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Research Article

Anti-Adipogenicity of Garcinia Indica Choisy. and Pterocarpus Marsupium Roxb. in 3T3-L1 cell line – A comparative study

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Abstract

Introduction: Obesity is a multifactorial metabolic disorder associated with several chronic diseases. Modulating adipocyte differentiation, particularly during the early phase of mitotic clonal expansion (MCE), is an important approach for limiting adipogenesis. *Garcinia indica Choisy. (Family: Clusiaceae)* and *Pterocarpus marsupium Roxb. (Family: Fabaceae)* are classical Ayurvedic plants described for their potential in managing *Medoroga* and related metabolic disorders. This study investigates and compares their anti-adipogenic effects using the 3T3-L1 preadipocyte model. Materials and Methods: Aqueous extracts of *Garcinia Indica Choisy and Pterocarpus Marsupium Roxb.* were prepared and assessed for cytotoxicity on Human Mesenchymal Stem Cells (hMSCs) to determine safe concentrations. Non-toxic doses were used to treat 3T3-L1 preadipocytes during differentiation. Lipid accumulation was quantified following induction. The influence of both extracts on MCE and related adipogenic signaling markers was evaluated to understand underlying mechanisms. Results: Cytotoxicity studies confirmed that both plant extracts were safe and non-toxic on hMSCs. Differentiation assays demonstrated a significant reduction in lipid accumulation in 3T3-L1 cells treated with either extract. *Garcinia Indica Choisy* produced a more substantial inhibition of adipogenesis, particularly by attenuating proliferation during the MCE phase. Molecular analyses supported these observations, revealing down regulation of adipogenic pathways in treated groups. Conclusion: Both *Garcinia Indica Choisy. and Pterocarpus Marsupium Roxb.* exhibit promising anti-adipogenic potential. *Garcinia Indica Roxb.* showed superior activity, likely due to its stronger regulatory effects on MCE and associated signaling mechanisms. These findings support the traditional *Ayurvedic* use of these plants and highlight *Garcinia Indica Roxb.* as a more potent natural candidate for obesity management.

Keywords: 3T3-L1 Cell line, *Garcinia Indica* Choisy., *Pterocarpus Marsupium* Roxb., Obesity

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Introduction

Obesity has emerged as one of the most pressing global health challenges of the 21st century, contributing significantly to the burden of chronic diseases such as type 2 diabetes, cardiovascular disorders, and metabolic syndrome. Characterised by excessive accumulation of adipose tissue, obesity results from a complex interplay of genetic, environmental, and lifestyle factors. Obesity management requires a multifaceted therapeutic approach targeting energy balance, adipogenesis, lipolysis, and metabolic regulation. In *Sthoulya* (obesity), aggravated *Kapha* and *Meda* lead to impaired *Agni* (digestive/metabolic fire) and the accumulation of excess fat tissue. *Ayurvedic* herbs acts by *Deepana* (enhancing digestion), *Lekhana* (scraping excess *Meda)*, and balancing *Vata* and *Kapha Doshas* to achieve *Samprapti Vighatana* (disruption of the disease pathway), thereby restoring normal metabolic function.

Among numerous *Ayurvedic* medicinal plants, *Vrukshamla* (*Garcinia Indica Choisy.)* and *Vijayasara* (*Pterocarpus Marsupium Roxb.*) are particularly noted for their traditional use in managing obesity and regulating lipid metabolism (3,4). Both *Vrukshamla* and *Vijayasara* are prominently cited in *Ayurvedic* texts and modern research for their anti-obesity potential, yet their mechanisms of action may differ significantly. *Vrukshamla* is known for its high hydroxycitric acid (HCA) content, which suppresses appetite and inhibits lipogenesis, whereas *Vijayasara* is

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traditionally used for its hypoglycemic, lipid-lowering, and anti-inflammatory effects. A comparative evaluation of these two botanicals provides insights into their unique and possibly synergistic roles in modulating adipogenesis.

The 3T3-L1 cell line, a well-established in vitro model for studying adipogenesis, provides a valuable platform to investigate the anti-adipogenic potential of herbal extracts. This study aims to compare the effects of *Vrukshamla* and *Vijayasara* on adipocyte differentiation and lipid accumulation in 3T3-L1 preadipocytes, thereby offering insights into their relative efficacy and potential mechanisms in combating obesity (6,7).

