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Evaluation of Anti-Adipogenic and Anti-Obesity Activities of *Ocimum americanum* L. Ethanolic Extract Using 3T3-L1 Mouse Pre-Adipocyte Cell Lines

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Abstract

Background: Obesity, a significant public health concern, is associated with various chronic diseases. Natural compounds, such as those derived from Ocimum americanum, may offer promising anti-obesity effects with fewer side effects compared to synthetic drugs.

Objective: This study aims to evaluate the anti-adipogenic and anti-obesity activities of a methanolic extract of inflorescence of Ocimum americanum (OA) using 3T3-L1 mouse embryo pre-adipocyte cell lines.

Methods: The study employed the MTT cytotoxicity assay to assess cell viability and the Oil Red O staining assay to measure lipid accumulation. The 3T3-L1 cells were treated with various concentrations of OA extract, and their effects on cell viability and lipid accumulation were compared to a control and a known anti-obesity agent, metformin.

Results: OA extract maintained high cell viability (>90%) at concentrations up to 200 µg/ml. It significantly reduced lipid accumulation in differentiated adipocytes compared to the control, demonstrating potent anti-adipogenic and anti-obesity effects.

Conclusions: The findings support the potential of OA extract as a natural therapeutic agent for obesity management, warranting further investigation into its molecular mechanisms and in vivo efficacy.

Keywords: Obesity; Ocimum; Herbal Medicine; MTT Assay; Oil Red O Staining; Adipocyte Differentiation

Abbreviations

OA: *Ocimum americanum*; AMPK: AMP-Activated Protein Kinase; IBMX: Isobutylmethylxanthine.

Introduction

Obesity is a major public health concern worldwide, characterized by excessive fat accumulation that poses significant health risks, including diabetes, cardiovascular diseases, and certain cancers. Plant-based therapies play a significant role in modern obesity management due to their potential efficacy, reduced side effects, and accessibility. Natural compounds, such as those derived from plants like Ocimum spp, demonstrate promising anti-adipogenic and anti-obesity effects by targeting key processes such as adipocyte differentiation and lipid accumulation. *Ocimum americanum* (OA), commonly known as Hoary Basil, is a

medicinal plant traditionally used in various cultures for its anti-inflammatory, antimicrobial, and antioxidant properties. Recent studies suggest that extracts from OA may also exhibit anti-adipogenic and anti-obesity effects, providing a promising avenue for obesity treatment [1,2].

Adipogenesis, the process of cell differentiation by which pre-adipocytes become mature adipocytes, plays a crucial role in the development of obesity. Inhibiting this process can be a viable strategy to prevent or reduce obesity. The 3T3-L1 cell line, derived from mouse embryo pre-adipocytes, is widely used as an in-vitro model to study adipogenesis and screen potential anti-obesity agents [3,4].

This study aimed to evaluate the anti-adipogenic and anti-obesity activities of a methanolic extract of inflorescence of OA using the 3T3-L1 mouse pre-adipocyte cell line. We employed two primary assays: the MTT cytotoxicity assay to assess cell viability and the Oil Red O staining assay to measure lipid accumulation within the cells. The MTT assay is a colorimetric technique that gauges cell proliferation and cytotoxicity by converting yellow tetrazolium salt (MTT) to purple formazan crystals by mitochondrial enzymes in living cells. The Oil Red O staining assay is used to detect intracellular lipid droplets, which are indicative of adipocyte differentiation and lipid accumulation [5,6].

Materials and Methods

Cell Culture and Maintenance

3T3-L1 cells were cultured in DMEM-high glucose media supplemented with 10% FBS and 1% antibiotic-antimycotic solution. Cells were maintained at 37°C in a 5% CO2 incubator.

MTT Cytotoxicity Assay

The MTT assay was conducted to determine the cytotoxicity and cell viability of the OA extract. Cells were seeded in a 96-well plate and treated with varying concentrations of OA extract for 24 hours. MTT solution was added, and the formazan crystals formed were dissolved in DMSO. Absorbance was measured at 570 nm, and cell viability was calculated [7,8].

