



Research Article

**HARNESSING THE THERAPEUTIC POTENTIAL OF CHORAKA (*ANGELICA GLAUCA* EDGEW.):
A HERBAL ALTERNATIVE FOR OBESITY IN AN IN VITRO MODEL**

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ABSTRACT

Aim- The study aims to evaluate the hypolipidemic and antioxidant potential of *Angelica glauca* Edgew. (*Choraka*) root and leaf extracts using an in vitro model with Caco-2 cells, a human intestinal epithelial cell line. **Objective-** To assess the ability of *A. glauca* extracts to reduce lipid accumulation, triglyceride content, and oxidative stress in Caco-2 cells, providing insights into their potential as natural therapeutic agents for metabolic disorders such as obesity and hyperlipidaemia. **Methods-** Hydroalcoholic extracts of *A. glauca* roots and leaves were prepared and tested for cytotoxicity using the MTT assay, establishing safe concentrations of 10 and 25µg/mL. Lipotoxicity and oxidative stress were induced in Caco-2 cells, and reactive oxygen species (ROS) levels were quantified using the DCFH-DA assay. Lipid accumulation and triglyceride content were also measured to determine the metabolic benefits of these extracts. **Results-** The root extracts demonstrated superior lipid-lowering effects, significantly reducing lipid accumulation and triglyceride content in treated cells. Meanwhile, leaf extracts exhibited stronger antioxidant properties, markedly decreasing ROS levels. Both extracts showed promising metabolic benefits, reinforcing their traditional use in Ayurvedic medicine. **Conclusion-** The study highlights *A. glauca*'s potential as a complementary therapeutic agent for metabolic disorders by effectively modulating lipid metabolism and oxidative stress. Root extracts may be more effective in lipid regulation, hence in obesity while leaf extracts show stronger antioxidant activity. These findings support further in vivo and clinical investigations to validate its efficacy and safety in human applications.

INTRODUCTION

Metabolic health conditions are prevalent all around the globe in recent time because of unwholesome lifestyle habits. Hyperlipidemia and oxidative stress are major contributors of metabolic diseases, including obesity and diabetes. Hyperlipidemia is common around the world affecting both developed and developing countries. The World Obesity Federation's 2025 Atlas, published on World Obesity Day, (4 March), projects that the total number of adults living with obesity will increase by more than

115% between 2010 and 2030, from 524 million to 1.13 billion. In this disease, blood level of lipids is elevated more than normal range. Elevation in the lipids viz cholesterol, triglycerides predispose the patient to develop further cardiovascular, hepatic and renal complications.^[1]

ROS, or reactive oxygen species, are byproduct of regular cellular metabolism. According to the definition of oxidative stress, which is "an imbalance between oxidants and antioxidants in favour of the oxidants," redox signalling and control are disrupted, and it results in molecular damage. ROS are a class of highly reactive, unstable chemicals that are produced by organisms from oxygen. ROS include hydroxyl radicals (OH•), hydroxyl ions (OH-), singlet oxygen (1O₂), hydrogen peroxide (H₂O₂), and superoxide anion (O₂•-).^[2] These conditions often result from an imbalance in lipid metabolism and management of

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reactive oxygen species (ROS), which damage cellular components. Conventional therapies, such as statins and antioxidants, often present limitations including adverse side effects and limited efficacy in specific populations. Consequently, this has led to a growing interest in natural products as potential therapeutic agents. Among these, medicinal plants have gained significant attention due to their diverse bioactive compounds, which exhibit various pharmacological properties, including lipid-lowering and antioxidant effects.

Ayurveda, being the ancient system of medicine, uses natural products as therapy tools. Plants are the main source of therapeutic agents for various ailments. One such plant is *Choraka*, (*Angelica glauca* Edgew.) mentioned in Ayurveda for treating various conditions. *Choraka* (*Angelica glauca* Edgew.) has been mentioned in Ayurveda in various texts including *Charaka Samhita*, *Sushruta Samhita*, *Madanpala Nighantu*, *Raj-Nighantu*, *Bhavaprakasha Nighantu* and so on. This ancient herb *Choraka* is identified as *Angelica glauca* Edgew. as per Ayurvedic Pharmacopoeia of India (API).^[3] The references in *Kaideva Nighantu*.^[4] and *Bhavaprakasha Nighantu*.^[5] has attributed many properties including *Medohara* (lowering excessive *Meda dhatu*) property to *Choraka* which served as a basis for the evaluation of hypolipidemic and further antioxidant caliber of *A. glauca* Edgew.

Angelica glauca Edgew., is a perennial herb from Apiaceae family. It is endemic to the Himalayan region where it is used by ethnic communities for several culinary and medicinal purposes including anti-inflammatory, anti-microbial, and digestive benefits.^[6] Recent studies have highlighted the potential of *Angelica glauca* Edgew. in the management of metabolic disorders, particularly in reducing lipid levels and combating oxidative stress.^[7] The native range of this species is E. Afghanistan to W. Tibet and W. Himalaya where it grows primarily in the temperate biome.^[8] In local dialects, this plant is known by the names *Chora*, *Chura*, *Gandhrayan* etc.

This study evaluates the lipid-lowering and antioxidant effects of *A. glauca* root and leaf extracts using Caco-2 cells, a human intestinal epithelial cell line. Caco-2 cells mimicks^[9,10,11,12,13] the human intestine cells and known for their enterocyte-like properties, provide an excellent model to simulate the intestinal absorption and metabolism of dietary compounds. By testing various compounds in Caco-2 model, researchers can identify those that are well absorbed and/or those that have a positive impact on lipid metabolism, suggesting potential anti-obesity activity.^[14-20]

MATERIALS AND METHODS

The plant was harvested from its native habitat in the temperate zone of the Himalayan region, at an elevation of 2000–4000 meters. Additional verification was carried out at Dravyaguna Department of R.G.G.P.G. Ayurvedic College, Paprola, and CSIR-IHBT, Palampur. The plant was subjected to *in vitro* experiments to explore its hypolipidemic and antioxidant potential. Caco-2 cell line was used for the purpose where extracts of leaves and roots were used for the experiments performed in the nutrition and dietetics division, CSIR-IHBT, Palampur.

