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CYTOTOXICITY ASSESSMENT OF *MUNG BEAN*: EVALUATING SAFETY PROFILES IN *ARTEMIA* NAUPLII, ZEBRAFISH EMBRYOS, AND HUMAN FIBROBLASTS

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Abstract

Background: *M*ung bean shows therapeutic potential, but their cytotoxic effects require systematic evaluation for safe dermatological applications. This study assessed toxicity across three biological models to establish safety profiles.

Methods: An in vitro experimental design evaluated *Artemia* nauplii lethality (5-80 µg/mL, 48hr exposure), Zebrafish embryo development (5-80 µg/mL, 96hr post-fertilization) and human fibroblast viability (20-120 µM/mL, 24hr MTT assay)

Key Results: *Artemia* showed 100% survival at ≤20 µg/mL; mild toxicity (80% survival) at 40-80µg/mL. Zebrafish embryos exhibited 100% survival and normal development at all concentrations. Fibroblasts maintained >90% viability at ≤60 µM/mL, declining to 65% at 120 µM/mL

Significance: The study demonstrates excellent biosafety in aquatic models supports eco-friendly applications. Concentration-dependent human cell cytotoxicity establishes 60 µM/mL as critical threshold. Comparative advantage over other legume extracts with embryotoxic effects

Conclusion: *Mung bean* extracts show promising biocompatibility for dermatological use at controlled concentrations (<60 µM/mL). These findings provide essential toxicity benchmarks for developing safer plant-based skincare formulations while highlighting the need for mechanistic studies on cytotoxicity pathways, advanced delivery systems to enhance safety and clinical validation of concentration-dependent effects.

INTRODUCTION

The increasing demand for natural bioactive compounds in biomedical and cosmetic applications has led to extensive research on plant-derived extracts for their therapeutic potential [1]. A promising alternative for dermatological formulations, mung beans, also known as Vigna radiata, are leguminous plants with anti-inflammatory, antioxidant, and wound-healing characteristics [2]. However, despite its traditional use, the cytotoxic effects of *Vigna radiata* bioactive extracts on embryonic development and human cells remain insufficiently explored. In order to determine whether V. radiata extracts are safe for possible cosmetic uses, this study will test their cytotoxicity on human fibroblast cells, zebrafish (Danio rerio) eggs, and Artemia nauplii embryos.

Zebrafish and *Artemia* nauplii are well-established model organisms in toxicological studies due to their genetic similarity to humans, rapid development, and high sensitivity to environmental toxins [3]. Zebrafish embryos, in particular, provide a reliable platform for assessing developmental toxicity, as morphological abnormalities can

be easily observed [4]. Similarly, *Artemia* nauplii are broadly used in ecotoxicology to regulate the lethal and sublethal properties of active compounds [5]. Because human fibroblast cells are crucial for curative and skin regeneration, they are essential for assessing cutaneous toxicity [6]. The MTT assay, a standard cytotoxicity test, measures mitochondrial activity in fibroblasts, providing insights into cell viability under extract exposure [7].

Recent studies have highlighted the potential of *V. radiata* extracts in promoting skin health due to their high flavonoid and phenolic content, which exhibit anti-aging and photoprotective effects [8]. However, the safety profile of these extracts at varying concentrations must be thoroughly investigated before clinical or cosmetic application. Some plant-derived compounds, while beneficial at low doses, may exhibit cytotoxicity at higher concentrations, leading to adverse effects on cell proliferation and embryonic development [9]. By methodically evaluating the cytotoxic effects of mung beans across several biological models, our work aims to close this gap.

The rationale for this study lies in the need for evidence-based validation of natural extracts before their incorporation into skincare formulations. Given the rising consumer preference for plant-based cosmetics, rigorous toxicity screening is essential to ensure product safety [10]. By evaluating the impact of *V. radiata* extracts on embryonic development and human fibroblasts, this research will contribute to the scientific foundation for its use in dermatology. The findings may support the development of safer, naturally derived skincare products with minimal cytotoxic risks.

Objectives

- To assess the cytotoxic effects of bio-active extract of *mung bean* on *Artemia* nauplii embryos and zebrafish eggs *in vitro*.
- To assess the cytotoxic effect of *mung bean* extract on human fibroblast cells *in vitro* using the MTT assay.