Oil Red O Staining

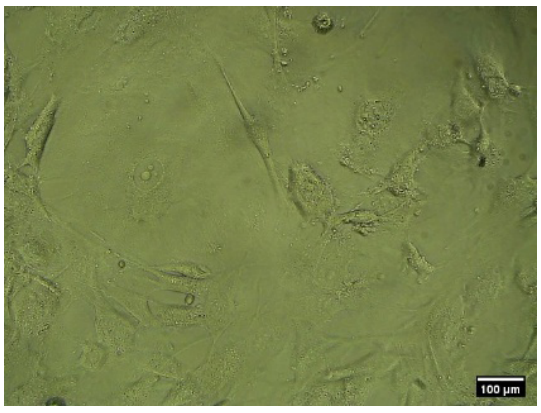
To assess lipid accumulation, 3T3-L1 cells were induced to differentiate using an adipogenic differentiation medium (MDI: IBMX, dexamethasone, insulin) and co-treated with OA extract or metformin. Post-differentiation, cells were fixed and stained with Oil Red O solution. Lipid content was quantified by extracting the dye with isopropanol and measuring absorbance at 520 nm [9-11].

Results

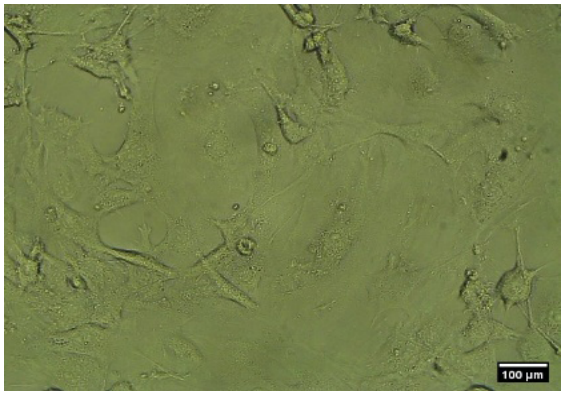
MTT Cytotoxicity Assay

Cell Viability: OA extract showed high cell viability (>90%) up to 200 µg/ml after 24 hours. Concentration Effect: At 200µg/ml, OA extract maintained greater than 91.52% cell viability.

Metformin with 100uM/ml was used as a std control for the study. Based on the obtained MTT assay results, the 200ug/ml as an optimum concentration for further assays which caused the greater than the 90% cell viability (Figures 1 & 2).



3T3-L1 METFORMIN-100uM (1)



