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IN-VITRO CYTOTOXIC STUDY OF NATURAL POLYSACCHARIDE ISOLATED FROM *T. CORDIFOLIA* FRUIT

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ABSTRACT: The polysaccharide-derived polymers can play an integral and versatile role in the novel drug delivery system for a variety of therapeutic agents in the biological system. In this research, natural polysaccharide isolated to evolve natural polymer-based innovative drug delivery carriers to provide maximum therapeutic effect and minimize clinical complications, which could also be a step towards biological environment-friendly drug delivery systems since the envisaged natural polymer could be a good substitute compared to the synthetic polymers currently used for novel drug delivery systems with modified drug delivery properties. *Tinospora cordifolia* is a tropical herb of the Menispermaceae family, also known as 'Guduchi', well-known for its therapeutic benefits in the Indian traditional system. This plant's fruits cluster in groups of one to three, and the mucilage contains complex polysaccharides. *In-vitro* cytotoxicity studies on fibroblast cells revealed about 80% cell viability, confirming its biocompatibility as a natural excipient with potential applications in green and advanced pharmaceutical drug delivery systems.

INTRODUCTION: Polymer-based drug delivery systems have emerged as a major focus in contemporary pharmaceutical research due to their capacity to enhance drug stability, regulate release profiles, and improve therapeutic efficacy [1]. Polymers are extensively employed as carriers in advanced delivery platforms such as nanofibers, nanoparticles, hydrogels, and microspheres. However, before these systems can be deemed suitable for biomedical applications, their biological compatibility must be rigorously assessed [2].

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In this context, *in-vitro* cytotoxicity studies serve as a critical step in evaluating the safety of polymers and drug-loaded formulations. *In-vitro* cytotoxicity testing involves examining the toxic effects of materials on cultured cells under controlled laboratory conditions [3, 4]. These studies provide essential insights into cell viability, proliferation, morphology, and metabolic activity following exposure to polymers, drugs, or drug-polymer combinations. Since polymers in drug delivery systems directly interact with biological tissues, it is imperative to determine their potential to induce cellular damage, inflammation, or growth inhibition [5, 6]. Primarily, cytotoxicity evaluation must consider not only the polymer itself, but also the final drug polymer formulation. Drug polymer interactions can significantly influence toxicity, either mitigating harmful effects through controlled release or exacerbating them due to chemical

incompatibility. Thus, *in-vitro* studies are indispensable for optimizing polymer concentration, drug loading, and formulation parameters to achieve maximal therapeutic benefit with minimal adverse cellular effects. Another key role of cytotoxicity studies is screening novel biodegradable and biocompatible polymers. Materials such as poly (lactic acid), polycaprolactone, chitosan, alginate, and polyethylene glycol are routinely tested to confirm their safety in supporting cell growth [7]. These evaluations also align with regulatory requirements, as cytotoxicity testing is a prerequisite before advancing to *in-vivo* studies and eventual clinical applications. Overall, *in-vitro* cytotoxicity studies are a foundational step in developing polymer-based drug delivery systems. They provide crucial information on safety, compatibility, and therapeutic potential, thereby guiding researchers in the rational design of effective and biocompatible formulations for pharmaceutical and biomedical use.

MATERIAL AND METHOD: Dulbecco's modified Eagle's medium (DMEM), trypsin-EDTA solution, penicillin–streptomycin–Amphotericin solution, and fetal bovine serum (FBS) were procured from Himedia Laboratories Pvt. Limited, India.

Tinospora cordifolia fruits were collected from a neem tree planted in the health center of the Indian Institute of Technology (IIT), Kanpur, Uttar Pradesh (India) in the months of April-June. Authentication of plant material was carried out by Dr. K.M. Prabhukumar, Senior Scientist and Herbarium Curator, Plant Diversity, Systematics and Herbarium Division of CSIR-National Botanical Research Institute, Lucknow (PDSH/LWG/Authentication/Ang./2024-25/06) dated 08 April 2024. Authenticated plant fruits were then washed and dried in the shade for further use.

