

Molecular Cytocompatibility and Morphomorphometric Analysis of a *Sesamum indicum*-*Rubus idaeus* Herbal Gel on 3T3-L1 Fibroblast Architecture

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ABSTRACT

Background: Chronic inflammation and oxidative stress lead to tissue damage, resulting in heightened interest in plant-based therapeutics with low toxicity. Sesame and raspberry contain bioactive compounds that are both anti-inflammatory and antioxidant. However, little research has been conducted on their combined formulation, particularly regarding cytotoxicity and biocompatibility. **Aim:** This study aimed to evaluate the molecular cytocompatibility and dose-dependent effects of a novel sesame root-raspberry herbal gel on the structural integrity and metabolic activity of 3T3-L1 fibroblast cells. **Materials and Methods:** An *in vitro* laboratory study was conducted over four months (March-June 2025) at Saveetha Medical College and Hospital, Chennai. Aqueous extracts of sesame root and raspberry were formulated into a bioactive gel. Cytotoxicity was quantified on 3T3-L1 fibroblast cells using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay at varying concentrations (50, 150, 250, 350, and 450 µg/mL). Alterations in cellular morphometry and cytoskeletal arrangements were evaluated using phase-contrast microscopy. Data were expressed as mean±standard deviation. One-way ANOVA with Tukey's *post hoc* test was used, with $p < 0.05$ considered significant. **Results:** Lower concentrations (50-250 µg/mL) demonstrated high cell viability (>90%) with no significant cytotoxicity. At 350 µg/mL, viability remained above 80%, indicating non-cytotoxicity, while 450 µg/mL showed mild cytotoxicity (~71%). Morphological analysis supported these findings, revealing minimal changes at lower concentrations and mild cellular alterations at higher concentrations. **Conclusion:** The sesame-raspberry gel exhibits excellent cytocompatibility at lower therapeutic concentrations, suggesting its potential as a safe natural agent for oral and biomedical applications. These findings highlight a dose-dependent interaction with fibroblast architecture, underscoring the importance of concentration optimization for preserving tissue-level homeostasis.

Keywords: Cytoskeletal stability, Cytotoxicity, Fibroblasts, Phase-contrast morphometry, Tetrazolium Salts.

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INTRODUCTION

Chronic inflammation and oxidative stress lead to tissue damage, generating interest in plant-based therapeutics with low toxicity. Sesame (*Sesamum indicum*) and raspberry (*Rubus idaeus*) contain bioactive compounds that have been shown

to reduce inflammation and fight free radicals (Gomathi *et al.*, 2024). Although the effectiveness of each component is well-established, research on combined formulations, especially sesame root-raspberry gels, is insufficient. This gap encompasses insufficient data regarding cytotoxicity and biocompatibility employing standard assays.

The therapeutic efficacy of *Sesamum indicum* and *Rubus idaeus* is attributed to their rich polyphenolic and anthocyanin profiles (Gomathi *et al.*, 2024; Ramalingam *et al.*, 2024). At a molecular level, these compounds are hypothesized to stabilize the mitochondrial membrane potential and scavenge Reactive Oxygen Species (ROS), which are primary drivers of cellular



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senescence and tissue degradation (Gomathi *et al.*, 2024; Cohen, 1988). Evaluating the histocompatibility of this combined gel is essential to ensure that the formulation supports cellular homeostasis without disrupting the cytoskeletal framework of connective tissue fibroblasts (Gomathi *et al.*, 2024; Cohen, 1988). Consequently, this study assesses the MTT-based cytotoxicity of a novel sesame root-raspberry gel on cell lines to ascertain its safety, dose-dependent effects, and potential biomedical applications.

MATERIALS AND METHODS

The current laboratory-based *in vitro* study was conducted over four months (March-June 2025) at Saveetha Medical College and Hospital, Chennai. The Institutional Ethics Committee (IEC Proposal Nos. HEC/SDC/PhD/OMED-1628/21/237 and IEC-FAC/MAR/2025/270) gave their approval, and all procedures followed the Declaration of Helsinki.

