

Research Article

Evaluation of Anti-Adipogenic and wound healing properties of *Coix lachryma* & *Hordeum vulgare* employing Human Mesenchymal Stem Cell model

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Abstract

Obesity, characterized by excessive accumulation of adipose tissue, is a burgeoning epidemic linked to various comorbidities including type 2 diabetes, cardiovascular diseases, and certain cancers. The role of natural compounds in regulating adipocyte differentiation has garnered attention as a potential therapeutic strategy for obesity. Natural products offer a rich reservoir of bioactive compounds with therapeutic potential. *Coix lachryma-jobi* (Job's Tears) and *Hordeum vulgare* (Barley) are two traditional cereals recognized for their diverse health benefits, including anti-inflammatory, antioxidant, and immunomodulatory properties. This explore a comprehensive experimental framework to evaluate the anti-adipogenic and wound healing capabilities of extracts from *Coix lachryma* and *Hordeum vulgare* using a human mesenchymal stem cell (hMSC) model. Dental Pulp Stem cells (DPSCs) were isolated and culture in cell culture media. DPSCs were characterized for surface marker CD70, CD105, CD73, CD45, CD34 and HLA-DR. Cytotoxicity of *Coix lachryma* & *Hordeum vulgare* were performed using different concentration, on DPSCs. Effect of both extracts on adipogenic differentiation of the DPSCs was observed. DPSCs expressed CD90, CD73, and CD105 stem cell surface marker and negative for CD34, CD45 and HLA-DR. *Coix lachryma* & *Hordeum vulgare* did not show any signs of cytotoxicity and wound healing prosperities either in alone or in combination. Combination of *Coix lachryma* & *Hordeum vulgare* inhibits the adipogenic differentiation in DPSCs. Our results demonstrate that Combination of extracts of *Coix lachryma* & *Hordeum vulgare* are capable of modulating adipogenic pathways, presenting them as promising candidates for obesity management.

Keywords: Anti-adipogenic, *Coix lachryma*, *Hordeum vulgare*, Mesenchymal stem cells, Obesity

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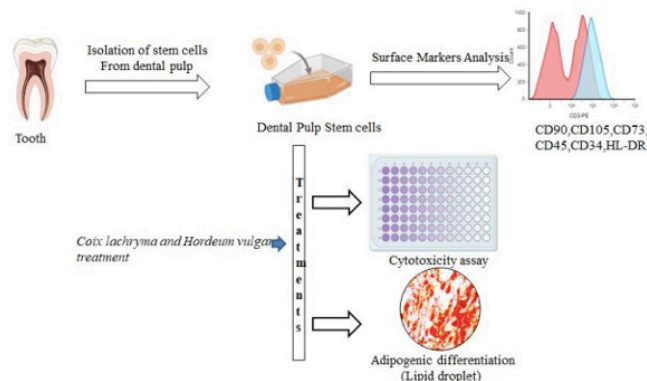


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Introduction

Obesity is a chronic difficult condition characterized by excessive fat deposits that may affect health; overweight is a state in which the body has excessive fat deposits.(1) Obesity raises the risk of heart disease and type 2 diabetes, harms bone health and reproduction, and increases the likelihood of some types of cancer. (2) Obesity also affects quality of life, which makes it a societal concern. Obesity is associated with abnormal growth of white adipose tissue in the form of hypertrophy and hyperplasia of adipocytes.(3) Accumulation of beyond the storage and functional capacity of adipose tissue leads to its ectopic deposition in important organs like liver, kidney, muscle, pancreas etc, thus affecting their functional capability.(4) In view of the

Graphical abstract-Inhibitory Activity of *Coix lachryma* & *Hordeum vulgare* on Adipocyte Differentiation in Human Mesenchymal Cell model.



aforementioned mechanisms, medicinal and dietary interventions focussing on the regulation of size and number of adipocytes can prove beneficial in therapeutic approach to obesity.

