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Cytotoxic Evaluation of a Novel Herbal Topical Anesthetic Gel on Human Mesenchymal Stem Cells: An In Vitro Study

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KEYWORDS

SpilanthesAcmella; Topical Anesthetic Gel; Cytotoxicity; Mesenchymal Stem Cells; Pediatric Dentistry

ABSTRACT:

Background: Herbal products are gaining attention in dentistry as safer alternatives to synthetic agents. Spilanthesacmella, a medicinal plant with known anesthetic properties, contains spilanthol, which produces a characteristic numbing effect. However, its cytotoxic profile on human cells must be established before clinical application.

Aim: To evaluate the cytotoxicity of a novel herbal topical anesthetic gel containing S. acmella on human mesenchymal stem cells (hMSCs).

Materials and Methods: An herbal gel was prepared using Soxhlet extraction of S. acmella whole plant powder, incorporated into a polyethylene glycol base, and flavored for palatability. hMSCs were cultured and exposed to varying concentrations (100–1000 µg/mL) of the gel for 24 hours. Cytotoxicity was assessed using the MTT assay, and absorbance was measured at 570 nm to determine cell viability.

Results: The gel demonstrated a concentration-dependent cytotoxic effect. At 100 µg/mL, cell viability was 90.34%, while at 200 µg/mL it declined to 78.98%. Further increases in concentration resulted in progressively lower viability, with only 39.02% survival at 1000 µg/mL. The half maximal inhibitory concentration (IC_{50}) was calculated as 803.52 µg/mL. Based on these findings, the maximum recommended safe concentration was 0.8 mg.

Conclusion: The S. acmella herbal anesthetic gel exhibited high biocompatibility at lower concentrations and dose-dependent cytotoxicity at higher concentrations. These results indicate its potential as a safe, natural topical anesthetic in pediatric dentistry, warranting further in vivo and clinical validation.

Introduction

Traditional medicine has long utilized herbal extracts for their therapeutic benefits, with such remedies being integral to healthcare practices for thousands of years. The World Health Organization reports that nearly 80% of populations in developing nations depend primarily on herbal medicines for their healthcare needs, underscoring their widespread acceptance and accessibility [1]. Within dentistry, herbal remedies have been applied in the management of conditions such as periodontitis and dental pain, owing to their analgesic and anti-inflammatory properties. The renewed global interest in herbal-based therapies has been driven by perceptions of enhanced safety and reduced systemic complications compared to synthetic formulations. Consequently, many botanicals are currently being investigated for potential applications in dental practice, particularly for their local anesthetic properties.

One such plant is *Spilanthesacmella*, also known as *Acmella oleracea*, Akarkara, Paracress, or the Eyeball

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Plant. Belonging to the Asteraceae family, this herb is widely cultivated in India, especially in southern and central regions [2]. Its therapeutic effects are attributed to the bioactive compound spilanthol, an alkylamide known to produce characteristic tingling, burning, and numbing sensations [2,3]. The plant demonstrates analgesic, anesthetic, antimicrobial, and anti-inflammatory actions, which make it a promising candidate for integration into modern dental materials. The potential development of topical anesthetic gels incorporating *S. acmella* offers a "green dentistry" approach, reducing reliance on synthetic anesthetics while providing effective pain management.

Local anesthetics are indispensable in pediatric dentistry, where fear of injections often complicates treatment delivery. Topical anesthetics serve as a valuable adjunct to minimize discomfort prior to needle insertion for nerve blocks or infiltrations [4,5]. However, despite the traditional use of *S. acmella* for its numbing properties, scientific evidence regarding its cytotoxic effects on human cells remains scarce. Establishing its biocompatibility is essential before clinical translation, particularly through in vitro studies using human mesenchymal stem cells (hMSCs). Such investigations are crucial to ensuring both safety and therapeutic efficacy. This study, therefore, evaluates the cytotoxic potential of a novel herbal topical anesthetic gel derived from *S. acmella* on hMSCs, providing a foundation for its possible use in pediatric dental practice.

Materials and Methods:

The present in vitro study was carried out in the Department of Pedodontics and Preventive Dentistry in collaboration with the Department of Pharmacy. The study protocol was reviewed and approved by the Institutional Ethical Review Board of the institute prior to commencement, ensuring adherence to ethical guidelines for biomedical research.

Preparation of Herbal Topical Anesthetic Gel

Spilanthesacmella plant powder was procured from Sidhara Betta Herbals (FSSAI No. 21218152000107). Fifteen grams of coarse powder were introduced into the thimble of a Soxhlet extraction apparatus (Figure 1), which consists of a muslin cloth or filter paper pocket serving as a semipermeable membrane.

Figure 1. Soxhlet extraction apparatus

A hydroalcoholic solvent prepared in a 50:50 ratio of water and ethanol was introduced through the solvent inlet above the condenser. The solvent was heated in a round-bottom flask, transformed into vapor, condensed in the condenser, and dripped over the crude drug until sufficient extraction was achieved. The concentrated solvent was filtered and evaporated to dryness at room temperature, yielding 4 g of crude extract. Phytochemical analysis confirmed the presence of N-isobutyl amide, the principal alkaloid responsible for anesthetic action.