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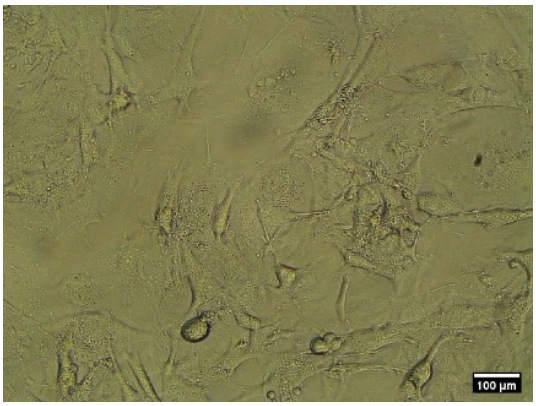
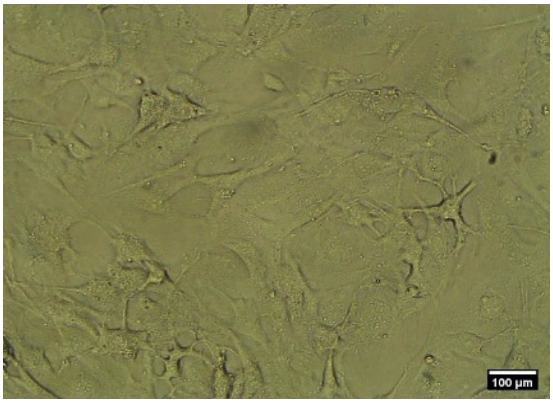
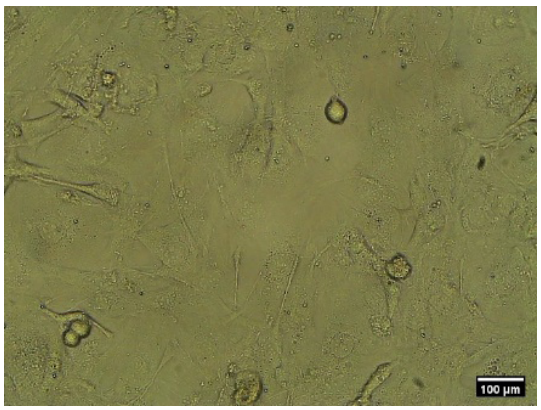
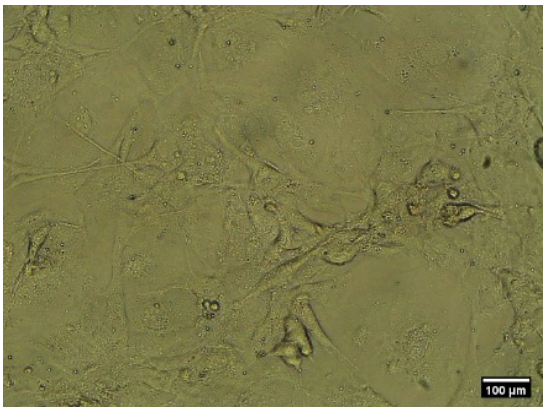
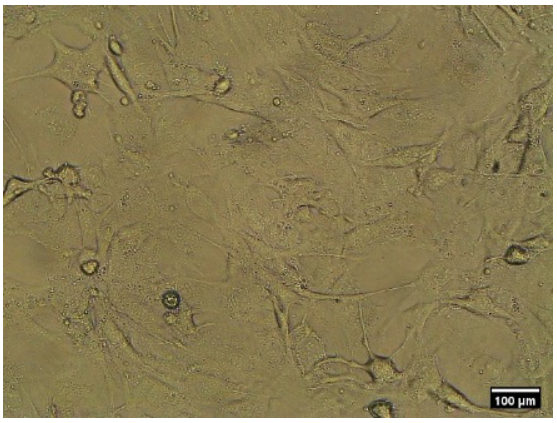
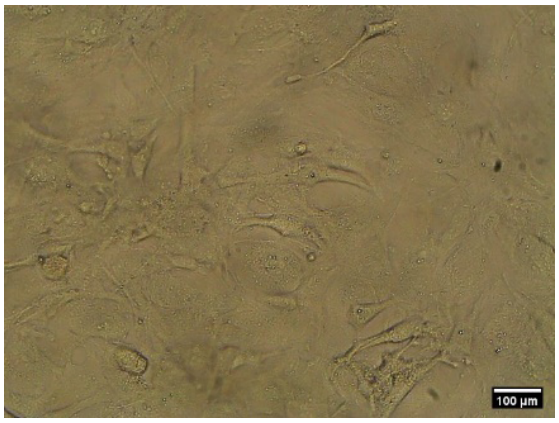
	