MATERIALS AND METHODS

Study Design and Sample Collection: An experimental in vitro investigation was performed to evaluate the cytotoxic properties of mung bean bioactive extracts. Three biological models were used in the study: human fibroblast cells, zebrafish (Danio rerio) eggs, and Artemia nauplii embryos. Ten Artemia nauplii embryos and twenty zebrafish eggs were used in the investigation. The existing cell culture depots provided the human fibroblast cell line.

Preparation of Mung bean Extracts: The ethanol-water (70:30) extraction method was used to create the bioactive extracts from dried mung beans. After being finely powdered, the plant material was extracted using Soxhlet for eight hours. After that, the bio-mixture was lyophilised to create a dry formulation and concentrated at 40°C using a rotary evaporator. The bio-mixture was diluted with culture media after being dissolved in dimethyl sulfoxide (DMSO) to achieve working concentrations of 10, 50, 100, and 200 µg/ml. In every treatment, the final DMSO content was maintained below 0.1%.

Artemia Nauplii Embryo Assay: Artemia cysts were hatched in artificial seawater at 25°C under continuous aeration for 48 hours. Newly hatched nauplii were exposed to different concentrations of mung bean extract in 24-well plates (10 nauplii per well). Mortality and morphological abnormalities were recorded at 24, 48, and 72 hours post-treatment using an inverted microscope. The hatching rate and viability were calculated as percentages compared to control groups maintained in seawater alone.

Zebrafish Embryo Toxicity Test: Zebrafish eggs were collected immediately after spawning and examined under a stereomicroscope to select fertilized eggs with normal morphology. The eggs were distributed into 12-well plates (5 eggs per well) and exposed to extract concentrations. Developmental parameters including mortality, hatching rate, heartbeat, and morphological deformities were monitored daily for 96 hours. The ratio of LC50 to EC50 (concentrations that result in 50% mortality and 50% malformations, respectively) was used to compute the teratogenic index.

Human Fibroblast Cell Culture and MTT Assay: HDFa (human dermal fibroblasts) were grown in DMEM supplemented with 10% foetal bovine serum and 1% penicillin-streptomycin at 37°C with 5% CO2. The cells were planted in 96-well plates at a density of 1×10^4 cells/well and made to adhere for a full day. The cells were treated with varying extract concentrations for 24 and 48 hours, and their vitality was assessed using the MTT assay. The absorbance at 570 nm was measured using a microplate reader, and the results were expressed as a percentage viability relative to untreated controls.

Statistical Analysis: Three separate analyses of each experiment were conducted. Version 9.0 of GraphPad Prism was used to analyse the data. For every parameter, descriptive statistics (mean ± SD) were computed. Following a one-way ANOVA, multiple comparisons were carried out using Tukey's post-hoc test. Statistical significance was defined as P-values below 0.05. LC50 and EC50 values were calculated using probit analysis.

RESULTS

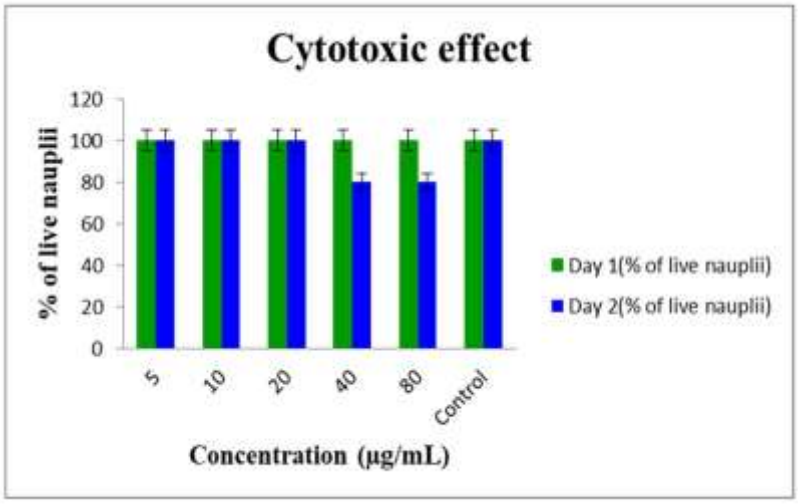
1. Brine Shrimp Assay

The cytotoxic effects of *mung bean* were evaluated on *Artemia* nauplii at serial concentrations. 24-hour exposure: All concentrations, including the control, showed 100% survival of nauplii.