Sample Size Estimation

Sample size was determined using G*Power software (version 3.1.9.4). An effect size of $f=0.40$ was selected based on preliminary data, indicating a moderate-to-large effect as per Cohen's criteria (Gomathi *et al.*, 2024; Gomathi *et al.*, 2024). The calculation was performed with a significance level (α) of 0.05 and a statistical power ($1-\beta$) of 80%. Although a smaller sample size per group was sufficient, each concentration was evaluated in triplicate ($n=3$) to ensure reliability and reproducibility of the results.

A total of 18 samples were included in this *in vitro* study and categorized into one control group and one test group. The control group consisted of untreated cells, while the test group comprised cells treated with the Sesame + Raspberry herbal gel. The test group was further subdivided into five subgroups based on concentration levels (50, 150, 250, 350, and 450 $\mu\text{g/mL}$). Each subgroup was analyzed in triplicate ($n=3$) using the MTT assay and designated as C (control) and T1-T5 to ensure standardized grouping and uniform comparison across all concentrations.

Methods

Extraction of Phytoconstituents from Raspberry and Sesamum Root

Locally sourced fresh raspberries and sesame roots were cleaned thoroughly with distilled water. Raspberries were shade-dried at $25\pm 2^\circ\text{C}$, while sesame roots were air-dried until constant weight. The dried materials were pulverized separately and stored in airtight containers. For extraction, 50 g of each powder was soaked in 200 mL of distilled water for 72 hr with periodic shaking. The extracts were filtered through Whatman No. 1 filter paper and concentrated under reduced pressure at temperatures below 40°C using a rotary evaporator. Equal volumes of both extracts were then combined (1:1) and stored at 4°C in amber containers until further use (Gomathi *et al.*, 2024).

Formulation of the Herbal Gel

The gel was formulated by dispersing 5% (w/w) Carbopol 940 and 5% (w/w) Carboxymethyl Cellulose (CMC) in distilled water under constant stirring to form a uniform gel base. The mixture was allowed to rest to remove trapped air and ensure complete hydration. Subsequently, 2 mL of the combined raspberry-sesame extract was gradually added to 10% of the gel base under homogenization to achieve a uniform, stable gel. The finished formulation was stored in sterile, airtight containers at room temperature for subsequent testing.

Assessment of Cell Viability via MTT Assay

Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12 (DMEM F-12), antibiotics (penicillin and streptomycin), trypsin-EDTA, Phosphate-Buffered Saline (PBS), and Fetal Bovine Serum (FBS) were obtained from Gibco (Invitrogen, USA). The MTT reagent (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) and Dimethyl Sulfoxide (DMSO) were procured from Sigma-Aldrich Chemicals Pvt. Ltd., USA. All other reagents used were of analytical grade.

The cytotoxicity of the Sesame + Raspberry herbal gel was assessed using the MTT assay on 3T3-L1 fibroblast cells. Cells were seeded in 96-well plates at a density of 5×10^3 cells per well in DMEM F-12 supplemented with 10% FBS and $1\times$ antibiotic solution, and incubated at 37°C in a humidified atmosphere containing 5% CO_2 .

After attaining appropriate confluence, the cells were washed with 100 μL of $1\times$ PBS and treated with the test gel at different concentrations. The plates were then incubated for 24 hr under standard culture conditions. Following treatment, the culture medium was aspirated, and 100 μL of MTT solution (0.5 mg/mL in PBS) was added to each well. The cells were further incubated for 4 hr at 37°C .

After incubation, the MTT-containing medium was removed, and the cells were gently washed with PBS. The resulting formazan crystals were dissolved in 100 μL of DMSO and mixed thoroughly. The absorbance was measured at 570 nm using a microplate reader, with the formation of a purple-blue color indicating viable cells.