Antioxidant Studies: The Antioxidant activity of the mucilage of *Tinospora cordifolia* fruit was evaluated by the radical-scavenging of the DPPH (diphenyl-2-picryl-hydrazyl) method. A 1mg/ml solution of mucilage with various concentrations (60-100 ug/ml) was mixed with an equal amount of 0.1 mM DPPH in methanol and incubated in the dark at room temperature for 30 minutes. The absorbance was measured at 517 nm against methanol as a blank. A control containing methanol and DPPH was prepared, and Ascorbic acid was used as a reference standard [8, 9, 10]. The percentage inhibition was calculated using the following formula:

$$\% \text{ inhibition} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$$

***In-vitro* Cytotoxic Study:** The biocompatibility of the *T. cordifolia* fruit mucilage was assessed using the indirect leach- out method according to ISO 10993-5 standards (Biological evaluation of medical devices—Part 5, 2009), employing NIH3T3 fibroblast cells. For preparing the conditioned medium, mucilage samples were incubated with serum- free Dulbecco's Modified Eagle's Medium (DMEM) at a concentration of 10 mg/mL for 24 hours at 37 ° C. After incubation, the mixture was centrifuged at 1000 rpm for 10 minutes at room temperature, and the supernatant was collected and filtered through a 0. 22 µm syringe filter. Two serial dilutions were made from the 10 mg/mL stock to obtain concentrations of 1 mg/mL and 0. 1 mg/mL. Each filtrate was supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin to prepare complete conditioned media.NIH3T3 fibroblasts were seeded at a density of 1. 1.5×10^4 cells per well in 48- well plates and incubated for 24 hours to promote cell attachment. The culture medium was then replaced with 200 µL of conditioned medium corresponding to each concentration, and the cells were further incubated for 48 hours at 37 ° C in a humidified atmosphere containing 5% CO_2. All experiments were performed in triplicate [11-15].

Cell viability was evaluated using the Alamar Blue assay. After exposure to the conditioned media, 200 µL of 0. 01 mg/mL Alamar Blue reagent was added to each well and incubated for 4 hours at 37°C. Fluorescence intensity was recorded at excitation and emission wavelengths of 530 and 590 nm, respectively, using a Biotek Gen 5 microplate reader. Results were expressed as the percentage of viability relative to untreated controls as shown in **Fig. 1.** For qualitative analysis, cytocompatibility was further assessed by live/dead staining with Calcein- AM and Propidium Iodide (PI).

Viable cells emitted green fluorescence (Calcein-AM positive), while non- viable cells showed red fluorescence (PI positive). Stained cells were observed using a fluorescence microscope, and images were analyzed with Image J software over 48 hours at 37°C in a humidified atmosphere with 5% CO_2. All experiments were conducted in triplicate.

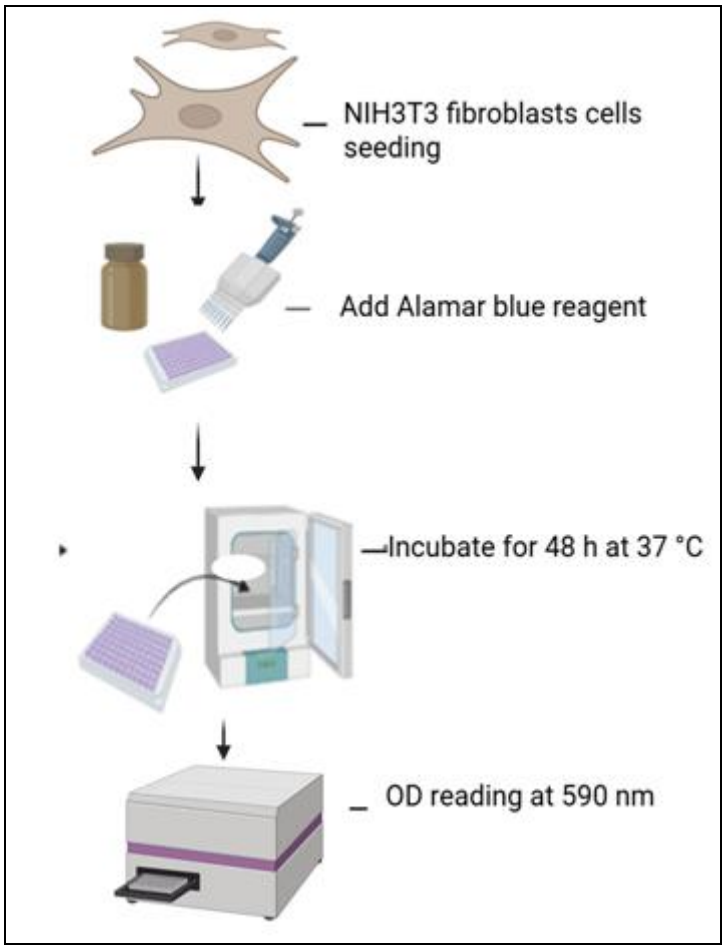

FIG. 1: SCHEMATIC DIAGRAM OF THE ALAMAR STUDY

RESULT AND DISCUSSION:

Antioxidant Activity: The antioxidant properties of the isolated fruit pulp mucilage were evaluated at concentrations ranging from 60 to 100 µg/mL, demonstrating a concentration-dependent increase in percentage inhibition for both ascorbic acid (R^2 = 0.9918) and the *Tinospora cordifolia* fruit pulp mucilage (R^2 = 0.9958). Ascorbic acid consistently showed higher inhibition percentages across all concentrations, increasing from 77% at 60 µg/mL to 90% at 100 µg/mL **Fig. 2.** In contrast, acetone-precipitated mucilage exhibited moderate antioxidant activity, with inhibition levels rising from approximately 30% at 60 µg/mL to 55% at 100 µg/mL. The IC_{50} value for ascorbic acid 1.64 µg/ml reflects its strong free radical scavenging ability, requiring a relatively low concentration to achieve 50% inhibition. Meanwhile, the IC_{50} value for *Tinospora cordifolia* fruit mucilage (10.54 µg/mL) indicates measurable antioxidant activity due to a reduction in the polyphenolic and flavonoid constituents, subsequently decreasing free radical scavenging activity.

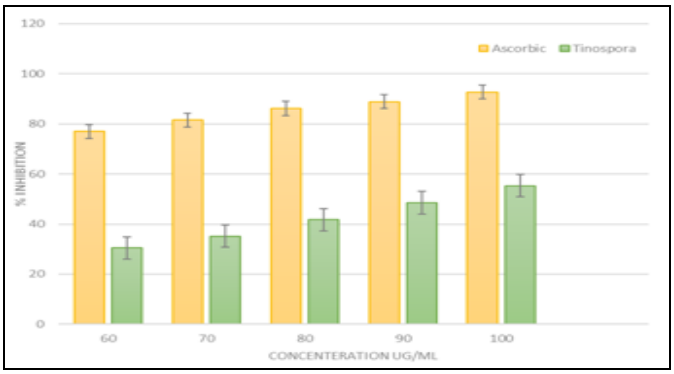

FIG. 2: ANTIOXIDANT ACTIVITY OF ISOLATED MUCILAGE

***In-vitro* Cytotoxic Study:** Cell viability of fibroblast (NIH3T3) cells exposed to mucilage-infused culture media was assessed using the Alamar Blue assay. It was observed that treatment with conditioned media at concentrations of 0.1, 1, and 10 mg/mL did not significantly reduce cell viability compared to the untreated control **Fig. 3**. In all tested concentrations, cell viability remained above 80%, indicating that the mucilage extract exhibited no cytotoxic effects, which is consistent with the ISO 10993-5 criteria for biocompatibility. Live/dead fluorescence imaging further confirmed these results, showing a predominance of green-fluorescent viable cells with minimal red-fluorescent dead cells across all treatment groups [15, 19, 20]. The results demonstrated that the mucilage is non-cytotoxic and biocompatible toward fibroblast cells, highlighting its potential as a polysaccharide-based carrier for drug delivery and as a regenerative component in biomedical formulations.