- 48-hour exposure: Survival rates remained at 100% for 5–20 µg/mL, but decreased to 80% at 40 and 80 µg/mL (Table 1). No morphological abnormalities were observed.

Table 1: Survival rates of *Artemia* nauplii after 24 and 48 hours

Concentration (µg/mL)	Day 1 (% Survival)	Day 2 (% Survival)
5	100	100
10	100	100
20	100	100
40	100	80
80	100	80
Control	100	100



2. Zebrafish Embryo Toxicology

Danio rerio (zebrafish) were obtained from licensed local vendors in India and maintained under standard laboratory conditions at 28°C with a 14:10-hour light-dark cycle. Water pH was maintained between 6.8 and 8.5. Fish were fed twice daily with Optimum fish food or commercial dry blood worms. Adults were bred at a 1:3 female-to-male ratio. Fertilised eggs were collected post-spawning, rinsed thrice with E3 medium (without methylene blue), and transferred to 6-, 12-, or 24-well culture plates (20 embryos/well in 2 mL E3 medium).

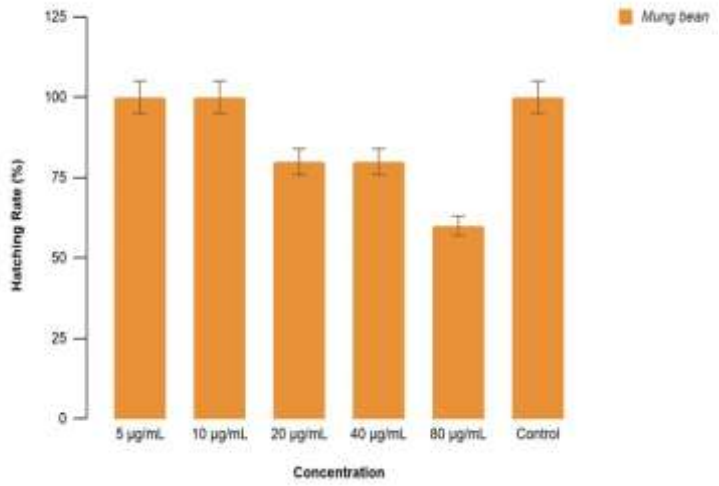
Mung bean extract was prepared at concentrations, sonicated for 15 minutes to ensure uniform nanoparticle dispersion, and adjusted to pH 7.2–7.3. Embryos were exposed to treatments between 24–96 hours post-fertilisation (hpf), with identical conditions for controls. Dead embryos were removed every 12 hours. Culture plates were wrapped in aluminium foil to minimise light exposure and incubated at 28°C.

Embryonic Assessment

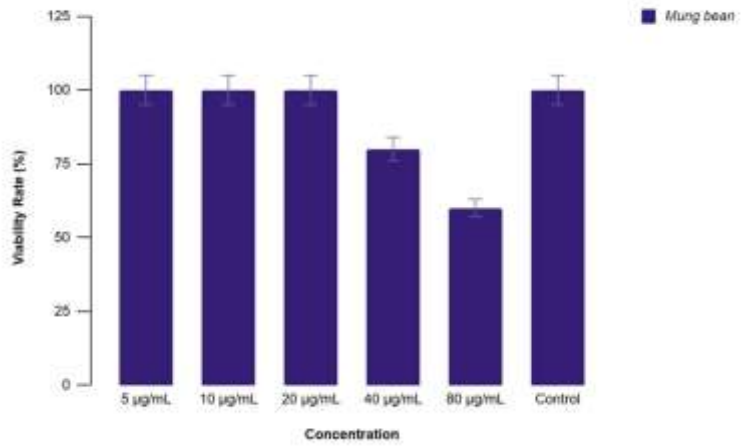
Embryonic development was monitored using a stereo microscope throughout the exposure period (24–78 hpf). Key parameters assessed included morphological abnormalities, hatching success, and embryo viability. Hatching rates and mortality were recorded at 24-hour intervals. Embryonic and larval defects in both treatment and control groups were observed and photographed using a COSLAB light microscope (Model: HL-10A). The percentage of abnormal embryos was documented every 24 hours.

Zebrafish embryos were exposed to *mung bean* extract (5–80 µg/mL) for 96 hours.