Materials and methodology

Collection of Drug

Both *Vrukshamla* and *Vijayasara* were collected from Pune, India. The samples were authenticated and standardized from pharmacognosy expert of our GMP certified pharamacy. The dried powder (50g) of both the herbs were extracted from 100% water (450ml) making it an aqueous solution by Soxhlet method. The 10mg of dried forms of extracts were dissolved in 1ml distilled water each. (figure1: Soxhlet technique)

Figure 1: Soxhlet technique

Cytotoxicity Assay

Cell viability was assessed using the MTT assay hMSCs cells were plated into 96-well plates and cultured until they reached approximately 80% confluence. Upon reaching the desired confluency, the cells were treated for 24 hours with various concentrations (2,5, 10, 15, 20, and 30 µg/mL) of *Vrukshamala* extract (*Garicinia Indica Choisy*.) and *Vijayasara* extract (*Pterocarpus Marsupium Roxb*.) prepared in aqueous solvents. Following the 24-hour treatment period, the culture media was removed, and 50 µL of MTT reagent [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added to each well. The plates were then incubated at 37°C with 5% CO_2 for an additional 4 hours to allow for the formation of formazan crystals. To solubilize the purple formazan crystals formed by metabolically active cells, 100 µL of dimethyl sulfoxide (DMSO) was added to each well. Absorbance was measured at 570 nm using an ELISA plate reader to quantify cell viability.

3T3-L1 Cell culture and differentiation

3T3-L1 cell line was purchased from National Centre for cell science (NCCS), Pune, India. 3T3-L1 preadipocytes were cultured in Dulbecco's Modified Eagle's Medium (DMEM), supplemented with 10% fetal bovine serum (FBS) and 1% antimycotic-antibiotic solution. Cells were maintained at 37°C in a humidified incubator with 5% CO_2. Upon reaching 80–90% confluence, the cells were induced to differentiate using a standard adipogenic differentiation cocktail.

To initiate differentiation (designated as Day 0), the confluent cells were treated with a combination of 1.0 µM dexamethasone (DEX), 0.5 mM isobutylmethylxanthine (IBMX), and 200 µM indomethacin (IT), in the presence of an aqueous extract of G. indica and P. marsupium. The cells were then incubated for 48 hours at 37°C with 5% CO_2. On Day 3, the differentiation media was replaced with adipogenesis progression media, comprising DMEM supplemented with 10 µL/mL insulin. At this stage, cells were exposed to varying concentrations (1, 10, and 100 µg/mL) of the aqueous extract of *G. Indica and P. Marsupium* incubated for an additional 48 hours.

On Days 5 and 7, the culture media was refreshed by carefully replacing the old media with freshly prepared media containing the respective treatments. Incubation was continued under standard culture conditions (37°C, 5% CO_2). On Day 8, cells were observed under a microscope to assess morphological changes indicative of adipocyte differentiation. Microscopic evaluation was used to determine the inhibitory or modulatory effects of the G. indica and P. marsupium extract on adipogenesis.

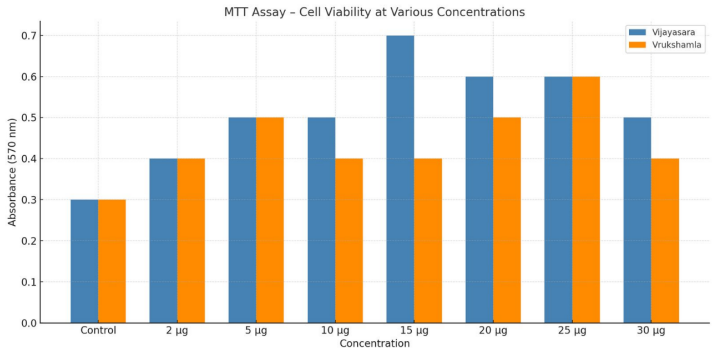
Oil Red O staining was performed to assess lipid accumulation in differentiated 3T3-L1 cells. After fixation with 4% formaldehyde, cells were stained with Oil Red O solution for 15 minutes. Excess stain was removed by PBS washes, and stained lipid droplets were visualized under a microscope. Quantitative analysis of lipid accumulation was done using ImageJ software.

Results

MTT assay Results Highlight the Non-Toxic nature of *Vijayasara* and *Vrukshamla*

Effect of *Vijayasara* and *Vrukshamla* on hMSCS Cell Viability – MTT Assay. After the treatment of both extracts of *Vrukshamla* and *Vijayasara* at concentration of 1,2,5,10,15,20, 25 and 30 µg/mL, the outcome was that there was no toxicity in cells, hence both the drugs were chosen for further adipogenesis evaluation. (Figure 2).