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Ayurveda, the age-old medical science of India, has used the word 'Ati Sthoola' for Obesity and also stated overeating, excessive intake of oily and sweet foods, lack of physical activity, excessive sleeps etc. as causes for the same. (5) These factors cause excessive accumulation of *Meda* (Fat) in the body, as compared to other tissues, leading not only to Obesity but other conditions like *Prameha* (Diabetes), impotency, debility etc. (6) In management of Obesity, Ayurveda has given equal importance to medicinal and dietary interventions wherein the inclusion of grains like *shyamaka*, *yavaka*, *yava*, *jurnahva* and *kodrava* has been advised. (7) The same grains, along with *Gavedhuka* (*Coix lachryma-Jobi Linn.*), have also been mentioned in the *Annapana vidhi* chapter of *Charak Samhita* for their *Kapha-Pitta* pacifying and *Meda* (fat) reducing property. *Gavedhuka* and *Yava* are known for their *Meda* (fat) reducing properties in Ayurvedic literature. However, their mechanism of action of *Gavedhuka* (*Coix lachryma*) and *Yava* (*Hordeum vulgare*) has not been explored in stem cell model.

Mesenchymal Stem cell models are a promising platform for screening and evaluating the potential, safety and mechanism of action of traditional medicines using modern research tools and parameters. (8) DPSCs are a type of mesenchymal stem cell (MSC) known for their high proliferative capacity, multilineage differentiation potential, and strong paracrine signalling capabilities. (9) Previously demonstrated that DPSCs can differentiate into adipogenic, osteogenic, and chondrogenic lineages, making them a relevant model for studying adipogenesis. (10) In addition, their origin from the craniofacial region and their secretion of trophic factors associated with tissue regeneration and angiogenesis position them as a promising model for wound healing research. (11)

Compared to other MSCs such as bone marrow-derived MSCs or adipose-derived stem cells, DPSCs offer practical advantages including non-invasive harvesting, higher proliferation rates, and reduced ethical concerns. Furthermore, DPSCs are increasingly recognized in regenerative medicine for their role in soft tissue repair, which aligns with the wound healing aspect of this study. (12)

In light of these properties, we selected DPSCs as a relevant and potentially superior in vitro model to investigate the anti-adipogenic and wound healing effects of *Coix lachryma* and *Hordeum vulgare*. The discovery of natural remedies for controlling adipogenesis has gained momentum, signalling a paradigm shift in obesity treatment paradigms. This study aims to systematically investigate the effects of *Coix lachryma* and *Hordeum vulgare* extracts on hDPMSC behavior, focusing specifically on their anti-adipogenic potential and their ability to promote wound healing-related processes. By employing a human-derived cellular model, this research seeks to provide relevant insights into the therapeutic application of these common cereals.

Materials and methods

Collection and authentication

The fruits of *Coix lachryma* were collected at Tamhini Ghat, Mangaon, Tal. Mulshi, Pune, Maharashtra (India), and fruits of *Hordeum vulgare* were collected from Padanur village, Tal. Indi, Dist. Vijapur, Karnataka (India) and identified by Botanical Survey of India, Pune (Specimen no- 1) and specimen no. SRRHV1 respectively. The fruits were air dried and subjected for analytical study at Quality Control Laboratory of institutional GMP and FDA approved Pharmacy.

Preparation of extract

To ensure thorough, effective, and standardised extraction of their water-soluble bioactive components, the aqueous extracts of *Coix lachryma-jobi* and *Hordeum vulgare* underwent Soxhlet extraction. Each sample was prepared using 50g of coarsely powdered dried fruit and 450ml of distilled water. A temperature range of 70–90 degree centigrade was maintained. The production of the aqueous extract took fourteen hours. A constant temperature of 60–85 degree centigrade. It took ten hours to finish the process. After being dried in a water bath, each extract was preserved.