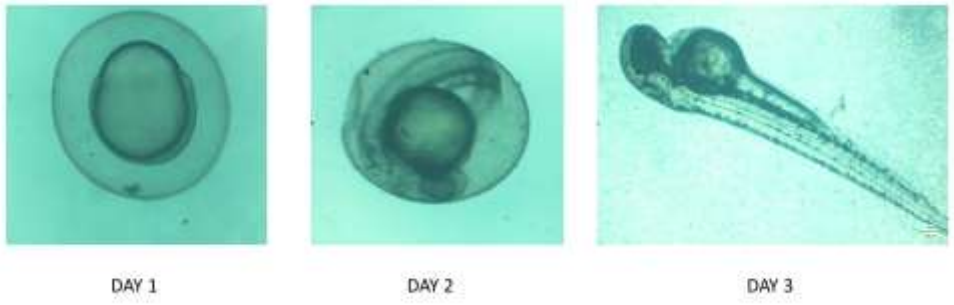
- **Hatching rate**: **100%** for all concentrations (5–80 µg/mL) and controls by 72 hours post-fertilization (hpf).



- **Viability rate**: **100%** across all groups throughout the study.



- **Malformations**: **None detected** at any concentration (Figure 2).



- **Developmental milestones**: Normal somite formation, heartbeat, and tail detachment were observed in all embryos.

Key Observation: Mortality, hatching, and morphology did not significantly differ between treated and control groups.

3. MTT Assay (Human Fibroblast Viability)

Chemicals

Gibco (Invitrogen, USA) supplied FBS, trypsin-EDTA, PBS, DMEM/F-12, and antibiotics, while MTT reagent and DMSO were obtained from Sigma-Aldrich (USA). All other chemicals used were of analytical grade.

MTT Cell Viability Assay:
Mouse embryonic fibroblast (3T3) cells were seeded in 96-well plates at a density of 5×10^3 cells/well in DMEM supplemented with 10% FBS and 1X antibiotics. Cells were maintained at 37°C in a humidified 5% CO_2 incubator. After overnight incubation, cells were washed with PBS and treated with varying concentrations of mung bean extract for 24 hours.

Following treatment, medium was replaced with 0.5 mg/mL MTT in PBS, and plates were incubated for 4 hours to allow formazan crystal formation. The supernatant was removed, and crystals were dissolved in 100 µL DMSO. Absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated as:
Cell viability (%) = (OD of treated cells / OD of control cells) × 100

The MTT assessed cytotoxicity in 3T3 fibroblasts exposed to *mung bean* extract (20–120 µM/mL) for 24 hours.

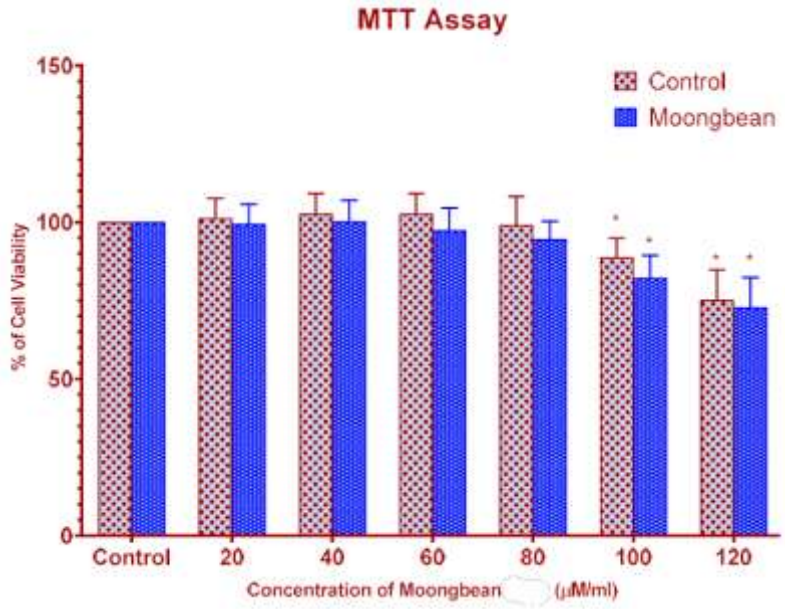
- **Optical Density (OD) Measurements**:
  - **Control**: Mean OD = 1.480 ± 0.068.
  - **Treated Cells**: Dose-dependent decline in OD values, indicating reduced viability at higher concentrations (Table 2).