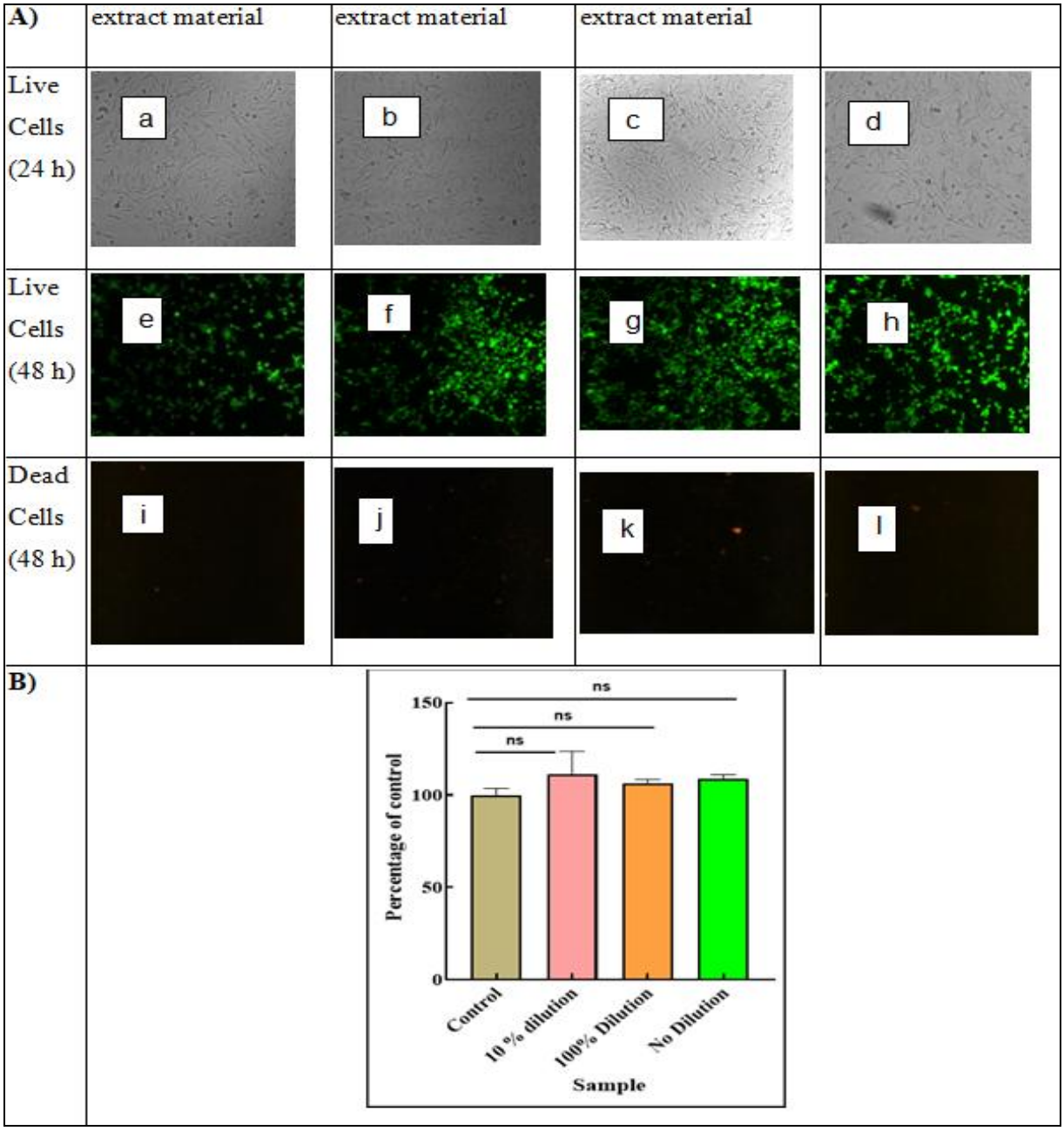

FIG. 3: (A). MICROSCOPIC IMAGES OF FIBROBLAST (NIH3T3) CELLS IN MUCILAGE DILUTIONS, NO DILUTION AND CONTROL (A, B, C, D) BEFORE ALAMAR BLUE ASSAY. FLUORESCENCE IMAGES OF LIVE CELLS (E, F, G, H) AND DEAD CELLS (I, J, K, L) ARE SHOWN IN GREEN AND RED, AND CONTROL. (B) RESULT OF THE INDIRECT LEACH-OUT METHOD USING ALAMAR BLUE ASSAY FOR 48 H

CONCLUSION: The present research successfully isolated a natural polymer from the fruit pulp mucilage of *Tinospora cordifolia*, a widely recognized medicinal herb in the Ayurvedic system. The extraction process yielded a polysaccharide-rich mucilage using alkaline extraction, followed by purification and characterization. In extraction and purification some solvents were used that can be toxic and cause cellular damage, beyond basic safety, these assays are vital for mapping complex dose-response dynamics, determining therapeutic windows, and understanding cellular mechanisms of action. The Alamar Blue assay and free radical scavenging activity demonstrated that the mucilage-based formulation maintained high cell viability, indicating its cytocompatibility and safety for biomedical applications. These findings demonstrate the potential of *Tinospora cordifolia* derived polymers as promising natural excipients for use in novel drug delivery systems. Their ability to provide controlled and/or targeted release of therapeutic agents makes them attractive alternatives to synthetic polymers. Moreover, this approach aligns with the growing demand for sustainable, biocompatible, and environmentally friendly materials in the pharmaceutical, cosmeceutical, and food industries. This study represents a significant step toward the development of innovative, nature-derived drug delivery platforms.

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CONFLICTS OF INTEREST: The authors declare no conflict of interest.

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