Isolation and culture of Human Dental Pulp Stem Cells (hDPSC)

hDPSCs were isolated from healthy tooth dental pulp. To extract the pulp, tooth-cutting discs were used to segment the extracted tooth. Phosphate Buffer Saline (PBS) solution was added to the pulp tissue. Following next, the pulp tissue was finely chopped into tiny pieces, seeded into tissue culture flasks containing 10% FBS + DMEM solution, and incubated at 37°C with 5% CO₂. The medium was replaced every two to three days. To increase the number of cells, the confluent cells were trypsinized and subcultured for further cell expansion.

Characterization of hDPSCs cells using flow cytometry and CFU-F

Using a flow cytometer, surface marker analysis of CD90, CD73, CD105, CD45, CD34, and HLA-DR markers was carried out upon confluence. The colony forming unit assay was performed for hDPSC. 2 x 10² cells were cultured in 6 well plates and incubated in CO₂ incubator with complete medium in each well. Under a microscope (which), the plate was examined to make sure that the isolated single cells were free of contamination. Each well was dyed with 0.3% Crystal Violet on the twelfth day of incubation, and the mixture was let to stand at room temperature for 20 minutes. The colonies were inspected using an inverted phase-contrast microscope as well as the human eye.

Cytotoxicity using MTT assay

The effect of aqueous extracts of *Coix lachryma*, *Hordeum vulgare*, and their combination on the viability of human dental pulp stem cells (hDPSCs) was assessed using the MTT assay. hDPSCs were seeded into 96-well plates at a density of 1,500 cells per well and allowed to adhere for 24 hours. Subsequently, the cells were treated with various concentrations (1, 2, 5, 10, 15, 20, and 25 µg/mL) of aqueous extracts of *Coix lachryma*, *Hordeum vulgare*, or a combination of both. After 24 and 48 hours of incubation, the culture medium was removed and replaced with 100 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) per well. Plates were incubated for an additional 4 hours to allow for formazan crystal formation. Following MTT incubation, the supernatant was discarded, and the formazan crystals were dissolved in DMSO. The absorbance was measured at 570 nm using an ELISA microplate reader to determine cell viability. In parallel, a trypan blue exclusion assay was performed at each time point to further assess the percentage of dead versus viable cells. All treatments were conducted in triplicate to ensure accuracy and reproducibility of the results.

Adipogenic differentiation of DPSCs

The DPSCs were cultured in a 24-well plate (2500/cm²). Cells were incubated after 24 hour in standard adipogenic induction media containing medium 1% AA, 1µM dexamethasone, 10µM insulin, 200µM indomethacin and 0.5mM isobutyl-

methylxanthine). The media was replaced twice a week for three weeks in presence and absence of *Coix lachryma*, *Hordeum vulgare* and combination of *Coix lachryma* + *Hordeum vulgare* extracts. The lipid droplets were confirmed by staining 0.3% oil red O.

Scratch assay for wound healing

The angiogenic capacity was found to be comparable among mitomycin C pre-treated mesenchymal stem cells (MSCs) treated with *Coix lachryma*, *Hordeum vulgare*, and a combination of both extracts. A scratch assay was performed to evaluate the wound healing and cell migration properties of these treatments. A linear scratch was introduced across the MSC monolayer, followed by treatment with either *Coix lachryma*, *Hordeum vulgare*, or their combination. Cells cultured in 10% DMEM served as the positive control for proliferation and migration. To specifically assess migration independent of proliferation, all cells were pre-treated with mitomycin C. Phase-contrast images were captured at 0, 6, and 24 hours post-treatment using a phase-contrast microscope. The extent of wound closure was quantified using ImageJ software.

Statistical analysis

Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

Results

DPSCs express stem cell surface markers

Dental pulp explant culture were showing outgrowth of elongated, spindle shaped fibroblast cells like morphology (Figure 1 A,B). Culture DPSCs show fibroblast like colony forming structure in culture dish stain with 0.1% crystal violet (Figure 1C). MSC-specific cell surface markers were used for characterization of cells by flow cytometer. DPSCs expressed CD73 (99.21%), CD90 (98.54%) and CD105 (94.54%) surface markers and were negative for hematopoietic markers CD34 (1.1%), CD45 (1.5%) and a component of MHCII complex HLA-DR (0.05%). (Figure 2)

