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*Research Article*

# Cytotoxic potential of high, medium and low molecular weight chitosan on L929 fibroblast and MCF-7 breast cancer cell lines: a comparative *in vitro* study

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**Background:** Chitosan, a commonly used biopolymer, has attracted a lot of attention because of its potential in various chemotherapeutic applications. Even though its biomedical properties have been thoroughly studied, systematic studies assessing how molecular weight affects its cytotoxic effects across various cell lines while holding other crucial parameters like pH and degree of deacetylation constant are still lacking. Information regarding the cytotoxic profiling of different molecular weight chitosan is necessary to optimize the therapeutic efficacy.

**Methods:** The current study aimed to evaluate the cytotoxic effects of high, medium, and low molecular weight chitosan on L929 fibroblast and MCF-7 breast cancer cell lines using the MTT assay. Different concentrations of low, medium, and high molecular weight chitosan were treated on both cell lines for 24 hours, followed by addition of MTT reagent. After 2 hrs incubation, the formazan crystals were dissolved in DMSO, and the optical density was measured to calculate cell viability.

**Results:** High molecular weight chitosan had the lowest cytotoxicity in L929 cells with an $IC_{50}$ of $496 \pm 37.46^{a}$ µg/mL, while low molecular weight chitosan exhibited the highest cytotoxicity with an $IC_{50}$ of $345 \pm 9.03^{c}$ µg/mL. Interestingly, in the case of the MCF-7 cell line, the high molecular weight chitosan exerted a higher cytotoxic effect with an $IC_{50}$ value of $217 \pm 5.72^{d}$ µg/mL, followed by $331 \pm 13.55^{c}$ µg/mL of medium and $479 \pm 14.75^{a}$ µg/mL of low molecular weight chitosan.

**Conclusion:** The current findings indicate that high molecular weight chitosan demonstrates greater compatibility compared to low and medium molecular weight chitosan in the L929 cell line. However, it also shows potential cytotoxic effects in the MCF-7 breast cancer cell line, suggesting its possible therapeutic application in drug delivery and cancer treatment.

***Keywords:*** *chitosan, L929 & MCF-7 cell lines, biocompatibility, cytotoxicity*

## Introduction

Cancer is a significant global health issue, and while chemotherapy remains a key treatment, its severe side effects highlight the need for targeted therapies and controlled drug delivery systems to improve efficacy and patient quality of life (Ding & Guo, 2022). Chitosan (Figure 1), a naturally derived biopolymer obtained from the deacetylation of chitin, and it has garnered significant interest in the biomedical field owing to its distinctive characteristics such as compatibility with biological systems, ease of degradation, and inherent antimicrobial activity. (Rafiq et al., 2024). The positively charged chitosan interacts effectively with negatively charged biological membranes, making it highly suitable for various biomedical applications such as wound repair, targeted drug delivery, and tissue engineering (de Sousa et al., 2020). Interestingly, the biological functions of chitosan are influenced by its physicochemical characteristics, such as molecular weight and degree of Deacetylation (Mikušová & Mikuš, 2021). This study evaluates the cytotoxic effects of high, medium, and low molecular weight chitosan using the MTT assay on two distinct cell lines: L929 fibroblasts (normal cells) and MCF-7 breast cancer cells. This investigation may provide more insight into cytocompatibility and cytotoxic effects of different molecular weights of chitosan on MCF-7 cancer cell line and L929 mouse fibroblast cell line. Also, clarifying these relationships may provide scientific insights relevant to the design of chitosan-based sustained-release drug formulations for therapeutic interventions, particularly in cancer treatments.

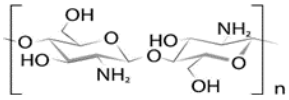



**Figure 1. Structure of chitosan**

## Materials and methods

### Preparation of different molecular weight chitosan

A precise amount of 0.25 g of low molecular weight (50-150 kDa), medium molecular weight (100-300 kDa), and high molecular weight chitosan (300-500 kDa) was measured using an analytical balance. Each chitosan sample was then dissolved in 25 mL of 1% acetic acid solution. The mixture was stirred continuously using a magnetic stirrer to ensure thorough dissolution of the chitosan into the acetic acid, facilitating uniform dispersion and the formation of a homogenous solution. The pH of all chitosan solutions was adjusted to 3.8 using acetic acid.