3T3-L1 UNTREATED (1)	**3T3-L1 UNTREATED (2)**
	
OA-12.5 ug (1)	**OA-12.5 ug (2)**
	
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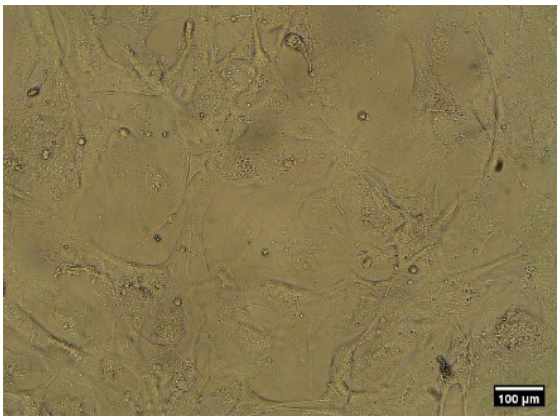
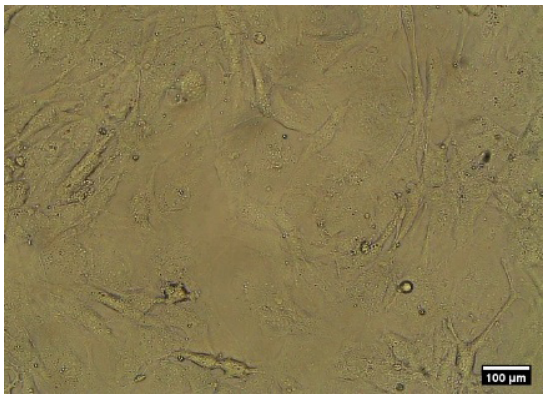
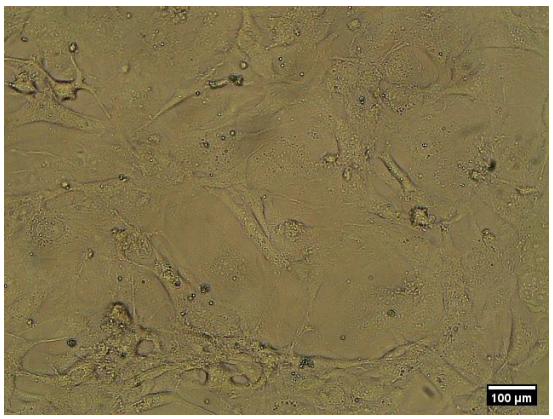


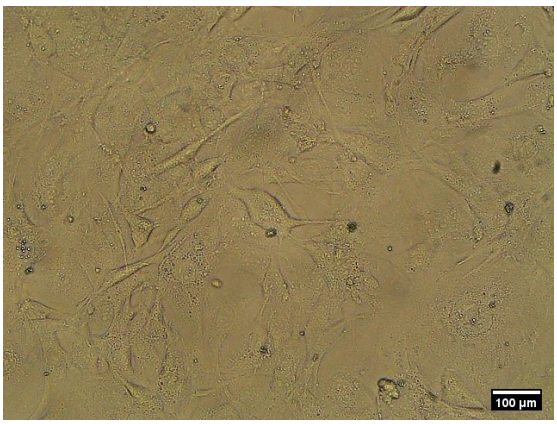
OA-50 ug (1)



OA-50 ug (2)



OA-100 ug (1)



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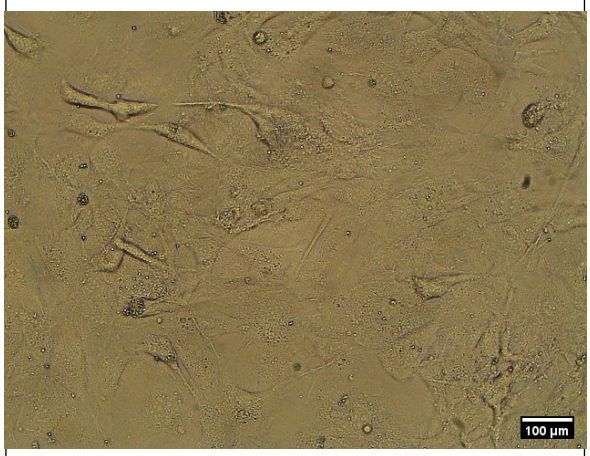
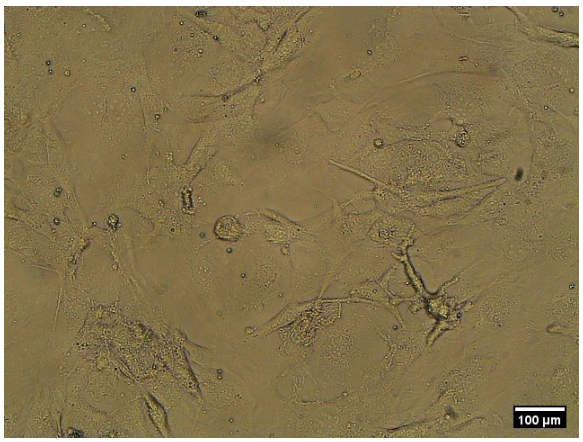


OA-200 ug (1)



OA-200 ug (2)

Figure 1: Photomicrographs of morphology of 3T3-L1 Cell lines.