Basic equipment used for the experiments were Cell culture hood (Biosafety cabinet) for sterile handling of cells cultures, Incubator (Galaxy 170S from New Brunswick), cell culture vessels (T-25, T-75 flasks, 6-well, 24-well, 48-well and 96-well plates), Microscope, pipette and pipette tips, centrifuge, refrigerator and freezer. Incubator Fluorescent Cell Imager (Bio-Rad), Microplate reader (Synergy H1 from BioTek).

Culture media and reagents- DMEM (Dulbecco's Modified Eagle Medium (Sigma-Aldrich), Bovine Serum Albumin (Sigma-Aldrich), DMSO (Dimethyl sulphoxide), PBS (Phosphate buffered saline), Trypsin-EDTA from Himedia were used frequently. For viability assays MTT (Thiazolyl blue tetrazolium bromide from Himedia), for ROS assay DCFH-DA (2,7-Dichlorofluorescein diacetate from Sigma-Aldrich) and for lipid assays Nile Red stain and Triglycerides des reagent from Transasia Bio-medicals Ltd. were used.

Preparation of Plant Extracts

The roots and leaves of *A. glauca* were shade-dried and powdered. Hydroalcoholic extracts were prepared by dissolving 25gm of each powder in a solvent mixture (200mL ethanol + 200mL distilled water) and incubating for 16 hours at 37°C in a shaker. The extracts were filtered, evaporated using a rotary vacuum evaporator (Equitron Roteva), and freeze-dried in a lyophiliser (Labconco) to obtain powdered form for experiments.

Cell Culture

The cell line chosen for experiment was Caco-2 cell line. Caco-2 cell line is an immortalized line of human colorectal adenocarcinoma cells. It was developed by Jorgen Fogh in 1977 at the Sloan-Kettering Institute. The name Caco-2 comes from 'Cancer coli' which means colon cancer. These cells spontaneously differentiate in culture to form a monolayer that exhibits many characteristics of enterocytes, the absorptive cells lining the small intestine, including the formation of tight junctions, microvilli, and the expression of enzymes and transporters typical of the intestinal epithelium.^[21] The Caco-2 human intestinal cell line has been widely

adopted to mimic the *in vivo* small intestinal epithelium for screening the characteristics and mechanisms of the uptake, metabolism and absorption of dietary compounds and drugs.^[22]

Thawing and Initial Culturing

A vial of frozen Caco-2 cells was retrieved from liquid nitrogen storage and quickly thawed in a 37°C water bath. The cell suspension was transferred into a 15mL centrifuge tube containing 10mL of pre-warmed DMEM supplemented with 10% FBS. The tube was centrifuged at 1000 rpm for 5 minutes to pellet the cells. After centrifugation, the supernatant was carefully aspirated, and the cell pellet was resuspended in 10ml of fresh culture medium. Caco-2 cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum (FBS), 1% penicillin-streptomycin, and 1% non-essential amino acids. The resuspended cells were transferred into a T-25 flask and placed in a 37°C incubator with 5% CO₂ and 95% humidity. Cells were sub-cultured upon reaching 70–80% confluence.

Subculturing Caco-2 Cells

When the cells reached approximately 70-80% confluence, the old medium was aspirated, and the cells were washed with 5mL of sterile PBS to remove residual serum. 2mL of trypsin-EDTA solution (0.25%) was added to the flask and incubated at 37°C for about 5 minutes until the cells detached. Once the cells detached, 8mL of fresh culture medium was added to neutralize the trypsin, and the solution was gently pipetted to create a single-cell suspension. The cell suspension was transferred to a 15mL centrifuge tube and centrifuged at 1000 rpm for 5 minutes. After centrifugation, the supernatant was aspirated, and the cell pellet was resuspended in 10 mL of fresh culture medium. The cells were split at a ratio of 1:3 by transferring 3mL of the cell suspension into a new T-25 flask, which was then filled with 7mL of fresh culture medium.

Seeding Cells into Microplates

Cells were cultured until they reached the desired confluence. The old medium was aspirated, and the cells were washed with sterile PBS. Trypsin-EDTA solution was added to detach the cells, and after incubation, fresh medium was added to neutralize the trypsin. The cell suspension was centrifuged, and the cell pellet was resuspended in fresh medium. Cell concentration was determined using a hemocytometer. Cells were seeded into microplates (e.g., 24-well, 48-well, 96-well) at the required density. The microplates were incubated at 37°C with 5% CO₂ and 95% humidity until the cells adhered and reached the desired confluence.

Differentiation and Maintenance

After seeding, cells were allowed to reach confluence in the microplates. Once confluence was achieved, the cells were maintained for 2-3 weeks to allow for differentiation into enterocyte-like cells. Medium was changed every 2-3 days to maintain cell health.

Cryopreservation

For long-term storage, cells were harvested at 70-80% confluence. Cells were detached using trypsin-EDTA, neutralized with fresh medium, and centrifuged. The cell pellet was resuspended in freezing medium (DMEM with 10% DMSO and 20% FBS). Cells were aliquoted into cryovials and gradually frozen at -80°C before transferring to liquid nitrogen storage.

Cytotoxicity Assay

The cytotoxicity of the extracts was assessed using the MTT assay. This assay was developed in 1983 by Mosmann et al. The MTT assay is done to assess the cellular viability, metabolic activity and treatment toxicity. Caco-2 cells were seeded into 96-well plates (10,000 cells/well) and treated with varying concentrations of root and leaf extracts (5–400µg/mL) for 24, 48, and 72 hours. Following treatment, 20µL of MTT solution (5mg/mL) was added to each well, and formazan crystals formed were dissolved in DMSO. The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) is a mono-tetrazolium salt which is yellowish in color. It consists of a positively charged quaternary tetrazole ring core which contains four nitrogen atoms surrounded by three aromatic rings including two phenyl moieties and one thiazolyl ring. MTT is reduced to violet coloured water insoluble formazan due to disruption of the core tetrazole ring.^[23] Absorbance was measured at 570nm. Concentrations with about 100% cell viability were considered safe for further experiments which were found 10 and 25mg/ml for both root and leaf of *Angelica glauca* Edgew. for Caco-2.

Likewise, the concentration of sodium palmitate that simultaneously caused oxidative stress along with lipid buildup in the cells was examined using MTT assay. Concentration with 70% viability i.e., 200µM was chosen for further experiments as it imparted both lipid accumulation and oxidative stress in cells.

Induction of Lipotoxicity and Oxidative Stress

Sodium palmitate (200µM) was used to induce lipotoxicity and oxidative stress in Caco-2 cells. This concentration was selected based on preliminary cytotoxicity assays, ensuring a balance between significant stress induction and cellular viability.