### MTT assay

MCF-7 and L929 cells were maintained in a $CO_2$ incubator set at 37 °C, with 5% $CO_2$ and 95% relative humidity. The cells were cultured in Dulbecco's Modified Eagle Medium (DMEM-LG), enriched with 10% fetal bovine serum and 0.01% ampicillin. To ensure proper osmolarity and facilitate routine washing of the cell cultures, 1X phosphate-buffered saline (PBS) with a pH of 7.4 was used. Cells were subcultured when they reached approximately 80% confluence, utilizing 1X Trypsin- EDTA. Trypsinized MCF-7 and L929 cells were seeded into a 96-well plate at a density of 10,000 cells per well and allowed to grow overnight to reach approximately 80% confluency. Following the incubation the spent media was discarded and replaced with serum free medium containing test substances. The cells were exposed in triplicates to different concentrations (200, 100, 50, 25, and 12.5 µg/mL) of low molecular weight chitosan, medium molecular weight chitosan, high molecular weight chitosan. The control group consisted of cells cultured in media without any test substances. Following a 24-hour incubation period, the media containing the test samples were discarded. Subsequently, 100 µL of MTT solution (0.5 mg/mL in 1X PBS) was added to each well, and the cells were incubated for 2 hours. Following this the MTT solution was removed, and the resulting purple formazan crystals were solubilized in 100 µL of 100% DMSO. The plates were incubated for an additional 10 minutes at room temperature. The absorbance of the resulting purple solution was measured at 570 nm to assess cell viability (Khorsandi et al., 2017). The cell viability was calculated using the formula

$$\text{Cell Viability} = \frac{\text{OD of the sample}}{\text{OD of the control}} * 100$$

A plot of concentration versus cell viability was drawn, and the data followed a straight-line equation, $y = m\,x + c$, where y is the percentage of cell viability, x is the concentration, m is the slope, and c is the intercept. The $IC_{50}$, or the concentration that reduces cell viability by 50%, was calculated by setting $y = 50$ and solving for x using,
$x = (50 - c)/m$

### Statistical analysis

The experiments were carried out in triplicates and One-way ANOVA was used to analyze the data. Significant differences between the mean value of the three replicates were determined by Duncan's multiple comparison test using the statistical software IBM SPSS2 (SPSS, Inc., Illinois, USA).