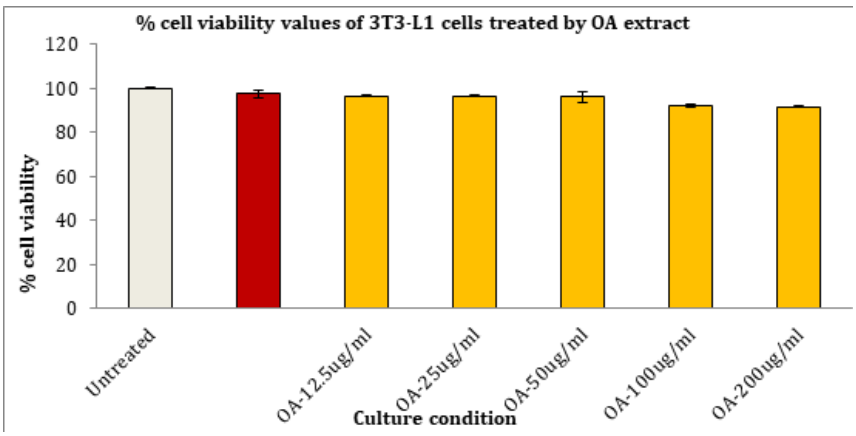


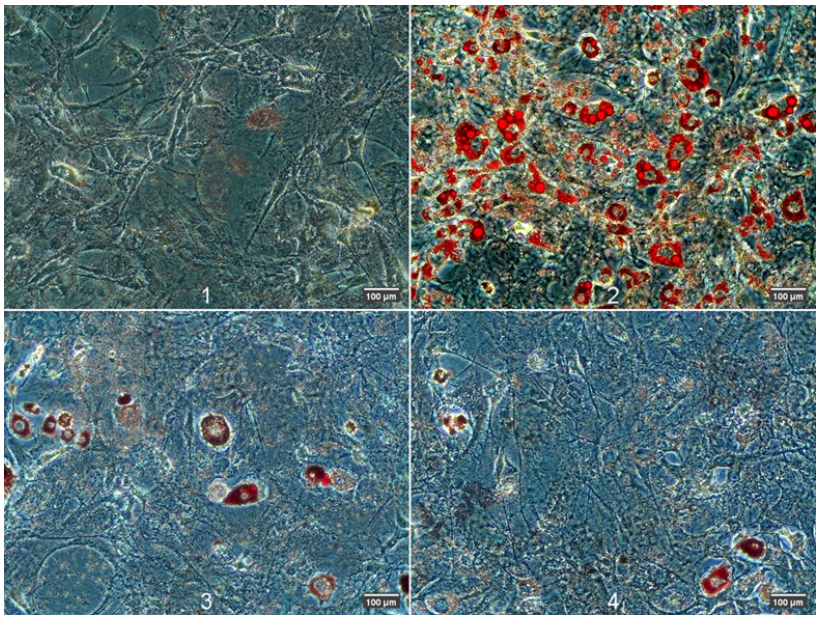
Figure 2: Overlaid Bar graph showed the % cell viability values of 3T3-L1 cell lines treated with different concentrations of OA extract by MTT study.