Lipid-Lowering Effect

Lipid accumulation was assessed using Nile Red staining and a triglyceride DES reagent assay. Cells were treated with safe concentrations (10 and 25 µg/mL) of root and leaf extracts for 6 hours, followed by sodium palmitate treatment for 16 hours. Stained cells were visualized under a fluorescence microscope (Zoe Fluorescent Cell Imager from Bio-Rad), and triglyceride content was assessed via a colorimetric assay using Synergy H1 Microplate Reader from BioTek at 505 nm.

Antioxidant Effect

ROS production was assessed using the DCFH-DA assay. Cells were pretreated with extracts for 6 hours, followed by sodium palmitate treatment for 16 hours. Post-treatment, cells were incubated with DCFH-DA (20 µM) for 30 minutes, and fluorescence intensity was measured using Synergy H1 Microplate reader from BioTek at excitation/emission: 488/535 nm.

RESULTS

In Ayurvedic literature, *Angelica glauca* Edgew. (*Choraka*) is classified under *Deepana*, *Pachana*, and *Vatanulomana* herbs. It possesses *Tikta* and *Katu rasa*, *Laghu* and *Ruksha guna*, *Ushna virya*, and *Katu vipaka*, contributing to its *Sangyasthapana*, *Kapha-Medohara*

and *Agnideepana* actions. These attributes align with its traditional use in managing *Sthaulya* (obesity) and *Prameha* (metabolic disorders)^[23], which was further evaluated in -vitro on scientific parameters as follows-

Cytotoxicity Assessment

Both extracts exhibited dose- and time-dependent cytotoxicity. Root and leaf extracts were deemed safe at concentrations of 10 and 25 µg/mL, maintaining ≥90% cell viability after 24 hours. (Figure 1), (Figure 2) and (Figure 3)

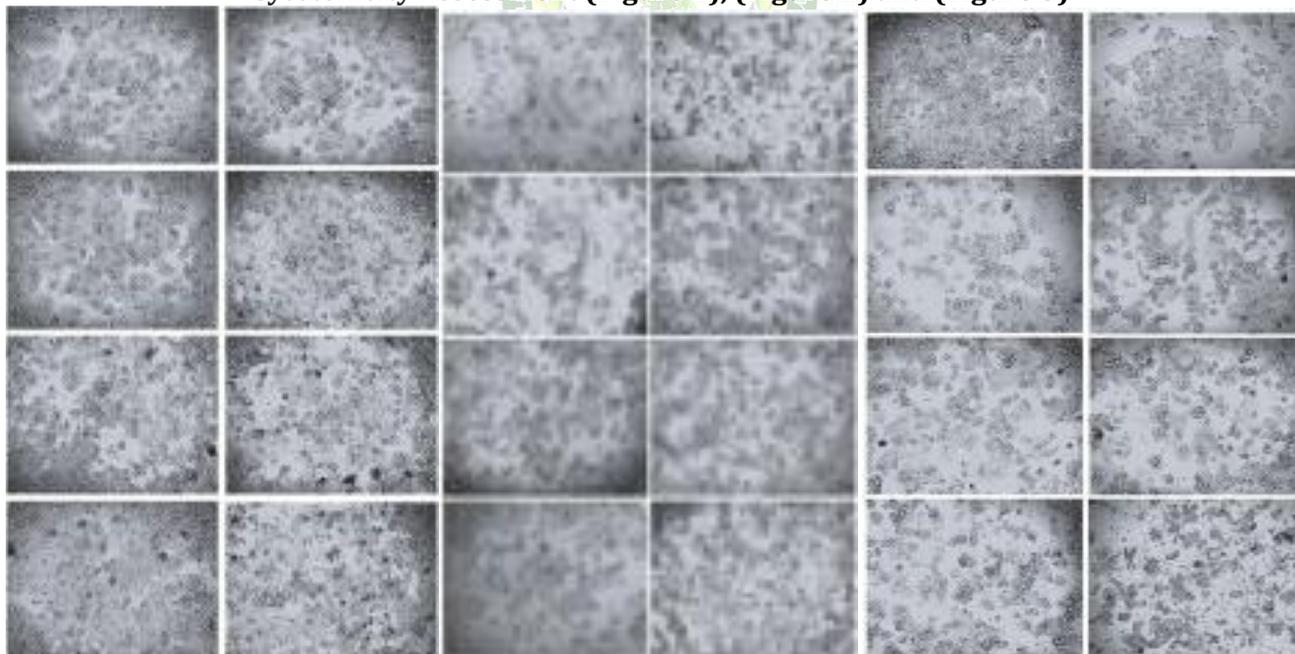
Lipid-Lowering Effect

Nile Red staining revealed significant reductions in lipid droplet accumulation in sodium palmitate-treated cells pretreated with *A. glauca* extracts. The triglyceride assay further confirmed these findings, with root extracts showing greater efficacy in reducing triglyceride content compared to leaf extracts. (Figure 4)