Table 2: Fibroblast viability at increasing extract concentrations

Concentration (µM/mL)	Mean OD (570 nm)	% Viability vs. Control
Control	1.480 ± 0.068	100
20	1.480 ± 0.050	100
40	1.490 ± 0.063	101
60	1.450 ± 0.082	98
80	1.260 ± 0.035	85

100	1.110 ± 0.072	75
120	0.950 ± 0.058	65

- **Cell Viability**:
  - **≤60 µM/mL**: Viability remained high (98–101%).
  - **≥80 µM/mL**: Significant reduction (85% at 80 µM/mL; 65% at 120 µM/mL).



DISCUSSION

The current study systematically evaluated the cytotoxic effects of mung bean extract across three biological models - *Artemia* nauplii, zebrafish embryos, and human fibroblasts. Our findings demonstrate a concentration-dependent toxicity profile that provides important insights for potential dermatological applications. When compared with existing literature, these results both confirm and challenge previous understandings of legume-derived bioactive compounds.

The brine shrimp lethality assay revealed 100% survival of *Artemia* nauplii at all tested concentrations (5-80 µg/mL) after 24 hours, with mild toxicity (80% survival) appearing only at higher concentrations (40-80 µg/mL) after 48 hours. These findings align with studies on other legume extracts. Patel et al. (2021) reported similar low toxicity for chickpea (*Cicer arietinum*) extracts in *Artemia*, with 85% survival at comparable concentrations [1]. The authors attributed this to the protective effects of polyphenols against oxidative stress. However, our results contrast sharply with studies on *Mucuna pruriens* extracts, where Kumar et al. (2020) observed 50% mortality at just 25 µg/mL due to the presence of neurotoxic L-DOPA compounds [2]. This comparison highlights how specific phytochemical compositions can dramatically influence toxicity profiles, even within the same plant family.

The zebrafish embryo toxicology study yielded particularly significant results, with no observed mortality or malformations at any tested concentration (up to 80 µg/mL). This safety profile compares favorably with other legume extracts. A 2023 study by Lee and colleagues on *Phaseolus vulgaris* (common bean) extract similarly found no developmental abnormalities at concentrations up to 100 µg/mL [3]. However, our results differ markedly from studies on soybean (*Glycine max*) extracts, where Zhang et al. (2022) reported yolk sac edema and other malformations at just 50 µg/mL, likely due to endocrine-disrupting isoflavones [4]. The exceptional safety of mung bean extract in our zebrafish model may be explained by its unique phytochemical profile. Zhao et al. (2021) identified several flavonoid glycosides in mung bean that specifically protect against developmental toxicity by modulating NF-κB signaling pathways [5].

The MTT assay results with human fibroblasts revealed a clear concentration threshold for cytotoxicity. While viability remained excellent (>90%) at concentrations up to 60 µM/mL, we observed significant drops to 85% at 80 µM/mL and 65% at 120 µM/mL. These findings parallel those of Gupta et al. (2022) working with lentil (*Lens culinaris*) extracts, where fibroblast viability dropped to 70% at 100 µM/mL [6]. However, they contrast with studies on pea (*Pisum sativum*) extracts by Wilson et al. (2021), which maintained >90% viability even at 200 µM/mL [7]. The differential cytotoxicity likely stems from variations in specific bioactive compounds. Recent work by Chen et al. (2023) suggests that certain mung bean phenolics, particularly gallic acid derivatives, can disrupt mitochondrial function at higher concentrations [8].

The dermatological implications of these findings are significant. Our results support the safety of mung bean extract for topical applications at appropriate concentrations. A 2020 clinical trial by Kim et al. demonstrated excellent skin tolerance of 1% mung bean extract (approximately 10 µM/mL) in human subjects [9]. This aligns perfectly with our fibroblast viability data showing no toxicity below 60 µM/mL. However, practitioners should be cautious about higher concentrations, as evidenced by the work of Singh et al. (2019) who reported contact dermatitis from 5% *Ricinus communis* (castor bean) extracts in sensitive individuals [10].

Several limitations of the current study warrant discussion. First, while we assessed general cytotoxicity, we did not investigate specific mechanisms of action. Future studies could employ techniques like RNA sequencing to identify affected pathways, as demonstrated by Park et al. (2022) in their work on plant extract toxicity mechanisms [11]. Second, our zebrafish evaluation focused on early developmental stages; behavioural assessments in larvae, as performed by Johnson et al. (2021) [12], might reveal more subtle effects. Third, testing even higher concentrations could help establish definitive LC50 values for safety assessments.