Further studies like, Lipolysis, Triglyceride estimation assay, Glucose Uptake study by Flow Cytometry using the 2-NBDG staining, Oil Red O Staining to measure the content of lipids or Triglycerides in MDI induced model, Bio assays like DPP-IV inhibition study etc need to be performed to confirm the molecular mode of action behind the anti-obesity effect of OA extract in mouse adipocytes

Oil Red O Staining

Lipid Accumulation: Untreated cells showed 100% lipid accumulation. MDI alone increased lipid accumulation by 70.39%. MDI + Metformin (100 µM) reduced lipid accumulation to 25.93%. MDI + OA (200 µg/ml) reduced lipid accumulation to 12.73%.

Quantitative analysis of Oil red O-stained lipids in Metformin with 100uM/ml and OA extract with 200ug/ml treated 3T3-L1 adipocytes (9, 10) showed that OA extract significantly reduced lipid accumulation to approximately 7 fold as compared to MDI alone induced cells whereas Metformin showed the 3 fold inhibition of lipid accumulation in cells in the same conditions (Figures 3 & 4).



Source: All the photos captured under Phase contrast inverted biological microscope at the 20 x magnification. Legend: 1-Untreated cells, 2-MDI alone, 3-MDI+Metformin with 100uM and 4-MDI+OA extract with 200ug/ml.

Figure 3: Overlaid montage photo represented the Oil Red O Staining for assessing the differentiation of 3T3-L1 adipocytes as well as Quantification of intracellular lipid accumulation in different culture condition after 10days.

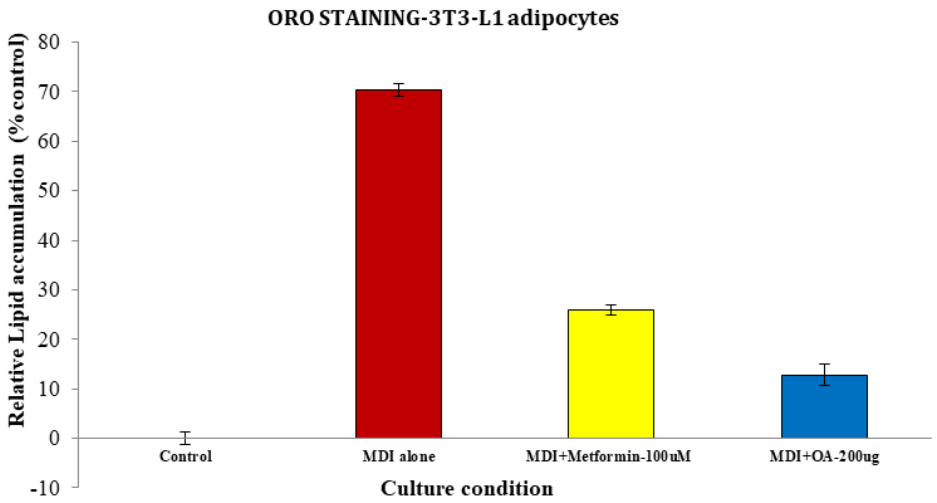


Figure 4: Quantification of intracellular lipid accumulation in MDI alone and Co treatment of MDI followed by either Metformin or OA extract treated cells after the treatment period of 10days.

Preliminary phytochemical examination of ethanolic extract of inflorescence of *Ocimum americanum* L

Preliminary phytochemical analysis by standard methods indicates the presence of Alkaloids, Carbohydrates, Glycosides, Tannins, Terpenoids, Steroids, Sterols & Triterpenoids, Flavonoids, Saponins, Proteins, and Phenols (Table 1).

S. No.	Type of Phytochemical	Alcoholic Extract of OA
1.	Alkaloids	+
2.	Carbohydrates	+
3.	Glycosides	+
4.	Tannins	+
5.	Terpenoids	+
6.	Resins	-
7.	Steroids	+
8.	Sterols & Triterpenoids	+
9.	Flavonoids	+
10.	Saponins	+
11.	Proteins	+
12.	Phenols	+

Source: '+' indicates Present, '-' indicates Absent

Table 1: Results of Preliminary Phytochemical Analysis of Ethanolic Extract of Inflorescence of *Ocimum americanum* (OA).