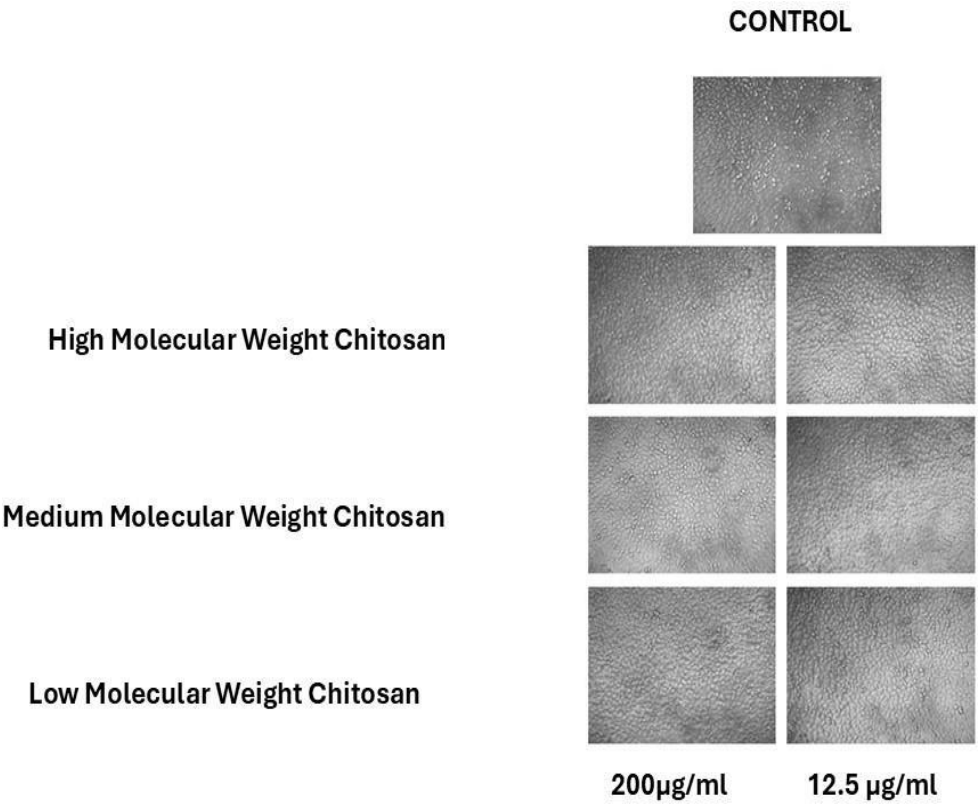
## Results

The half-maximal inhibitory concentration ($IC_{50}$) values for the different chitosan molecular weights were determined in L929 (Figure 2) fibroblast and MCF-7 (Figure 3) breast cancer cell line. In L929 cells high molecular weight chitosan exhibited a higher $IC_{50}$ value of 496 ± 37.46$^{a}$ µg/mL in comparison with medium molecular weight chitosan (409 ± 26.31$^{b}$ µg/mL), and low molecular weight chitosan (345 ± 9.03$^{c}$ µg/mL). These findings highlight the varying effects of chitosan molecular weight on cell viability. However, an intriguing exception to this trend emerged within the MCF-7 breast cancer cell line. High molecular weight chitosan showcased an impressive efficacy, marked by the lowest recorded $IC_{50}$ value of 217 ± 5.72$^{d}$ µg/mL (Table 1). This was followed by medium molecular weight chitosan at 331 ± 13.55$^{c}$ µg/mL and low molecular weight chitosan at 479 ± 14.75$^{a}$ µg/mL.

**Table 1. $IC_{50}$ values of different molecular weight chitosan using MTT assay in MCF-7 & L929 cell lines**

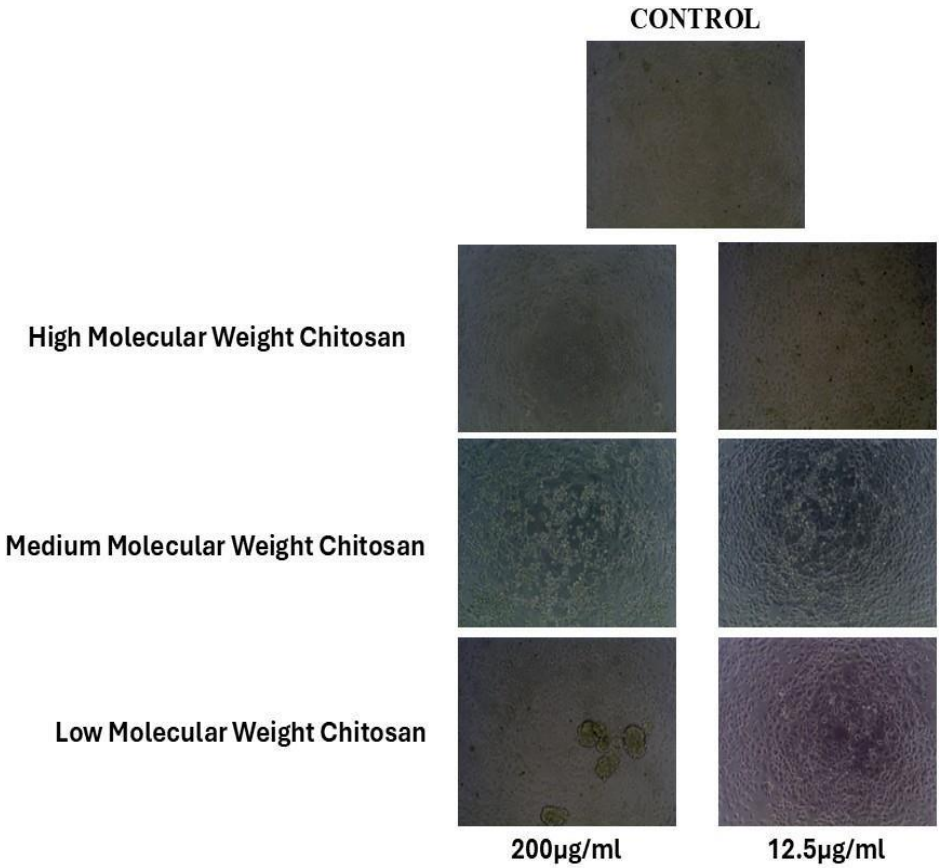
| Cell Line | Sample description | $IC_{50}$ value (µg/mL) |
|---|---|---|
| **L929** | High Molecular Weight Chitosan | 496 ± 37.46$^{a}$ |
| | Medium Molecular Weight Chitosan | 409 ± 26.31$^{b}$ |
| | Low Molecular Weight Chitosan | 345 ± 9.03$^{c}$ |
| **MCF 7** | High Molecular Weight Chitosan | 217 ± 5.72$^{d}$ |
| | Medium Molecular Weight Chitosan | 331 ± 13.55$^{c}$ |
| | Low Molecular Weight Chitosan | 479 ± 14.75$^{a}$ |