Cell viability was calculated using the following formula

$$\text{Cell viability} = \frac{\text{O.D of treated cells}}{\text{O.D of control cells}} \times 100$$

Statistical Analysis

All experiments were conducted in triplicate ($n=3$), and the results are expressed as Mean $\pm$ Standard Deviation (SD) to reflect variability within each experimental group. Optical Density (OD) readings obtained from the MTT assay at 570 nm were

used to calculate cell viability percentages for each concentration. Statistical analyses were performed using IBM SPSS Statistics software version 23.0 (IBM Corp., Chicago, IL, USA). One-Way Analysis of Variance (ANOVA) was employed to compare differences between the control and treatment groups. *post hoc* multiple comparisons were conducted using Tukey's test to identify statistically significant differences among individual concentrations. A *p*-value <0.05 was considered indicative of statistical significance. This approach ensured rigorous evaluation of the concentration-dependent cytotoxic effects of the test formulation while accounting for intra-group variability and reproducibility.

RESULTS

Optical Density (OD) readings at 570 nm exhibited a concentration-dependent trend, with higher OD values observed at lower concentrations across all experimental groups. The Optical Density (O.D) values of the sesame-raspberry gel at different concentrations were analyzed and expressed as mean±Standard Deviation (SD), along with Standard Error (SE) and *p*-values in comparison with the control group. The control group showed a mean O.D of 1.2850±0.0370 (SE=0.0214). At 50 µg/mL, the mean O.D was 1.2609±0.0985 (SE=0.0569) with a *p*-value of 0.7118, indicating no statistically significant difference from the control. Similarly, at 150 µg/mL and 250 µg/mL, the mean O.D values were 1.2063±0.1230 (SE=0.0710; *p*=0.3485) and 1.1927±0.1142 (SE=0.0659; *p*=0.2535), respectively, which were also not statistically significant. However, at 350 µg/mL, the mean O.D decreased to 1.1010±0.1040 (SE=0.0601) with a *p*-value of 0.0447, indicating a statistically significant reduction (*p*<0.05). A more pronounced effect was observed at 450 µg/mL, where the mean O.D further decreased to 0.9160±0.0521 (SE=0.0301) with a highly significant *p*-value of 0.0006 (*p*<0.01). Overall, the results demonstrate a dose-dependent decrease in O.D values, with significant activity observed at higher concentrations of the gel.

Guideline for Cytotoxicity Evaluation

The MTT assay was used to test the cytotoxicity of the Sesame + Raspberry herbal gel on 3T3-L1 mouse fibroblast cells. The results were compared to ISO 10993-5 guidelines for *in vitro* cytotoxicity of herbal gel (International Organization for Standardization, 2009).

According to ISO 10993-5, the observed cell viability results indicate a concentration-dependent cytotoxic response of the herbal gel across all experimental groups. The control group exhibited 100% viability, confirming optimal cell proliferation under baseline conditions. According to ISO 10993-5 classification, cell viability >80% is considered non-cytotoxic, 70-80% indicates mild cytotoxicity, 50-70% indicates moderate cytotoxicity, and <50% indicates severe cytotoxicity. For the

Sesame + Raspberry herbal gel, lower concentrations (50, 150, and 250 µg/mL) maintained cell viability above 90% (98.04±5.22%, 93.78±7.67%, and 92.73±7.08%, respectively) with no significant difference from the control (*p*>0.05), demonstrating excellent biocompatibility. At 350 µg/mL, viability decreased to 85.58±5.82% (*p*=0.021), remaining within the non-cytotoxic range, whereas the highest concentration (450 µg/mL) reduced viability to 71.50±6.05% (*p*=0.009), indicating mild cytotoxicity. These findings highlight a dose-dependent cytotoxic response of the herbal gel, underscoring the need for concentration optimization to ensure safe therapeutic application while retaining its bioactive properties.