Figure 2: Effect of *Vijayasara* and *Vrukshamla* Extracts on hMSC Viability Assessed by MTT Assay



Vijayasara and *Vrukshamla* reduces Adipogenesis in 3T3-L1 cell line (Oil red o staining)

To investigate the potential lipid-lowering activity of *Vijayasara* and *Vrukshamla*, 3T3-L1 preadipocytes were induced to differentiate into adipocytes over a period of eight days using a standard hormonal induction protocol. Experimental groups were treated with *Vijayasara* or *Vrukshamla* extract at a concentration of 10µg/ml during the differentiation phase, while the control group received no extract treatment.

After differentiation, intracellular lipid accumulation was assessed using Oil Red O staining. The control group was assigned a baseline lipid accumulation value of 100%. Cells treated with *Vrukshamla* showed a significant reduction in lipid content, with an average decrease of 44.8% ± 2.1 compared to control ($p < 0.001$). *Vijayasara* treatment also resulted in a reduction in lipid accumulation, with an average decrease of 24.6% ± 1.8 ($p < 0.05$), though the effect was less pronounced than that of *Vrukshamla.*

Greater Oil Red O staining intensity reflects increased intracellular lipid deposition and enhanced adipogenic differentiation, while reduced staining intensity indicates inhibition of adipogenesis. (Figure 3).

Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparison test. These results suggest that both extracts exert anti-adipogenic effects in the 3T3-L1 model, with *Vrukshamla* demonstrating stronger inhibition of lipid accumulation.

Figure 3. Microscopic images of *Vrukshamla and Vijayasara*-adipogenic Differentiation in 3T3-L1 cells stained with 0.3% Oil Red O stain

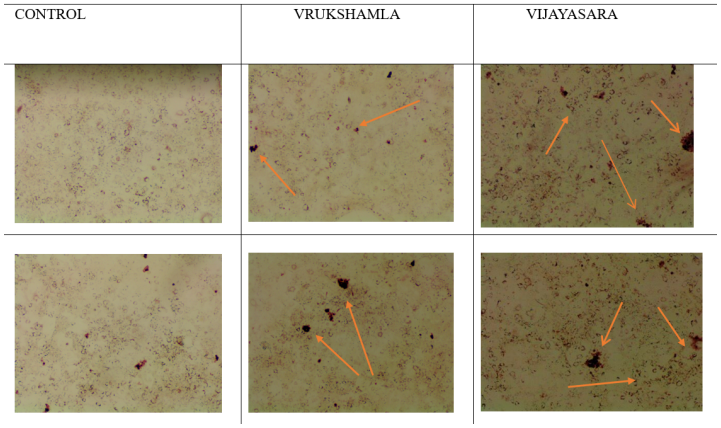
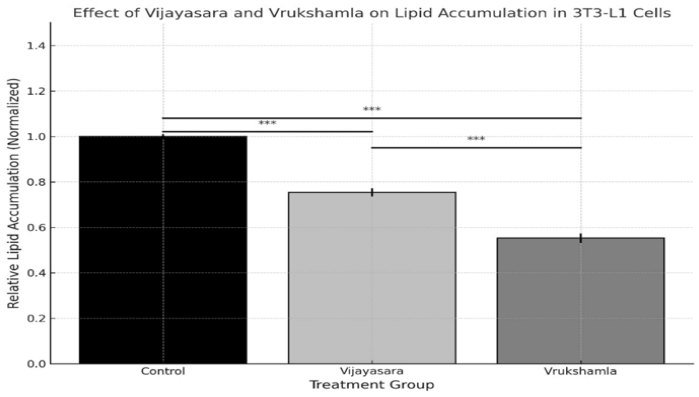


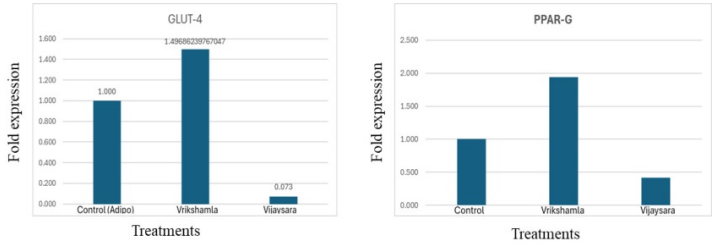
Figure 4: Quantitative analysis of *Vijayasara* and *Vrukshamla* Differentiated 3T3-L1 Adipocytes



Vrukshamla Enhances, While *Vijayasara* Suppresses GLUT-4 and PPAR-γ Expression

Vrukshamla treatment led to a substantial increase in both GLUT-4 and PPAR-γ expression compared to the adipogenic control group, indicating strong enhancement of adipocyte-associated marker activity. GLUT-4 expression increased to approximately 1.49-fold, while PPAR-γ rose to nearly 2-fold over control levels. In contrast, *Vijayasara* markedly suppressed these markers, showing a drastic reduction in GLUT-4 expression (0.07-fold) and a notable decrease in PPAR-γ levels. These findings suggest that *Vrukshamla* promotes adipogenic signaling, whereas *Vijayasara* exerts significant inhibitory effects on key adipogenic regulators, highlighting their contrasting biological actions in the 3T3-L1 adipogenesis model.