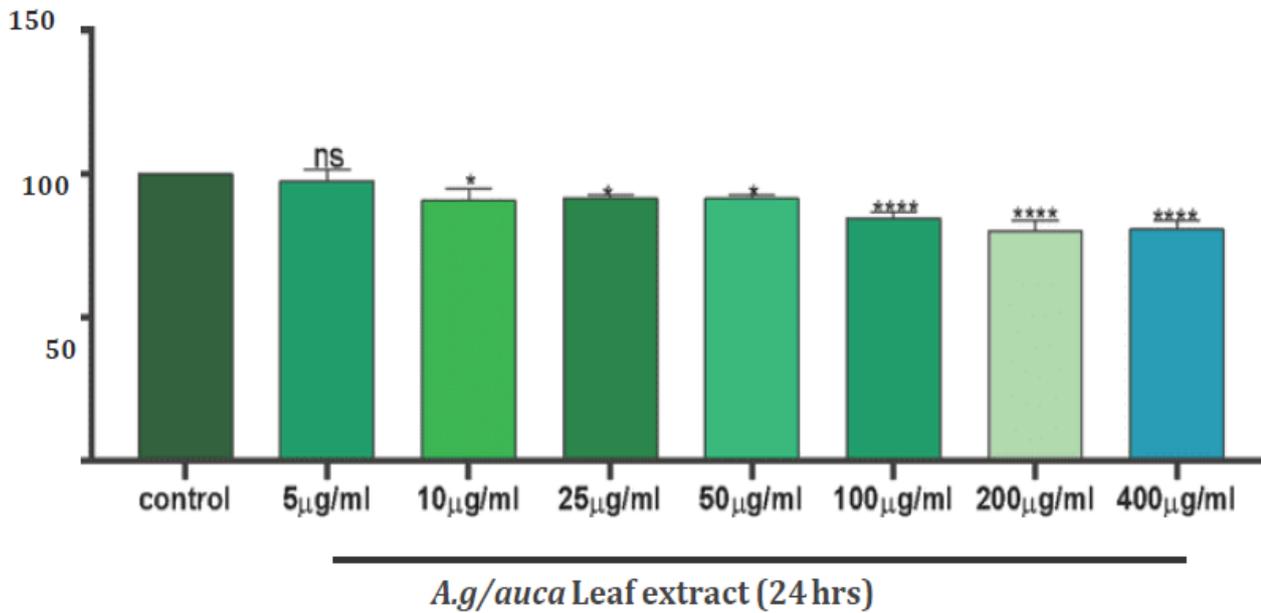
Antioxidant Effect

Extract pre-treatment significantly reduced ROS levels in sodium palmitate-treated cells, as indicated by lower fluorescence intensity. Leaf extracts demonstrated stronger antioxidant effects, with the 25 µg/mL concentration producing the most significant reduction. (Figure 5)

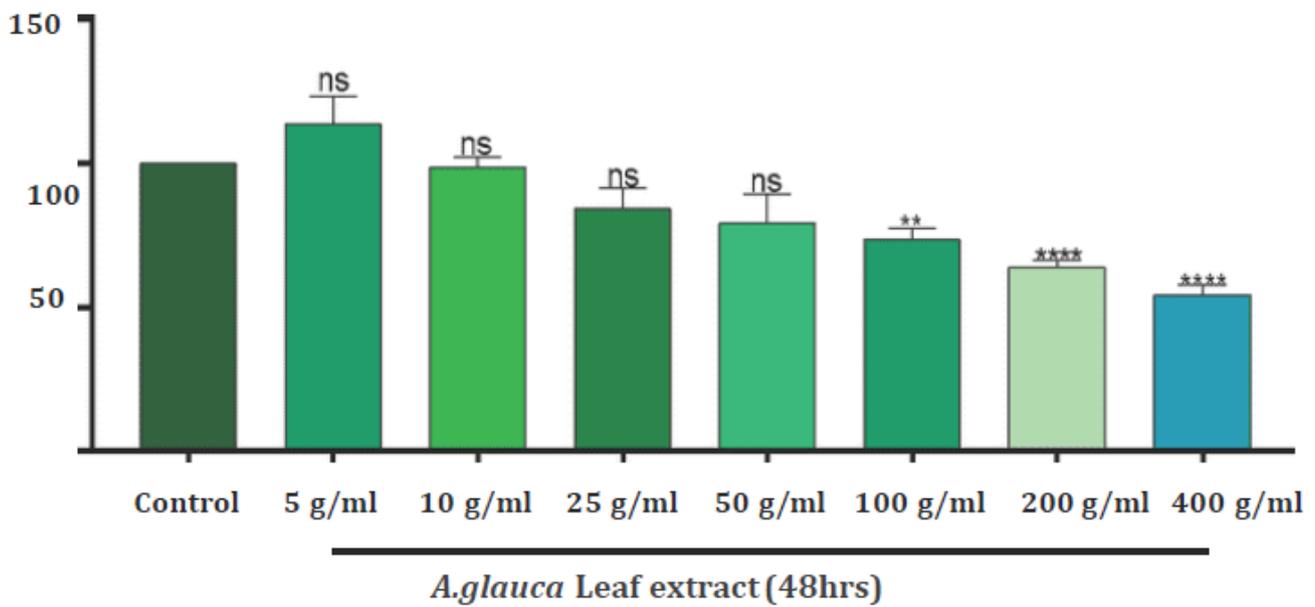
Cytotoxicity Assessment (Figure 1), (Figure 2) and (Figure 3)