Discussion

In-vitro Anti-adipogenic Activity Screening Using 3T3-L1 Mouse Embryo Pre-adipocyte Cell Lines

The anti-adipogenic potential of various compounds was investigated through an in-vitro study using 3T3-L1 mouse embryo pre-adipocyte cell lines. The MTT assay was employed to assess cytotoxicity and cell viability following treatment with different concentrations of a methanolic extract of *Ocimum americanum* (OA). The assay demonstrated that OA extract exhibited significant cell viability, maintaining over 90% viability even at the highest concentration tested (200µg/ml). Metformin, used as a standard control at 100 µM, displayed a comparable viability rate of 97.3%.

The MTT assay results suggest that OA extract has a substantial anti-adipogenic effect, indicated by its ability to maintain high cell viability while presumably inhibiting adipocyte differentiation. The results indicate that the OA extract at 200µg/ml is an optimal concentration for further detailed studies due to its significant cell viability maintenance. These findings highlight the potential of OA extract as a promising agent for anti-adipogenic applications, which warrants further investigation into its molecular mechanisms and long-term effects on adipogenesis.

In-vitro Anti-obesity Activity Screening Using 3T3-L1 Mouse Embryo Pre-adipocyte Cell Lines

The anti-obesity potential of OA extract was further examined using the Oil Red O staining technique to quantify intracellular lipid accumulation in differentiated 3T3-L1 adipocytes. The study compared the lipid accumulation

in cells treated with OA extract, Metformin, and a disease control group treated with MDI (Isobutyl methyl xanthine, Dexamethasone, and Insulin).

The quantitative analysis revealed a significant reduction in lipid accumulation in cells treated with OA extract. Specifically, OA extract at 200 µg/ml reduced lipid accumulation by approximately 7-fold compared to the MDI alone group. Metformin, used as a positive control, showed a 3-fold reduction in lipid accumulation under the same conditions. This substantial decrease in lipid content underscores the potent anti-obesity effects of OA extract.

The findings from the Oil Red O staining study support the anti-adipogenic results from the MTT assay, demonstrating that OA extract not only maintains cell viability but also significantly inhibits lipid accumulation in adipocytes. This dual action of OA extract—preserving cell health while reducing adipogenesis—highlights its potential as an effective anti-obesity agent. Metformin, a widely used anti-diabetic drug with known weight-reduction benefits, was chosen as the standard for comparison due to its well-established mechanism in reducing lipid accumulation and improving insulin sensitivity. Metformin acts by activating AMP-activated protein kinase (AMPK), which regulates energy homeostasis, inhibits adipogenesis, and enhances glucose uptake. By comparing OA extract to Metformin, the study effectively validated OA's potential in targeting obesity-related pathways while offering a natural alternative with possibly fewer side effects. MDI, comprising isobutylmethylxanthine (IBMX), dexamethasone, and insulin, is specifically designed to promote differentiation of pre-adipocytes into mature adipocytes by mimicking physiological signals. IBMX elevates intracellular cyclic AMP (cAMP) levels, triggering adipogenic transcription factors. Dexamethasone, a glucocorticoid, enhances the expression of adipogenic genes, while insulin activates the insulin signaling pathway to facilitate glucose uptake and lipid storage. This optimized combination ensures robust and reproducible differentiation, providing a controlled baseline to assess the inhibitory effects of OA extract on adipogenesis and lipid accumulation.

The Presence of Various Secondary Metabolites like Alkaloids, Carbohydrates, Glycosides

Tannins, Terpenoids, Steroids, Sterols & Triterpenoids, Flavonoids, Saponins, Proteins and Phenols (Table 1) may be the responsible for the observed activity. These findings indicate that OA extract has potential as an anti-obesity agent by inhibiting lipid accumulation in adipocytes.

Conclusion

Ocimum americanum extract has shown considerable potential in inhibiting adipogenesis and reducing lipid accumulation, highlighting its promise as a natural anti-obesity agent. Further research is essential to confirm these findings and explore the extract's therapeutic applications.

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