The results are expressed as mean ± SD (n=3). The values having different superscripts (a, b, c, d) are significantly ($p<0.05$) different from each other.




Images of L929 cells under different treatment conditions, captured at 10× magnification

**Figure 2. MTT assay: microscopic images of L929 fibroblast cells treated with high, medium, and low molecular weight chitosan.**




Images of MCF 7 cells under different treatment conditions, captured at 10× magnification

**Figure 3. MTT assay: microscopic images of MCF 7 breast cancer cells treated with high, medium, and low molecular weight chitosan**

## Discussion

The key factor influencing the biocompatibility and cytotoxic potential of chitosan in biomedical applications such as cancer drug delivery, tissue engineering, and wound healing is related to its molecular weight. In the present study, we have evaluated the effect of chitosan with varying molecular weights on the cytocompatibility and cytotoxicity profile in L929 cell line and MCF-7 breast cancer cell line, which are commonly used in vitro models for assessing cellular and molecular toxicity of anticancer drugs. To evaluate this, we employed MTT assay, a well-established and reliable method for measuring cellular metabolic activity, which reflects cell viability. The findings of the present study may provide insights for optimizing chitosan-based system in cancer therapeutics. This present research has shown that the cytocompatibility of chitosan is inversely proportional to its molecular weight in L929 cell line. This important observation concurs with previous studies conducted by (Chae et al., 2005) which highlighted that high molecular weight chitosan degrades very slowly, resulting in diminished cellular absorption compared to its low molecular weight counterpart. Chitosan has attracted considerable interest for its potential anticancer potential, functioning through multiple pathways including triggering apoptosis, promoting reactive oxygen species (ROS) production, and inhibiting cell proliferation (Adhikari & Yadav, 2018). The larger polymeric structure of high molecular weight chitosan likely facilitates prolonged and effective interactions with cellular surface and thereby inhibiting cellular uptake, membrane integrity, and downstream signaling pathways, and enhance its therapeutic capabilities. The lower $IC_{50}$ value of high molecular weight chitosan in MCF-7 cells reflects its comparatively higher anticancer potential over low and medium molecular weight chitosan. This enhanced activity is likely associated with its longer polymeric chains and greater abundance of protonated amino groups, which intensify electrostatic interactions with the negatively charged phospholipid components of cancer cell membranes. Such strong interactions promote closer adhesion, facilitate cellular uptake, and may disturb membrane integrity, thereby inducing cytotoxic effects. Our present finding concurs with previously reported experimental investigations (Shakil et al., 2021; Kean & Thanou, 2010; Park et al., 2011). The biofunctional properties of chitosan may be influenced by its molecular weight, as evidenced by high-molecular-weight fractions that exhibit superior inhibitory effects on cellular proliferation in cancer cell lines due to their higher charge density and stronger membrane affinity.

## Conclusion

In conclusion, high molecular weight chitosan inhibits the growth of MCF-7 breast cancer cells, demonstrating the lowest $IC_{50}$ value while maintaining excellent biocompatibility with fibroblast cells. This suggests its potential effectiveness for cancer treatment. The molecular weight-based efficacy of chitosan as biomaterials for drug delivery applications can be effectively utilized in tissue engineering or cancer therapy. The present study was limited to in vitro experiments, and further in vivo investigations are required to validate the anticancer potential of different molecular weight chitosan.

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## Author contributions

All authors contributed equally.

## Conflict of interest

The authors declare no conflict of interest.

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## Ethics approval

Not applicable.

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