Caco - 2



Caco- 2



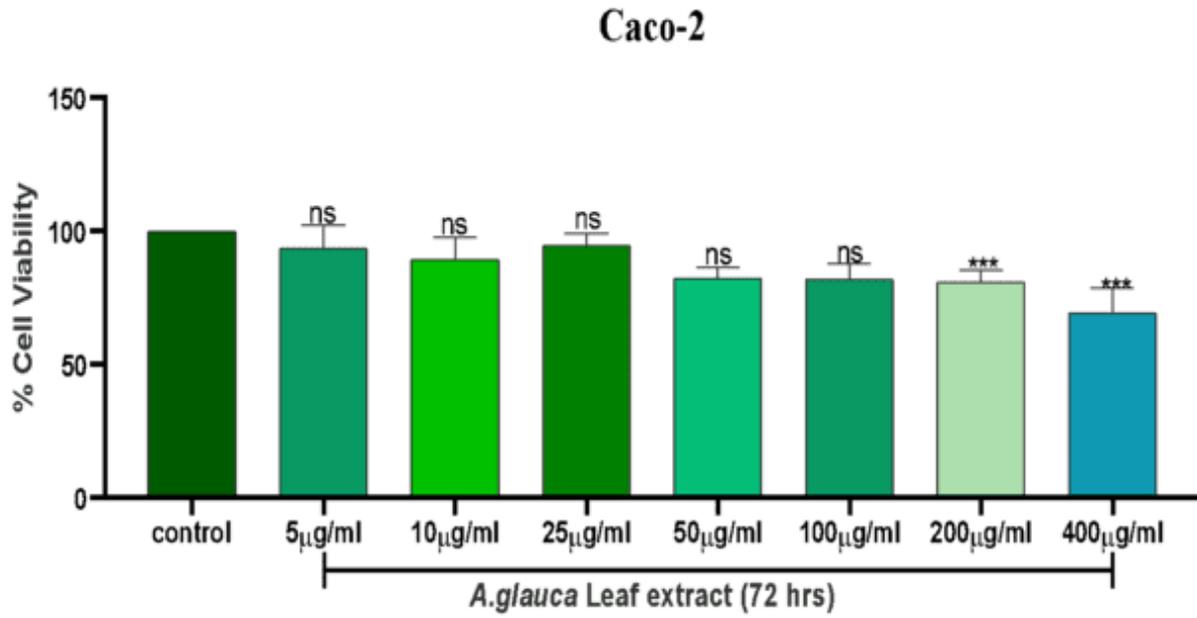
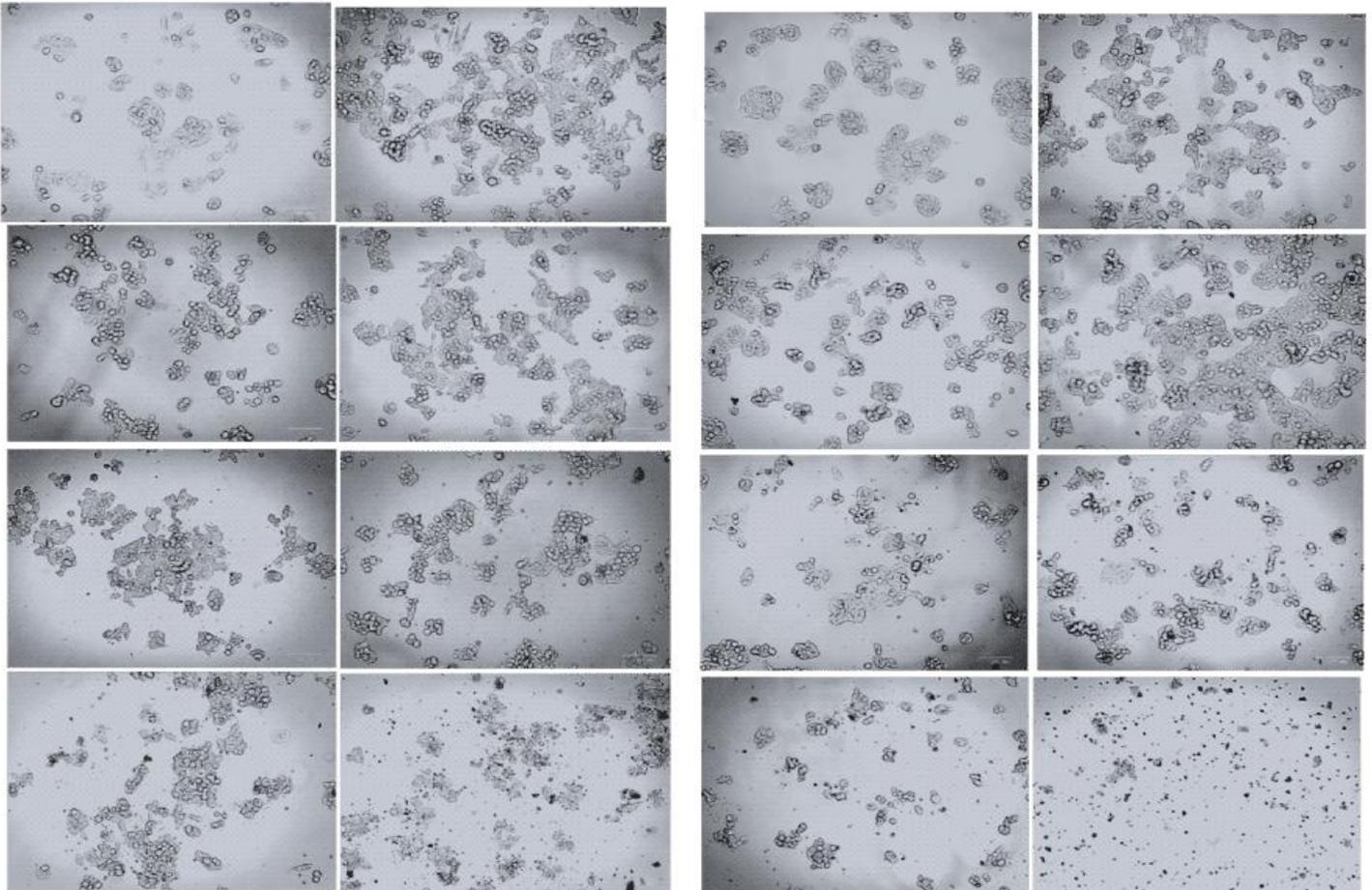
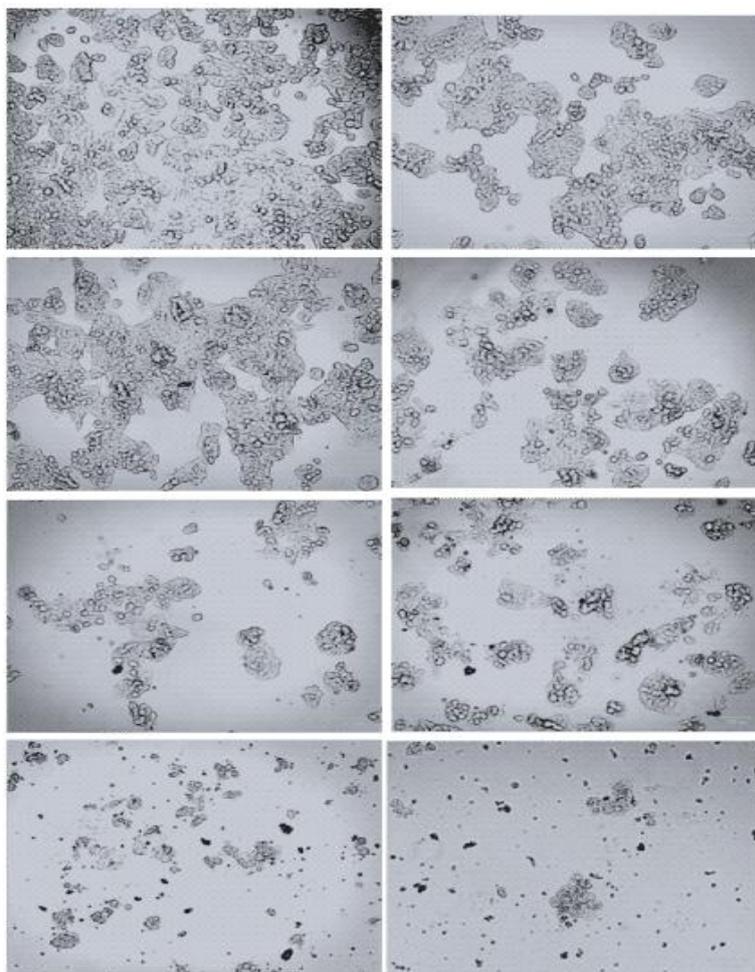
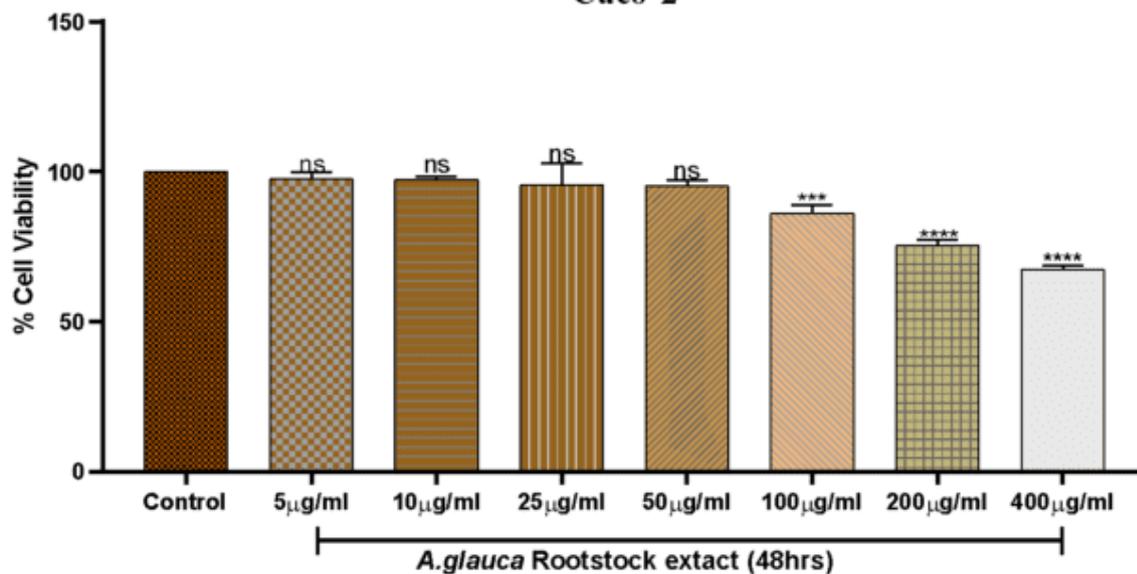