Following one-way ANOVA, Tukey's multiple comparison test was performed to evaluate intergroup differences in cell viability. The analysis revealed that there were no statistically significant differences between the control group and the lower concentrations of 50 µg/mL (*p*=0.294), 150 µg/mL (*p*=0.145), and 250 µg/mL (*p*=0.105), indicating that these concentrations did not significantly affect cell viability.

In contrast, a statistically significant reduction in cell viability was observed at 350 µg/mL (*p*=0.021) when compared to the control group, marking the onset of cytotoxic effects. At 450 µg/mL, the reduction in viability was highly significant (*p*=0.009) compared to the control and also showed significant differences when compared with all lower concentrations, including 50, 150, and 250 µg/mL (*p*<0.05), indicating a pronounced cytotoxic response.

Intergroup comparisons among the lower concentrations (50, 150, and 250 µg/mL) did not show any statistically significant differences (*p*>0.05), suggesting a similar level of cellular response within this range. Furthermore, the comparison between 350 µg/mL and 450 µg/mL revealed a significant difference (*p*<0.05), indicating a further decline in cell viability at the highest concentration.

Overall, the *post hoc* analysis confirms that significant cytotoxic effects are primarily observed at higher concentrations, particularly at 350 µg/mL and above, while lower concentrations remain relatively safe and biocompatible (Table 1) (Graph 1).

Cell Morphology Analysis

Based on the MTT assay results; the optimal concentrations of the Sesame + Raspberry gel (350 and 450 µg/mL) were selected for further evaluation. Morphological changes in the cells were assessed using phase-contrast microscopy. A total of 2×10^5 cells were seeded in 6-well plates and allowed to adhere. The cells were then treated with the test formulation and incubated for 24 hr under standard culture conditions. At the end of the incubation period, the culture medium was removed, and the cells were gently washed once with phosphate-Buffered Saline (PBS, pH 7.4). Subsequently, the cells were observed under a

Phase-contrast microscope to evaluate morphological alterations indicative of cytotoxic effects.

The control group of 3T3-L1 fibroblast cells exhibited normal, healthy morphology with well-spread, elongated, spindle-shaped cells and high confluency. Cells showed intact cell-to-cell adherence and uniform distribution, with no signs of cytotoxicity such as shrinkage, rounding, or detachment. The dense, well-defined cytoplasm indicates active and viable cells, confirming optimal growth conditions and serving as a baseline for comparison (Figure 1).

At 350 µg/mL, 3T3-L1 fibroblast cells largely retained normal morphology, appearing well-spread, elongated, and spindle-shaped, similar to the control. Mild changes such as slight cell rounding and reduced spreading were observed, but no significant cytotoxic features were noted. Overall, the gel demonstrated good cytocompatibility with minimal cellular stress at this concentration (Figure 2).

At 450 µg/mL, 3T3-L1 fibroblast cells showed noticeable morphological changes, including partial rounding, reduced spreading, and decreased cell-to-cell adhesion, with a slight reduction in cell density. Although some cells retained normal morphology, mild cellular stress was evident without severe cytotoxic features. Overall, the gel demonstrated moderate cytocompatibility with concentration-dependent effects at this level (Figure 3).

DISCUSSION

The present study supports the growing interest in plant-based therapeutics for oral health management, highlighting the combined benefits of sesame and raspberry. The bioactive compounds in sesame contribute antioxidant, anti-inflammatory, and antimicrobial effects (Gomathi *et al.*, 2022), while raspberries, rich in polyphenols such as ellagitannins and anthocyanins, enhance cellular protection against oxidative stress (Ramalingam *et al.*, 2024). These properties collectively promote improved tissue health and disease prevention. The findings of the current study, demonstrating favorable cytocompatibility at lower concentrations, are in agreement with existing literature emphasizing the safety and efficacy of natural products.