In vitro cytotoxicity assay

Different concentrations (1–50 mg/ml) of soxhlet extracts of *Coix lachryma* & *Hordeum vulgare* were added on to DPSCs (0.1×10^6 cells/ml) in the 96-well plate and were analyzed by MTT based cytotoxicity assay. The IC₅₀ of Soxhlet extracts was found to be 15 mg/ml and 25 mg/ml, respectively. *Coix lachryma* & *Hordeum vulgare* extracts were non-toxic at all concentrations alone and in combination, but highest proliferation was seen at 25 µg/mL of water-soluble extract (Figure 3).

Control p-values are constant (0.2445), serving as the baseline. G and Y Shows a decreasing trend in p-value at higher concentrations, with the lowest at 25 µg/mL ($p = 0.0846$), but not statistically significant ($p > 0.05$). Combination (G + Y) decreases with dose and approaches significance at 25 µg/mL ($p = 0.0667$), suggesting potential additive or synergistic effects.

Coix lachryma and *Hordeum vulgare* inhibit adipogenic differentiation

The differentiated adipocytes from DPSCs showed lipid droplets stained with 0.3% oil red O (Figures 4). The adipocyte differentiation from DPSCs was inhibited in the presence of *Coix lachryma* (33.97%), *Hordeum vulgare* (28.9%) and combination of *Coix lachryma* and *Hordeum vulgare* (23.32%) whereas the control showed 57.36% differentiation. (Figure 5))

The combination treatment (Y + G) exhibited the lowest IC₅₀ value, indicating the strongest inhibitory effect among the tested groups, with statistically significant differences compared to control ($p = 0.0082$). Both individual treatments G and Y also showed significant reductions in IC₅₀ values relative to control ($p < 0.05$) (Table 1).

Figure 1: (A) Cell outgrowth from dental pulp explant (B) hDPSCs showing fibroblast-like morphology (mesenchymal stem cells) (C) Single Colony of DPSCs like fibroblast

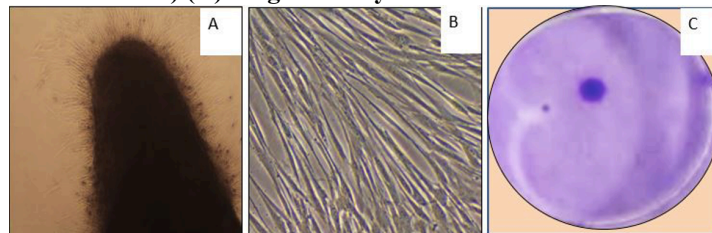


Figure 2: Surface marker analysis of DPSCs (Positive for CD105, CD73, CD90 and negative for CD34, CD45, HLA-DR.

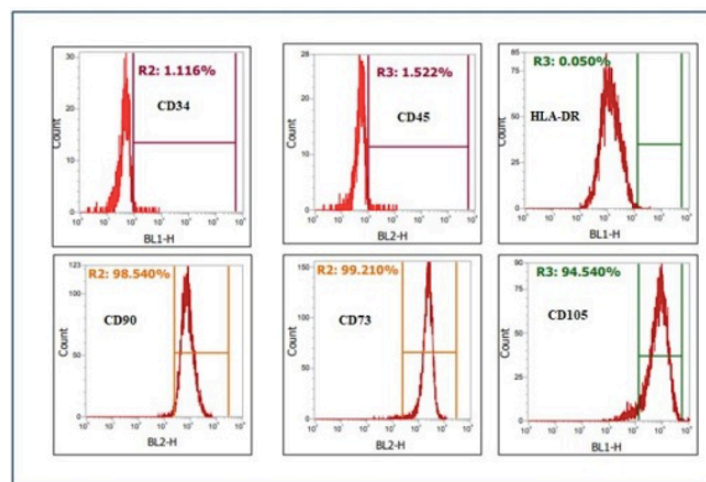


Figure 3: Cytotoxicity of Control (C), *Coix Lachryma*(G), *Hordeum Vulgare*(Y), and combination of *Coix lachryma* and *Hordeum Vulgare*(G+Y) fruit extract on DPSCs by MTT assay.

