) controls the expression of PPAR $\gamma$  gene leads to reduce the lipidemic effect than Orlistat encapsulated silver nanoparticles (60%). The anti- adipogenetic activity by epigenetic studies of *Persea americana* yet have not been investigated. The literature survey proves that, phytochemicals present in *Persea americana* controls the adipocytes proliferation and differentiation<sup>(28)</sup>. Our findings let us believe that the compounds present in *Persea americana* controls sequential expression of key transcription factor PPAR $\gamma$  gene that involved in the adipocyte differentiation program<sup>(29)</sup>. Like the similar expression mechanism of PPAR  $\gamma$ 2 on 3T3L1 adipocytes was observed by<sup>(30)</sup>.

#### 4. CONCLUSION

There are several herbs, and plant- based products were reported for weight management. But so far, a systematic, well designed and proper screening of compound from the herbal source for anti-obesity treatment is not attempted. Consequently, by using the knowledge from existing evidence- based science on natural products, we are attempting a qualitative research in obesity management that will be attracting the final users with effective benefits. In this research work, we are endeavors to separate the specific compound which is responsible for effective anti-obesity agent. In this probe, *Perseana americana* are used to develop active formulation (Silver nanoparticle) for the management of obesity. The primary and secondary metabolites in *Perseana americana* exert their activity in inhibition of PPAR $\gamma$  (Peroxisome Proliferator activated receptor  $\gamma$  expression. The mechanism of action at genetic level is explained in order to generate conviction amongst users as dynamic agent for weight management.

#### ACKNOWLEDGMENT

We Acknowledge ACS AMRI Unit of Dr. MGR towards financial support for this research.

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