However, the results also reflect the dual nature of herbal formulations, as increasing concentrations showed a gradual decline in cell viability, indicating a dose-dependent cytotoxic response. This observation aligns with previous reports that certain phytochemicals, although beneficial, can adversely affect cellular functions at higher concentrations by inducing oxidative stress, mitochondrial dysfunction, or cellular damage (Bairagee *et al.*, 2025). Variability in dosage, extraction methods, and phytochemical composition may further influence the safety profile of such formulations.

The results of the present study are in accordance with previous investigations by Rangasamy *et al.* (2023), who evaluated the cytotoxic effects of plant-derived extracts on various cell lines. Studies involving *Solanum trilobatum*, *Spathodea campanulata*, *Syzygium jambos*, and *Tylophora indica* demonstrated significant anti-proliferative activity, indicating the strong influence of extraction methods on biological activity (Rangasamy *et al.*, 2023). These findings support the current observation of dose-dependent cytotoxicity, emphasizing that phytochemical composition and concentration play a crucial role in determining cellular responses.

The cytotoxicity and biocompatibility evaluation of this novel sesame root-raspberry herbal gel on 3T3-L1 fibroblast cells demonstrates a clear concentration-dependent response. Lower concentrations (50-250 µg/mL) exhibit excellent cell viability and minimal cytotoxic effects, while higher concentrations (350 and 450 µg/mL) show a gradual decline in cell viability. These observations are consistent with the general principle that phytochemical-rich formulations, although beneficial at optimal doses, may exert cytotoxic effects when administered at higher concentrations due to increased bioactive compound activity.

The findings of the present study are further supported by investigations into the cytotoxic potential of commercially available herbal products, which demonstrated selective and concentration-dependent antiproliferative activity against various cell lines (Languon *et al.*, 2018). These findings align with the present results, where plant-based formulations exhibited dose-dependent cytotoxicity while the control group of 3T3-L1 fibroblast cells maintained normal baseline viability (Languon *et al.*, 2018).

Table 1: Effect of Sesame and Raspberry Herbal gel on 3T3-L1 Cell Viability After 24 Hr using MTT Assay.

Concentration (µg/mL)	Triplicate Values (%)	Mean Cell Viability (%)	Standard Error (SE)	p-Value vs. Control
Control	100,100,100	100.00	0.00	-
50	100.77,101.32,92.02	98.04	5.215	0.294
150	95.68,100.31,85.33	93.78	7.668	0.145
250	94.00,99.06,85.09	92.73	7.075	0.105
350	89.71,88.09,78.92	85.58	5.817	0.021
450	64.75,73.30,76.44	71.50	6.052	0.009

Effect of sesame-raspberry gel on 3T3-L1 cell viability after 24 hr (MTT assay). Values are mean±SE; $p < 0.05$ indicates significance.

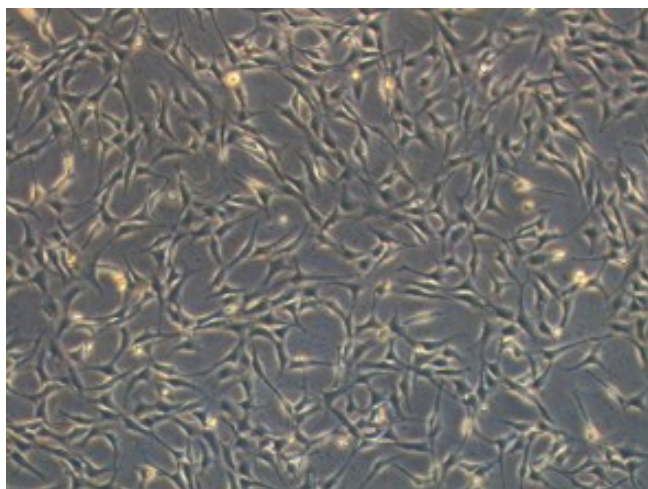


Figure 1: Untreated 3T3-L1 fibroblast cells showing normal morphology with well-spread, elongated, spindle-shaped cells and intact cell-to-cell adhesion under phase-contrast microscopy.