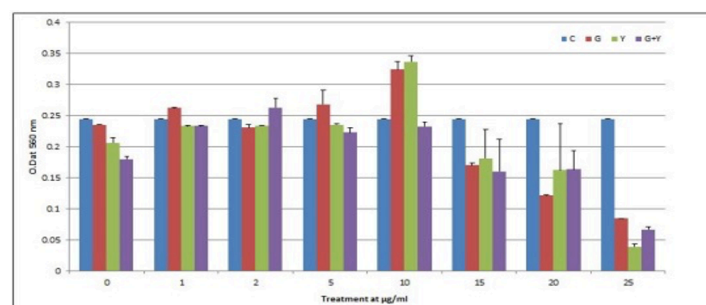


Figure 4: Differentiation of DPSCs after 21 days of Adipogenic induction showing Oil red O-stained adipocytes (A – Control, B- *Coix lachryma* treated, C- *Hordeum Vulgare* treated, D – treated with combination)

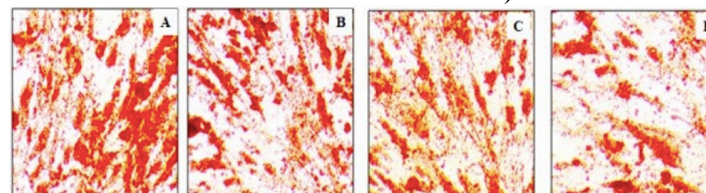
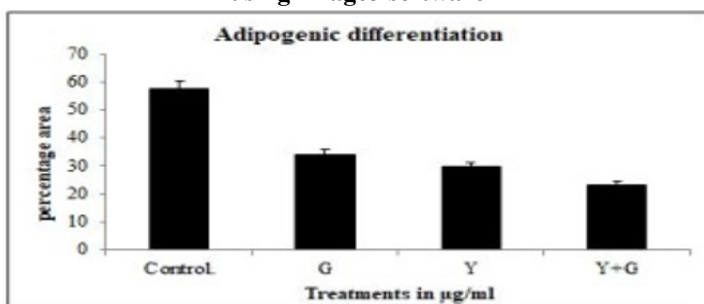
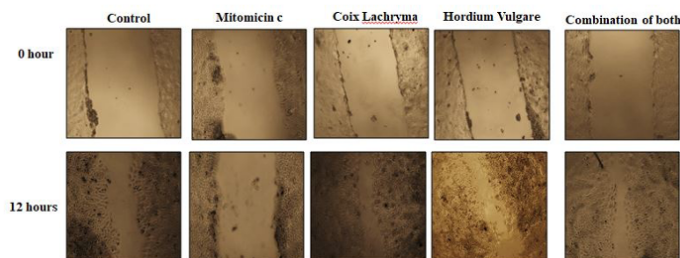


Figure 5: Quantitative analysis for Adipogenic differentiation using ImageJ software**Table 1: Adipogenic differentiation analysis**

Group	Mean IC ₅₀ (% Area)	SD/SEM	p-value (vs. Control)
Control	57.365	2.86825	—
Coix lachryma	33.973	1.69865	0.0197
Hordeum Vulgare	29.829	1.49145	0.0135
Coix lachryma + Hordeum vulgare	23.329	1.16645	0.0082

Wound healing properties of *Coix lachryma* and *Hordeum Vulgare*

Non-stimulated scratch closure (α MEM treated) was scratch area 20.4% \pm 0.4% (n = 5), and cell migration area is 79.6% \pm 0.3 while the *Coix lachryma* and *Hordeum vulgare* treated scratches closed by 12.3% , 11.7% and cell migration area 87.7% 88.3% respectively (**Table 2**). Scratches treated with combination of *Coix lachryma* and *Hordeum vulgare* encouraged cell movement compared to the control group, yet there were no significant differences in closure percentages between the two experimental groups when evaluated using ImageJ software. Representative images of the scratches at the final time point illustrate the extent of scratch closure for each treatment group (**Figure 6**). **Coix lacryma-jobi and Hordeum vulgare** both significantly enhance cell migration. **Combination treatment** yields the best result, though marginally. This data supports the potential **therapeutic value** of these plant extracts in wound healing or tissue regeneration applications.