Figure 1: Cellular viability using Angelica glauca leaf extracts





Caco-2



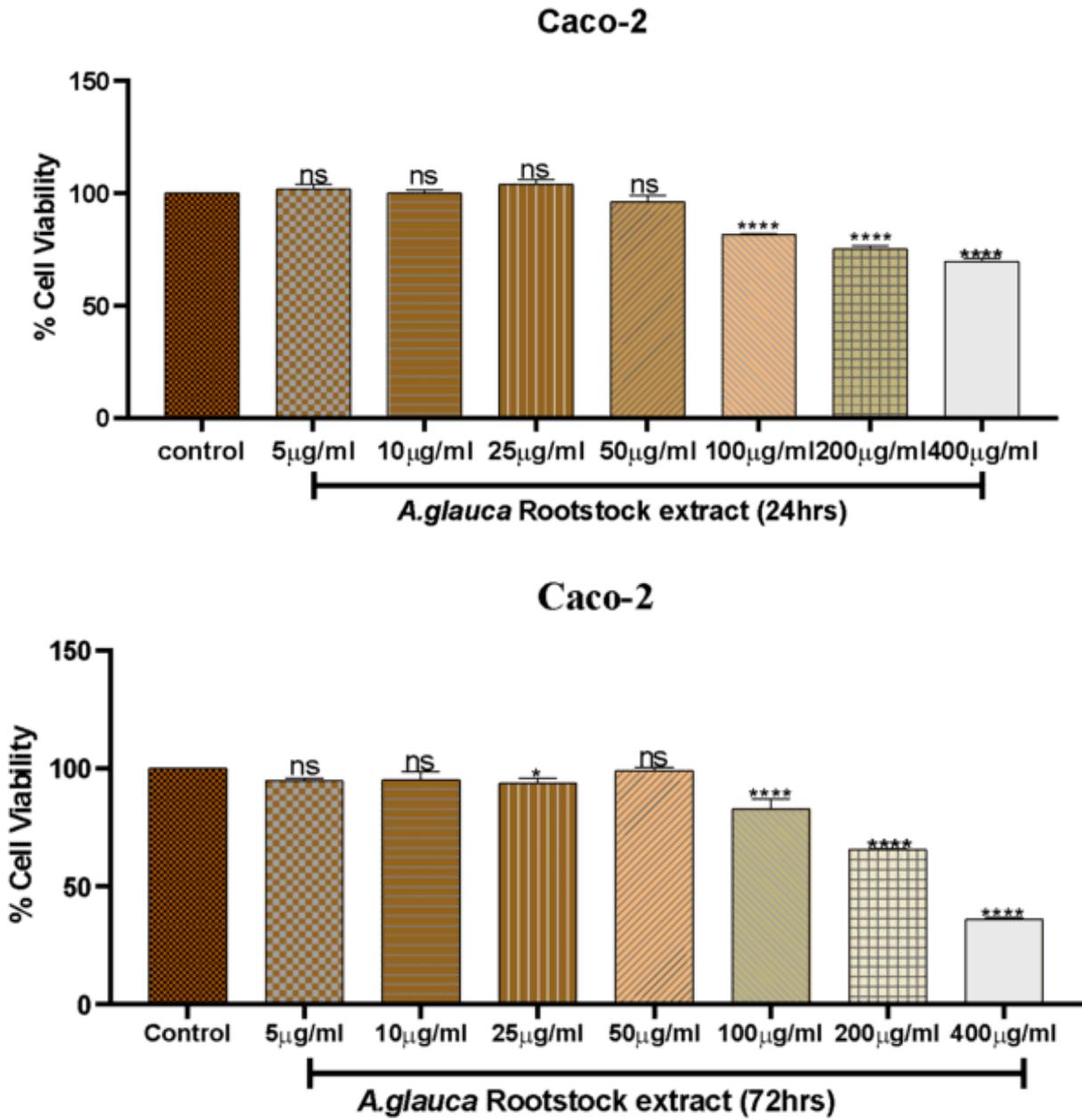


Figure 2: Cellular viability using Angelica glauca root extracts