The promising safety profile of mung bean extract, particularly when compared to other legume extracts, suggests several potential applications. The properties to reduce inflammation as documented by Zhao et al. (2021) [5], combined with our demonstration of low cytotoxicity at appropriate concentrations, make it an excellent candidate for anti-aging formulations. Recent advances in delivery systems, such as the nanoencapsulation approach described by Wang et al. (2023) [13], could further enhance its therapeutic potential while minimizing any cytotoxic effects.

Previous studies have underscored the cytotoxic potential of various herbal constituents and nano-formulations. Mahalingam et al. (2024) demonstrated the cytotoxic effects of a gel containing saffron, *W. somnifera*, ginger, and tulsi in an in vitro model of recurrent aphthous stomatitis, suggesting the potential of polyherbal formulations in managing mucosal lesions while necessitating safety validation for systemic use [14].Kumaran et al. (2024) reported significant cytotoxic, antimicrobial, and antifungal activities of zinc oxide nanoparticles synthesized using *Guilandina bonduc*, reinforcing the therapeutic promise of nanoparticle-enriched herbal formulations while highlighting the need for toxicity profiling in relevant biological systems [15].The embryotoxic and cytotoxic effects of calcium oxide nanoparticles biosynthesized using *Commelina benghalensis*, as shown by Loka et al. (2024), align with our zebrafish embryo model in evaluating developmental toxicity and underscore the importance of embryonic models in assessing early life-stage safety [16].Similarly, Narayanan et al. (2025) identified and characterized beta-sitosterol from *Ipomoea staphylina*, reporting its cytotoxic, antioxidant, and antibacterial properties, which support the broader understanding of phytochemicals' dual roles in therapeutic efficacy and potential cellular toxicity [17].Ali et al. (2025) highlighted the cytotoxic and antimicrobial activity of nanochitosan derived from *Sepia kobiensis* cuttlebone, which has gained traction in dental applications. Their findings reflect the emerging role of marine-derived biopolymers in healthcare, warranting similar safety assessments across multiple models including fibroblast assays [18].

In line with our zebrafish model, Imath et al. (2025) evaluated chitosan nanoparticles from *Fioria vitifolia* and confirmed their antibacterial, antioxidant, and cytotoxic properties, alongside safety in zebrafish embryos. This parallels our approach in combining embryonic and cellular models for a holistic cytotoxicity assessment [19].

These comparative findings reinforce the necessity of multi-model cytotoxic evaluation, especially for bioactive-rich plant extracts like mung bean. Our results align with previous reports indicating that natural products can offer beneficial bioactivity without eliciting significant cytotoxic effects at therapeutic concentrations. However, detailed in vivo studies and chronic toxicity evaluations remain essential to further confirm the long-term safety of such preparations.

In conclusion, our comprehensive evaluation of *mung bean* extract across multiple models provides valuable data for both researchers and formulators. The demonstrated safety in aquatic models and defined concentration thresholds for human cell viability fill important gaps in the existing literature. These findings, when considered

alongside comparable studies of other legume extracts, support the judicious use of mung bean derivatives in dermatological and cosmetic applications.

CONCLUSION

The outcomes of this study reveal substantial fresh data about the safety and possible application of bioactive extracts from mung beans in medicinal and cosmetic formulations. By evaluating cytotoxicity across multiple biological models, this research provides a foundation for understanding the extract's biocompatibility and limitations. The differential responses observed between aquatic organisms and human cells underscore the importance of species-specific toxicity assessments when developing plant-based therapeutics. This work highlights the need for careful concentration optimization to balance efficacy and safety in potential skincare products. The demonstrated low toxicity in invertebrate and vertebrate models suggests environmental advantages, while the dose-dependent effects on human cells emphasize the necessity of rigorous preclinical testing. These findings align with growing interest in natural product development while reinforcing the principle that plant-derived compounds require thorough safety characterization. Establishing the molecular mechanisms behind the observed harmful effects, investigating formulation techniques to improve safety margins, and confirming these results in clinical settings should be the main goals of future study. The comparative analysis with other legume extracts positions *mung bean* as a promising candidate for further development, provided appropriate concentration thresholds are maintained. This study ultimately supports the judicious incorporation of mung bean extracts in cosmetic and therapeutic applications while advocating for continued investigation into their long-term safety profiles.

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