Polyethylene glycol 300 and 1500 were weighed in a ratio of 48% to 52% and melted at 20–25°C. The extract was incorporated into the molten mass, cooled to room temperature, and orange flavor drops were added to improve palatability. The final herbal anesthetic gel was stored under sterile conditions, and 2 g of the prepared gel was transported to Cell Tech Life Sciences, Surat, for cytotoxic evaluation.

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Cell Culture and Exposure Protocol

hMSCs were procured from the National Centre for Cell Science (NCC), Pune. The cells were maintained under standard laboratory conditions and exposed to 20% *S. acmella* extract in a stepwise concentration manner for 24 hours. Cytotoxicity assessment was performed using the MTT assay, which is widely used to measure the viability of cells exposed to herbal extracts [5].

MTT Assay Procedure

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) assay [7] was employed as a colorimetric method for determining cell viability and cytotoxicity. Approximately 1×10^4 hMSCs were seeded into each well of a 96-well plate (Figure 2) and incubated at 37°C for 24 hours (Figure 3).

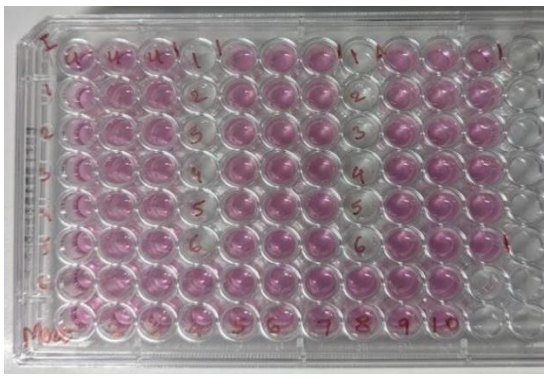

Figure2:CellPlatewithhMSCs

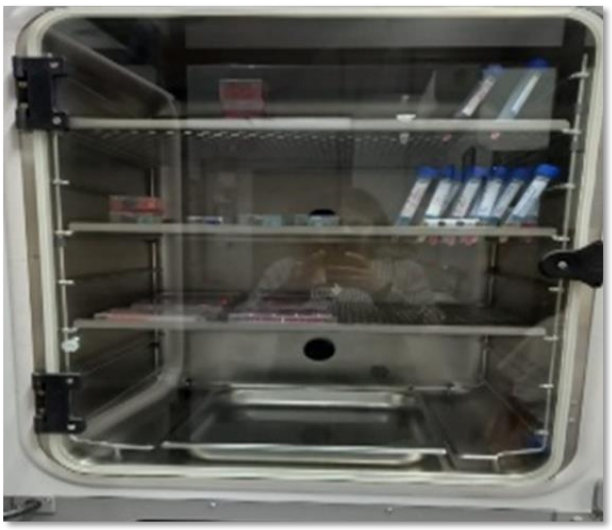

Figure. 3 Incubator

Following incubation, 5 mg/mL of MTT reagent was added to the wells, and the plates were incubated further for 3–4 hours. Viable cells metabolized the MTT reagent into insoluble formazan crystals, which appeared purple. These crystals were dissolved in 100 µL of dimethyl sulfoxide (DMSO), and absorbance was measured at 570 nm using a multiplate reader (Epoch, Bio-Tek) (Figure 4).

Figure 4. Cell plate reader

Absorbance levels corresponded to the quantity of formazan formed and thus reflected the number of metabolically active cells. Only viable cells can reduce MTT, making this a reliable indicator of cytotoxicity.

Calibration and Reliability

Calibration procedures were undertaken prior to experimentation using validated standards, with periodic recalibration to ensure consistency. To evaluate reliability, three independent operators performed repeated measurements on the same samples at different times. The results demonstrated a high consistency rate, confirming the reproducibility and accuracy of the cytotoxicity data generated by Cell Tech Life Sciences.

Results

The cytotoxic effect of the novel herbal topical anesthetic gel on hMSCs was evaluated by exposing the cells to different concentrations of the extract in a stepwise manner for 24 hours. At a concentration of

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100 µg/mL, cell viability remained high at 90.34%, with only 9.66% inhibition observed. Increasing the concentration to 200 µg/mL resulted in a reduction of viability to 78.98%, with a corresponding increase in inhibition to 21.02% (Table 1).

Table 1. The inhibitory effect of the gel determined by exposing hMSCs to 20% of the *S. acmella* extract in a stepwise concentration manner for 24 hours

Prepared gel quantity in Microgram/mL	Absorbance at wavelength 570 nm	% Cell viability	% Cell inhibition
100	0.954	90.34%	9.66%
200	0.834	78.98%	21.02%
500	0.689	65.25 %	34.75%
800	0.547	51.80%	48.20%
1000	0.412	39.02%	60.98%
Untreated	1.056	-	-

At higher concentrations, the gel demonstrated a progressively greater inhibitory effect. At 500 µg/mL, viability decreased to 65.25%, while inhibition rose to 34.75%. When the cells were exposed to 800 µg/mL, viability further declined to 51.80%, approaching the half maximal inhibitory concentration (IC_{50}), with inhibition recorded at 48.20%. At the maximum concentration tested, 1000 µg/mL, cell viability dropped sharply to 39.02%, with inhibition increasing to 60.98%. In comparison, untreated cells maintained an absorbance value of 1.056, serving as the baseline control with no inhibition.