Figure 5: Effect of *Vrukshamla* and *Vijayasara* on Adipogenic Marker Expression (GLUT-4 and PPAR-γ) in 3T3-L1 Cells



Statistical analysis

All experiments were performed in triplicates, and data are presented as mean ± standard deviation (SD). Statistical significance was assessed using one-way ANOVA followed by Tukey's multiple comparison test to evaluate differences between treatment groups. A *p*-value < 0.05 was considered statistically significant.

Discussion

The present study explored and compared the anti-adipogenic potential of *Garcinia Indica Choisy* (*Vrukshamla*) and *Pterocarpus MarsupiumRoxb.(Vijayasara*) using the 3T3-L1 cell model, a gold-standard in vitro system for adipogenesis. Both plant extracts demonstrated strong biocompatibility in hMSCs, as evidenced by MTT assay results indicating no cytotoxicity across a wide concentration range (up to 30 µg/mL), reinforcing their therapeutic safety for anti-obesity applications.

Treatment with both herbal extracts led to a significant reduction in lipid accumulation, a key marker of adipocyte differentiation. This observation was further validated using Oil Red O staining. Among the two, *Garcinia Indica* demonstrated a more pronounced inhibition of adipogenesis compared to *Pterocarpus Marsupium.* The superior anti-adipogenic effect of *Garcinia Indica* corresponds with earlier research emphasizing its high hydroxycitric acid (HCA) concentration. (13) Hydroxycitric acid (HCA) acts by blocking ATP citrate lyase, a key enzyme in converting citrate to acetyl-CoA, thus limiting the biosynthesis of lipids, HCA is also linked to appetite regulation through the enhancement of serotonin levels, providing a complementary pathway for its weight management benefits. (14)

On the other hand, Gene expression analysis revealed that both *Garcinia Indica (Vrukshamla) and Pterocarpus Marsupium (Vijayasara)* modulate key adipogenic markers, albeit with differing intensities The contrasting effects of *Vrukshamla* and *Vijayasara* on adipogenic marker expression highlight their

distinct biological roles in adipocyte differentiation. The marked upregulation of GLUT-4 and PPAR-γ in the *Vrukshamla*-treated group suggests that this extract actively promotes adipogenic signaling pathways, potentially facilitating enhanced glucose uptake and adipocyte maturation. (15) In contrast, *Vijayasara* demonstrated a strong suppressive effect, with dramatic downregulation of GLUT-4 and a significant reduction in PPAR-γ expression. (16) This inhibitory profile indicates that *Vijayasara* may interfere with early transcriptional events essential for adipogenesis, thereby limiting lipid accumulation and adipocyte formation. (17) Together, these findings underscore the opposing regulatory actions of the two extracts and support the potential use of *Vijayasara* as an anti-adipogenic agent, while *Vrukshamla* may possess adipogenesis-promoting properties under similar conditions.

Taken together, these findings validate *Ayurvedic* claims regarding the utility of *Vrukshamla* and *Vijayasara* in the management of *Sthoulya* (obesity). The superior effect of *Garcinia Indica Choisy*. on inhibiting adipocyte maturation, as demonstrated here, supports its classical recommendation for fat metabolism enhancement.

Conclusion

The study demonstrates that *Vrukshamla* and *Vijayasara* exert distinctly opposite effects on adipogenic regulation in 3T3-L1 cells. *Vrukshamla* significantly inhibits adipogenesis and intracellular lipid accumulation, indicating its ability to suppress pre-adipocyte differentiation and limit triglyceride storage within the cells by enhancing the markers. In contrast, *Vijayasara* markedly suppresses GLUT-4 and PPAR-γ expression, reflecting strong anti-adipogenic potential too. These findings highlight that *Vrukshmala* has anti- adipogenic activity but also shows *Vijayasara* as a promising natural inhibitor of adipogenesis. Study also emphasizes the divergent biological actions of these two traditional extracts. Further mechanistic studies are warranted to explore their therapeutic relevance in metabolic and obesity-related disorders.

Further research is warranted to:

Elucidate molecular mechanisms through gene expression profiling, validate findings in animal models and clinical settings, and explore potential synergistic effects when used in combination.

This integrative approach combining *Ayurveda* and modern biomedicine may pave the way for safe, natural, and effective interventions for obesity management.

Conflict of Interest

No conflict of interest

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