Figure 6: Scratch assay: Cell migration ability of mytocyin C pre-treated DPSCs (n=3) at 0, 6 and 24 hours (40 \times magnification) treatment with *Coix lachryma*, *Hordeum vulgare* and combination of *Coix lachryma* & *Hordeum vulgare* extract.**Table 2: Wound Healing scratch assay analysis using ImageJ software**

Treatments	Scrach area%	Cell migration area%
Control	20.4	79.6
Mytomicin C	57.1	42.9
<i>Coix lachryma</i>	12.3	87.7
<i>Hordeum vulgare</i>	11.7	88.3
<i>Coix lachryma</i> + <i>Hordeum vulgare</i>	11.1	88.9

Discussion

The present study investigated the effects of *Coix lachryma* (Job's tears) and *Hordeum vulgare* (barley) on DPSCs.

The combination of these two extracts was based on preliminary evidence suggesting complementary bioactivities. *Coix lachryma* has been reported to exert strong anti-adipogenic effects through modulation of lipid metabolism and PPAR γ signaling, while *Hordeum vulgare* has demonstrated notable wound healing properties, including promotion of fibroblast migration and angiogenesis. We hypothesized that combining the two may provide a broader therapeutic effect by simultaneously targeting multiple aspects of tissue repair and regeneration—specifically, by suppressing undesired adipogenesis while enhancing wound healing.

While mechanistic synergy has not yet been fully elucidated, the goal of this study was to explore potential additive or complementary effects at the cellular level, using DPSCs as a model. To our knowledge, this is the first study to systematically evaluate the combined effects of *Coix lachryma -jobi* and *Hordeum vulgare* extracts, rather than assessing each plant individually. The observed combine effects on adipogenesis inhibition and enhanced wound healing in our model systems suggest a novel combinatorial potential that warrants further exploration.

Our findings demonstrate that both the extracts significantly inhibited adipocyte differentiation in hMSCs, as evidenced by reduced lipid accumulation. These results align with previous studies that highlighted the potential of natural plant extracts in modulating adipogenesis. (13,14) Adipogenesis is a multi-faceted process through which pre-adipocytes convert into mature lipid containing adipocytes which involves the role of diverse factors Adipocyte differentiation is a biological process influenced by various nutritional and genetic factors(15). *Coix lachryma* , commonly referred to as Job's tears, and *Hordeum vulgare*, known as barley, are two traditional plants that have gained attention for their potential health benefits. (16) Ours study showed evidence regarding the effects of *Coix lachryma* and *Hordeum vulgare* on reducing adipocyte differentiation in stem cells. Our data revealed the mechanisms of action and potential applications of these plant extracts in addressing obesity and wound healing.

Coix lachryma has been traditionally used in various conditions for its medicinal properties, including weight management. Previously it is reported that *Coix* extracts could inhibit adipocyte differentiation in 3T3-L1 cells, supporting our findings.(17) The mechanisms proposed include the modulation of signalling pathways associated with energy metabolism and fat storage. However, modern scientific validation of Ayurvedic practices remains sparse. Mesenchymal stem cells (MSCs), with their unique properties, present a promising avenue for drug screening in Ayurveda. Our study extends this knowledge by demonstrating the efficacy of Coix in a human stem cell model, emphasizing its potential therapeutic applications in obesity management.