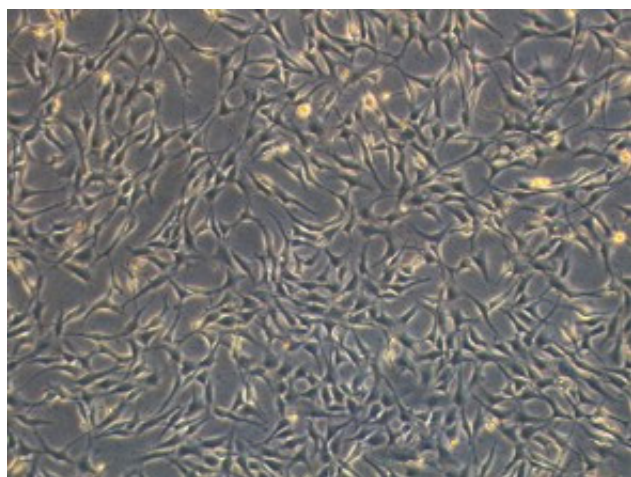


Figure 2: 3T3-L1 fibroblast cells treated with sesame-raspberry gel (350 µg/mL) for 24 hr showed largely preserved morphology with mild changes such as slight cell rounding and reduced spreading.

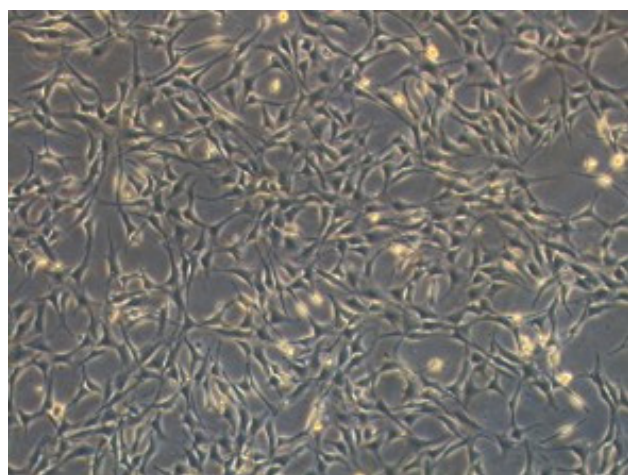


Figure 3: 3T3-L1 fibroblast cells treated with sesame-raspberry gel (450 µg/mL) for 24 hr showed noticeable morphological alterations, including partial rounding, reduced spreading, and decreased cell density.

Previous studies on plants from the *Asteraceae* family demonstrate varying cytotoxic and antioxidant effects depending on species and concentration, particularly in the management of gastrointestinal diseases. Extracts of *Tanacetum vulgare* and *Achillea millefolium* exhibited significant cytotoxicity at higher concentrations, whereas *Solidago gigantea* showed a supportive effect on cell proliferation at lower doses (Sowa *et al.*, 2020). Similarly, the present study highlights a concentration-dependent response, emphasizing the importance of dose optimization in the use of herbal formulations for oral diseases.