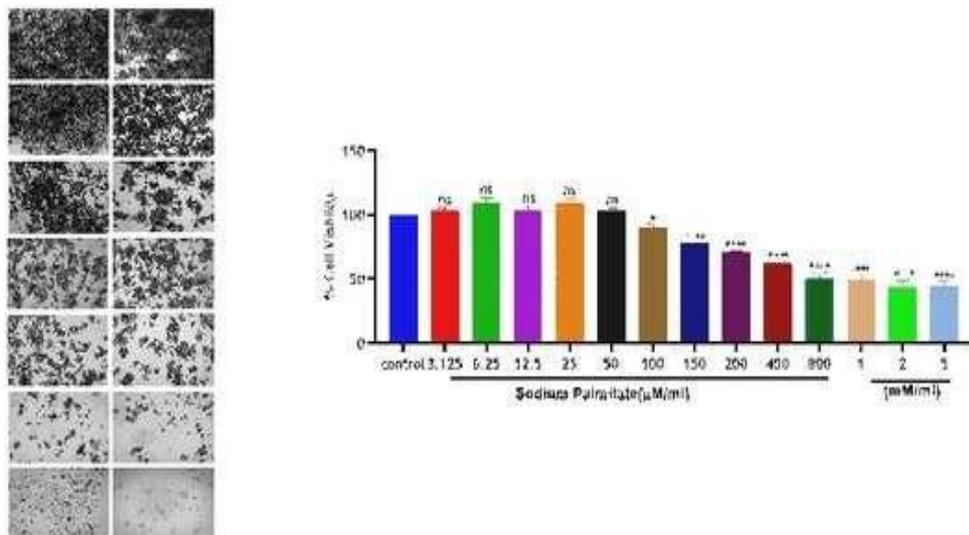


Figure 3: Cellular viability using Sodium palmitate

Lipid-Lowering Effect-Figure-4

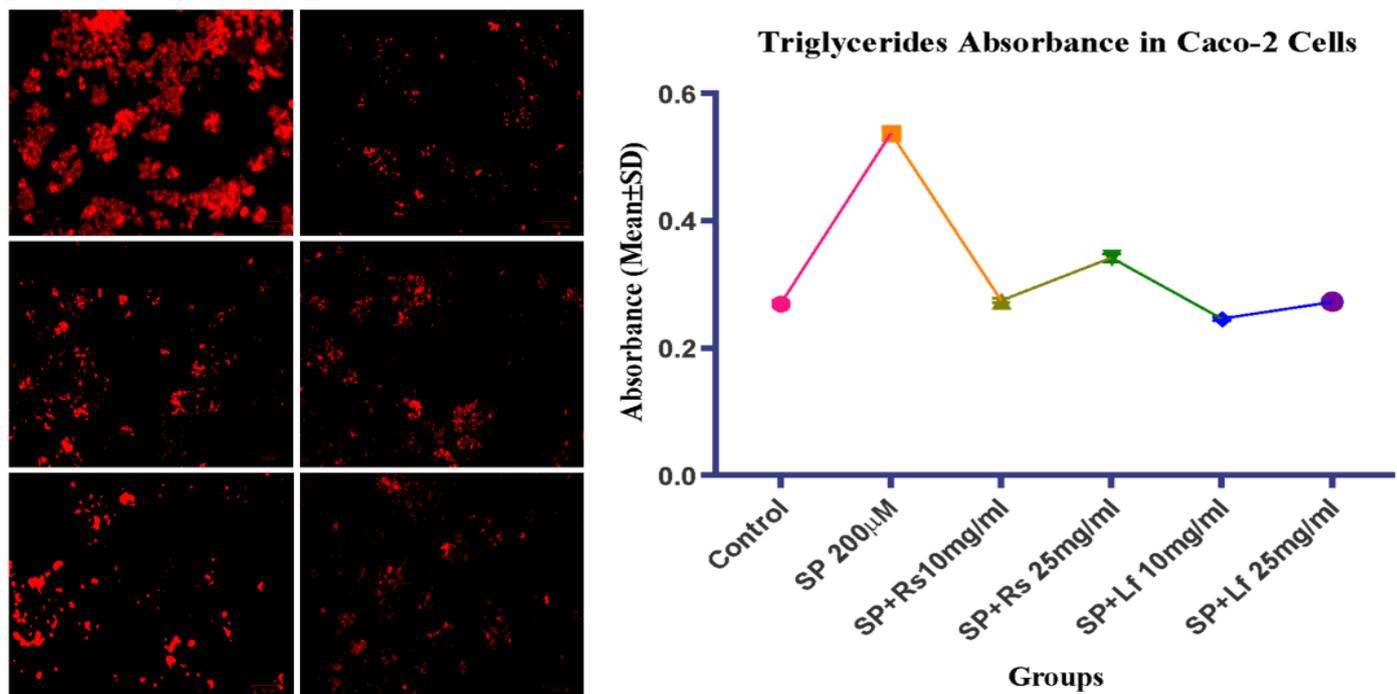
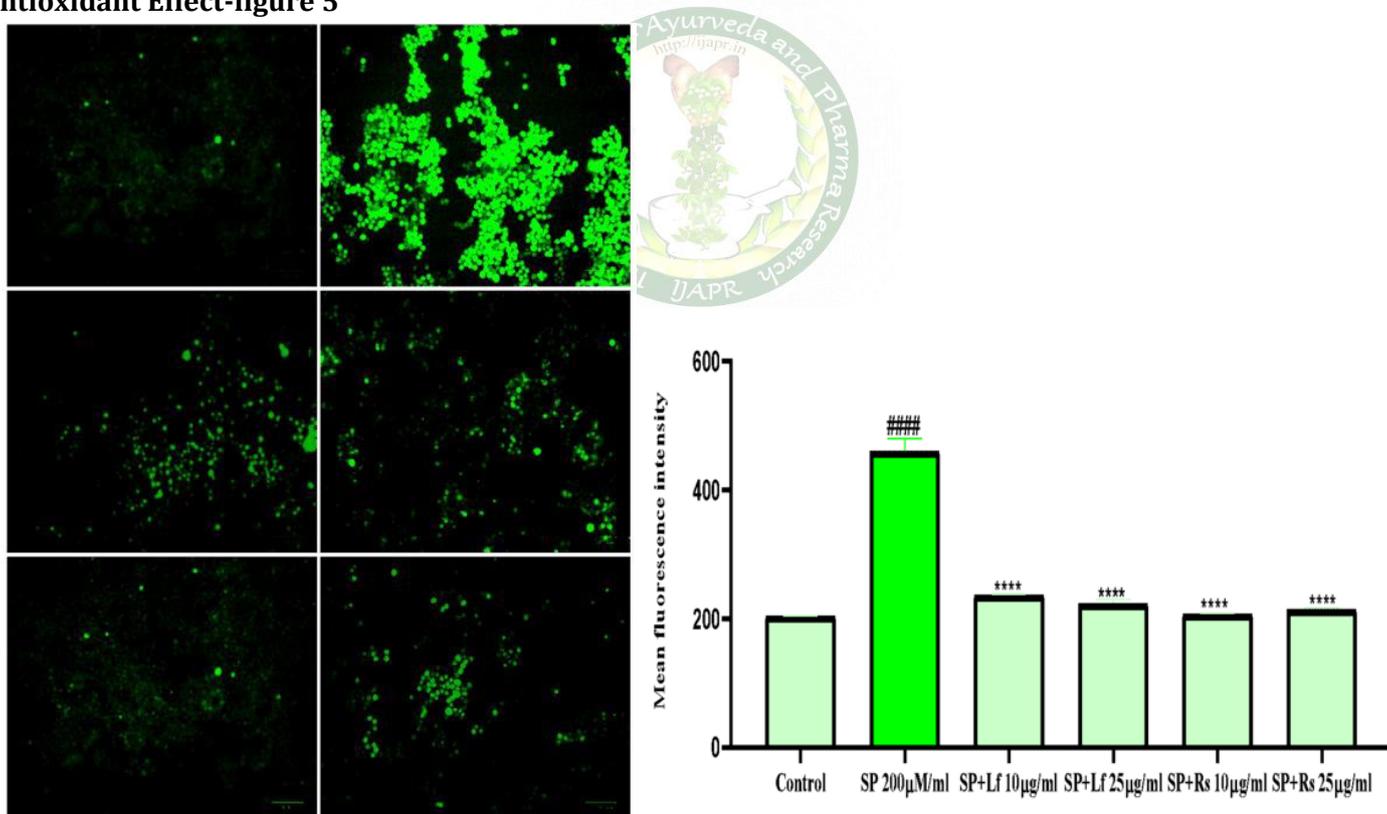