Similarly, *Hordeum vulgare* has garnered attention for its health benefits, particularly in metabolic regulation. A study by Kim et al. (2020) indicated that barley extract could suppress adipogenesis in vitro through the activation of AMPK signalling. (18) In our experiments, treatment with *Hordeum vulgare* also led to significant reductions in lipid droplet formation. This suggests that barley may exert its anti-adipogenic effect through similar or complementary mechanisms, potentially enhancing energy expenditure and fatty acid oxidation. *Hordeum vulgare* prescribed

for many health conditions such as anti-inflammatory, wound-healing, anti-diabetic, anti-obesity, etc.(19) Our study first time showed that wound healing properties of *Coix lachryma* and *Hordeum vulgare* using scratch assay.

Previously, the toxicity of herbal extracts or traditional medications was assessed using immortalized animal cell lines. (20,21) hMSCs obtained from dental pulp are normal cells (not immortalized).(9) They exhibit typical diploid chromosomal counts like Homo sapiens. They have a limited life and show signs of senescence as the number of passages increases. hDPMSCs are plastic adherent and show ability to differentiate into various cell types such as osteocyte, chondrocyte and adipocyte etc.(22) Cultured cells show specific surface markers for MSCs, including CD90, CD73, and CD105, but lack expression of CD34, CD45, and HLA-DR (MHC class II).

Herbs collected naturally contain phytochemicals as well as a plethora of other unidentified and unknown chemical compounds that enhance MSC proliferation.(23) MSCs have been utilized in vitro to test the efficacy, cytotoxicity and anti-senescence activity of traditional drugs.(24) By exposing MSCs to herbal extracts, researchers assess their effects on cell proliferation, differentiation, and gene expression.(25) The response of MSCs to these drugs can elucidate their potential mechanisms of action, providing insight into therapeutic potential of traditional medicinal formulations. Our data showed that *Coix lachryma*, *Hordeum Vulgare*, and combination of *Coix lachryma* and *Hordeum vulgare* fruit extract enhanced the DPSCs proliferation at higher concentration without cytotoxicity.

In conclusion, our findings provide compelling evidence for the anti-adipogenic and wound healing effects of *Coix lachryma* and *Hordeum vulgare* on human mesenchymal stem cells. The results not only corroborate previous studies but also open new avenues for research into natural products as potential interventions for obesity. This evidence based study support the hypothesis that extracts of *Coix lachryma* and *Hordeum vulgare* possess inhibitory effects on adipocyte differentiation in hMSCs. While our findings indicate that *Coix lachryma -jobi* and *Hordeum vulgare* extracts exhibit anti-adipogenic and wound-healing activity in vitro, these results should be interpreted as preliminary. Additionally, the specific phytochemicals responsible for the observed effects remains to be identified, warranting future investigations into the bioactive compounds present in *Coix lachryma* and *Hordeum vulgare*. The observed effects in hDPMSCs suggest potential bioactivity, but do not confirm therapeutic efficacy. Importantly, the study lacks in vivo validation and detailed mechanistic insights, such as pathway-specific modulation or receptor interactions, which are essential to establish causality and translational relevance. Future studies involving animal models and molecular pathway analyses are necessary to substantiate these in vitro effects and assess safety, bioavailability, and pharmacodynamics in a physiological context.

Conclusion

This study highlights the potential of *Coix lachryma* and *Hordeum vulgare* as natural inhibitors of adipocyte differentiation in human Dental pulp mesenchymal stem cells. Future research should focus on the isolation of active compounds and elucidating their mechanisms of action. These findings warrant further investigation to substantiate the therapeutic potential of these plant extracts in combating obesity and related metabolic disorders.

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Conflict of interest: The authors share no conflict of interest.

Ethical Statement: The study was approved by Institutional Ethics Committee (Ref. No. DYPV/IEC/475/2020) and Institutional Committee for Stem Cell Research (Ref. No. IC-SCR/RM/31/20) of Dr. D. Y. Patil Dental College and Dr. D. Y. Patil College of Ayurveda & Research Centre, Pimpri, Pune.

Abbreviations:

- AA- Antibiotic -Antimycotic
- DPSCs- Dental Pulp Stem Cells
- CD- Cluster differentiation
- FDA- Food Drug Administration
- GMP - Good Manufacturing Practice
- hMSCs- Human Mesenchymal Stem cells

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