The IC_{50} value was calculated using the regression equation $y = Mx + C$, where y represents percentage inhibition, M the slope, x the IC_{50} value, and C the intercept. Substituting the known values (y = 50, M = 0.0532, C = 7.2525), the IC_{50} was determined to be 803.52 µg/mL. This finding was corroborated by the observed data, as the 800 µg/mL concentration produced approximately 51.80% viability and 48.20% inhibition. Accordingly, the maximum recommended safe concentration of the gel for topical use was established as 0.8 mg.

Overall, the results demonstrated a clear dose-dependent cytotoxic response, wherein increasing concentrations of the *S. acmella* gel corresponded to reduced absorbance values, decreased cell viability, and increased inhibition. These findings suggest that the gel exhibits acceptable biocompatibility at lower concentrations, while higher concentrations exert a cytotoxic effect on hMSCs.

Discussion:

Newly developed dental materials are required to undergo rigorous in vitro and in vivo evaluation before they can be recommended for clinical application. In the present study, the cytotoxicity of a novel herbal topical anesthetic gel prepared from *Spilanthesacmella* was assessed using hMSCs. The results demonstrated a clear dose-dependent relationship, with lower concentrations showing high cell viability and higher concentrations exerting cytotoxic effects. These findings are consistent with the principle that while herbal extracts may have therapeutic potential, their safety profiles must be carefully characterized prior to human use.

Evidence from earlier research supports the relative safety of *S. acmella*. Ponpornpisit et al. conducted a toxicological study on zebrafish embryos using aqueous crude extracts of *S. acmella* leaves and observed no lethal effects even at the highest concentration tested (20%) [8]. In addition, its local anesthetic properties have been established not only in vitro but also in animal studies such as intracutaneous wheal tests in guinea pigs and plexus anesthesia in frogs, reinforcing its pharmacological potential [4]. Chakraborty et al. (2010) further suggested that the anesthetic effect of *S. acmella* is comparable to that of 2% xylocaine, attributing the activity to the presence of alkylamides, particularly spilanthol [9]. More recently, Mohite et al. (2020) demonstrated that a novel herbal anesthetic gel containing *S. acmella* and *Anacyclus pyrethrum* was as effective as 2% lignocaine gel in reducing needle-prick pain during inferior alveolar nerve block in children, further supporting its clinical relevance [5].

The inverse relationship between gel concentration and cell viability observed in this study highlights the importance of determining safe and effective dosages. While lower concentrations maintained cell survival above acceptable thresholds, higher concentrations

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reduced viability significantly, indicating a risk of cytotoxicity. This suggests that therapeutic use of the gel should be optimized to ensure maximal efficacy with minimal adverse cellular effects.

Beyond its anesthetic properties, *S. acmella* offers multiple therapeutic applications in dentistry. The alkylamides in the plant provide potent local anesthetic action [10], while spilanthol induces a numbing effect that functions as a natural analgesic [11]. Its antibacterial and antiseptic activity adds value in preventing and managing oral infections such as gingivitis and periodontal disease [12]. Furthermore, its anti-inflammatory properties render it useful in the management of oral ulcers and gum abscesses [13]. These diverse pharmacological effects suggest that *S. acmella* could be integrated into various dental formulations, not only as a topical anesthetic but also as an adjunct in infection control and pain management.

The findings of the present study emphasize the need for careful standardization of the concentration of *S. acmella* in commercial formulations. Although the herbal gel demonstrated promising biocompatibility at lower concentrations, higher concentrations were cytotoxic to hMSCs. Therefore, identifying the threshold at which cytotoxicity begins is crucial in order to establish safe dosage ranges for clinical use. Future research should focus on refining formulations, conducting in vivo animal studies, and ultimately designing controlled clinical trials to validate the therapeutic safety and efficacy of *S. acmella*–based anesthetic gels.

Conclusion

Within the limitations of this in vitro study, the novel herbal topical anesthetic gel formulated from *Spilanthesacmella* demonstrated promising biocompatibility with human mesenchymal stem cells, maintaining over 90% cell viability at 100 µg/mL, while higher concentrations exhibited a dose-dependent cytotoxic effect with an IC_{50} value of approximately 803.52 µg/mL. These findings indicate that the gel may be considered safe for topical use at concentrations up to 0.8 mg, highlighting its potential as a natural alternative to conventional topical anesthetics in pediatric dentistry; however, further in vivo studies and well-designed clinical trials are necessary to confirm its safety, efficacy, and clinical applicability.

Conflicts of Interest: The authors declare no conflicts of interest**.**

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