Figure 4: Nile red staining and triglycerides assay interpretation in different groups

Antioxidant Effect-figure 5



SP- Sodium Palmitate, Rs- Rootstock extract, Lf- Leaf extract

Figure 5: Depicting Antioxidant effect

DISCUSSION

From the Ayurvedic perspective, *Angelica glauca* Edgew., traditionally known as “*Choraka*” or “*Choura*”, is categorized under *Deepana*, *Pachana*, and *Vatanulomana dravyas*- denoting its ability to enhance digestion, correct impaired *Agni* (digestive/metabolic fire), and regulate vitiated *Vata dosha*. In the context of *Sthaulya* (obesity) and lipid disorders, Ayurveda attributes the pathogenesis to vitiation of *Medo-dhatu* and impaired *Agni*, which result in improper assimilation and excessive accumulation of *Kapha* and *Meda*. The herb’s intrinsic *Tikta* (bitter) and *Katu* (pungent) *Rasa*, along with *Laghu* (light) and *Ruksha* (dry) *Gunas*, contribute to its *Kapha-Medohara* and *Shoshana* (fat-reducing) effects. These classical actions resonate with the observed outcomes in the current study, where *A. glauca* extracts demonstrated inhibition of lipid deposition and oxidative stress at the cellular level. Therefore, the present findings offer experimental validation for the classical Ayurvedic use of *Choraka* in managing *Medoroga*, a condition encompassing obesity and related metabolic imbalances.

From a modern scientific viewpoint, the present study demonstrates that *Angelica glauca* root and leaf extracts exhibit potent lipid-lowering and antioxidant properties in sodium palmitate-induced Caco-2 cells. This cell line serves as a physiologically relevant model for mimicking intestinal lipid absorption and oxidative stress associated with metabolic dysfunctions. The significant reduction in intracellular lipid accumulation, as evidenced by Nile Red staining and triglyceride quantification, highlights the hypolipidemic potential of the extracts, likely due to their rich content of polyphenols and flavonoids. Notably, the leaf extract showed a greater capacity to attenuate reactive oxygen species (ROS), indicating robust antioxidative potential, which is crucial in mitigating adipocyte dysfunction and low-grade chronic inflammation- both hallmarks of obesity.

Thus, this study bridges traditional Ayurvedic principles with modern molecular evidence, establishing *Angelica glauca* as a promising botanical agent for managing obesity, hyperlipidemia, and oxidative stress-related metabolic disorders. Further in vivo and clinical evaluations are warranted to explore its full therapeutic potential, synergistic efficacy, and safety profile, promoting its application in evidence-based integrative medicine

CONCLUSION

In classical Ayurvedic literature, *Angelica glauca* Edgew. (*Choraka*) is recognized for its potent therapeutic attributes. It possesses *Tikta* (bitter) and *Katu* (pungent) *Rasa*, *Laghu* (light) and *Ruksha* (dry) *Guna*, and *Ushna veerya* (hot potency), which contribute to its *Kapha-Vata shamak* effects. These

properties make it effective in stimulating *Agni* (digestive/metabolic fire), facilitating *Ama pachana* (detoxification), and cleansing *Srotas* (bodily channels). Traditionally, it has been employed in the management of *Medoroga* (lipid disorders), *Sthoulya* (obesity), and other *Santarpanoththa vikara* (diseases caused by excessive nutrition), due to its *Medohara* (fat-reducing) and *Shothahara* (anti-inflammatory) actions.

The current study supports these traditional applications by scientifically validating the anti-obesity, lipid-lowering, and antioxidant potential of *Angelica glauca* through an in vitro model using Caco-2 human intestinal epithelial cells. The observed reduction in intracellular lipid accumulation and ROS levels demonstrates its promise in combating metabolic dysfunctions related to obesity and oxidative stress.

Thus, this integrative research reinforces the value of *Angelica glauca* as a natural therapeutic agent rooted in Ayurvedic wisdom and confirmed by modern science. Further in vivo studies, clinical trials, and mechanistic analyses are warranted to establish its therapeutic efficacy, safety, and formulation potential for addressing metabolic disorders within the framework of Ayurveda-inspired evidence-based medicine.

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