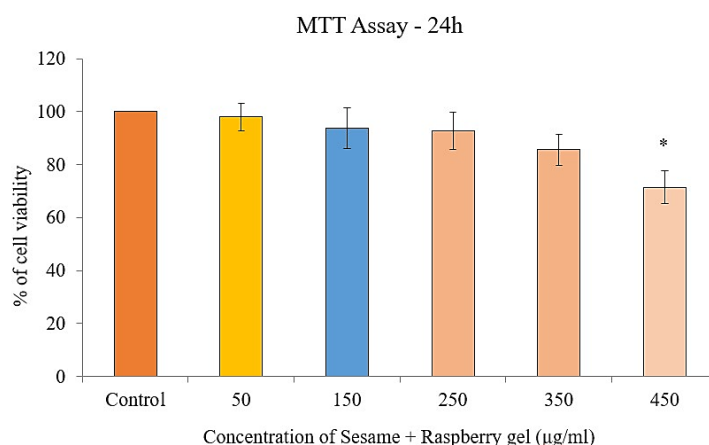
The high cell viability (>90%) observed at 50, 150, and 250 µg/mL confirms the excellent biocompatibility of the formulation at lower concentrations. This can be attributed to the synergistic action of bioactive compounds present in sesame and raspberry, such as lignans (sesamin, sesamol) and polyphenols (ellagic acid, anthocyanins), which are known for their antioxidant and anti-inflammatory properties. These compounds help in neutralizing reactive oxygen species and maintaining cellular homeostasis, thereby promoting cell survival and proliferation. The absence of statistically significant differences between these concentrations and the control group further reinforces their safety profile, making them suitable for potential therapeutic applications, especially in oral formulations where prolonged tissue contact is expected.

At 350 µg/mL, although a significant reduction in cell viability was observed, values remained above 80%, indicating non-cytotoxicity as per ISO 10993-5 standards and acceptable biocompatibility. Mild morphological changes suggested early cellular stress without irreversible damage. At 450 µg/mL, cell viability decreased to ~71%, indicating mild cytotoxicity, supported by morphological changes such as cell rounding, reduced adhesion, and decreased cell density, though without severe cytotoxic features.

These findings demonstrate a dose-dependent cytotoxic response, consistent with previous studies where natural compounds exhibit antioxidant effects at lower concentrations and pro-oxidant effects at higher doses. Morphological observations further supported MTT assay results, confirming cellular integrity at lower concentrations and gradual cytotoxic effects at higher levels.

LIMITATIONS OF THE STUDY

The present study is limited by its *in vitro* design, which may not fully replicate complex *in vivo* biological responses. The use of a single cell line (3T3-L1 fibroblasts) restricts the generalizability of the findings across different tissue types. Additionally, variations in phytochemical composition due to extraction methods were not extensively characterized. The study also evaluated short-term exposure (24 hr), which may not reflect long-term effects. Furthermore, the lack of molecular-level analysis limits understanding of the exact mechanisms underlying cytotoxicity.



Graph 1: Effect of sesame-raspberry gel on 3T3-L1 fibroblast cell viability after 24 hr, assessed by MTT assay. Cells were treated with increasing concentrations (50-450 µg/mL). A significant reduction in viability was observed at 450 µg/mL.

FUTURE PERSPECTIVES

Future studies should include *in vivo* models and clinical trials to validate the safety and efficacy of the sesame-raspberry gel. Investigations involving multiple cell lines and longer exposure durations are recommended for broader applicability. Detailed phytochemical analysis and standardization of the formulation are essential. Mechanistic studies exploring pathways of cytotoxicity and antioxidant activity would provide deeper insights. Additionally, optimizing dosage and exploring targeted drug delivery systems could enhance therapeutic potential.

CONCLUSION

The sesame root-raspberry herbal gel demonstrated excellent cytocompatibility at lower concentrations and mild, dose-dependent cytotoxicity at higher levels. The findings highlight its potential as a safe and effective natural alternative for oral and biomedical applications. However, careful dose optimization and further validation through *in vivo* and clinical studies are necessary to ensure its safe clinical use.

ABBREVIATIONS

MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **DMEM:** Dulbecco's Modified Eagle Medium; **PBS:** Phosphate-Buffered Saline; **FBS:** Fetal Bovine Serum; **DMSO:** Dimethyl Sulfoxide; **ANOVA:** Analysis of Variance; **SD:** Standard Deviation; **SE:** Standard Error; **ISO:** International Organization for Standardization; **ROS:** Reactive Oxygen Species; **IEC:** Institutional Ethics Committee; **CMC:** Carboxymethyl Cellulose; **OD:** Optical Density.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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