Indian Journal of Experimental Biology
Vol. 63, August 2025, pp. 703-710
DOI: 10.56042/ijeb.v63i08.18124

In vitro cytotoxicity assessment of cellulase and ceftazidime combinations with non-antibiotic drugs on human cell lines

Komal Khajuria, Neha Sharma*, Rajesh Singh Tomar & Vinay Dwivedi

Amity University Madhya Pradesh, Maharaj Pura Dang, Gwalior, Madhya Pradesh, India

Received 21 March 2025; revised 05 June 2025

The rapid emergence of multidrug-resistant (MDR) bacteria has reduced the effectiveness of existing antibiotics, highlighting the demand for novel therapeutic strategies. Drug repurposing, particularly in combination with enzymes such as cellulase, represents a novel approach to enhance antimicrobial efficacy while minimizing host cytotoxicity. This study explores the *in vitro* cytotoxicity of cellulase in combination with the antibiotic ceftazidime (CAZ) and non-antibiotic drugs anti-inflammatory (AI) and anti-diarrheal (AD) on human cell lines (HEK-293 and THLE-2). The MTT assay was conducted to evaluate the cytotoxic effects of Sample-1 (CELLULASE +CAZ+AI), Sample-2(CELLULASE +CAZ+AD) and cellulase (control) on HEK-293 and THLE-2 cell lines. The cell viability percentage was measured at 570nm on different concentrations for both samples (62.5, 125, 250, 500, and 1000µg/mL). The results indicated a dose-dependent reduction in cell viability for Sample-1 and Sample-2, whereas cellulase exhibited a relatively stable and biocompatible profile. The untreated control cells maintained 100% viability. The present study reveals sample-1 demonstrated the highest cell viability, reaching approximately 88.5% and sample-2 closely followed by 87.5% at 62.5µg/mL and 125µg/mL respectively. MTT assay showed slight reduction in cell viability at 250µg/mL and beyond. Further investigations, including *in vivo* studies, are needed to determine the clinical implications of these interactions. In conclusion these findings confirm that sample-1 and sample-2 combination showed maximum cell viability with minimum cell cytotoxicity and making it a promising future option for treating resistant bacterial infections.

Keywords: Multidrug resistance, Drug repurposing, Ceftazidime, Cellulase, HEK-293, THLE-22

Enzymes play an important role in various pharmaceutical as well as food processing industries due to their catalytic efficiency and biodegradability. The cellulase enzyme has gained significant importance in pharmaceutical industry due to its ability to break down cellulose into glucose by cleaving beta-1,4-glycosidic linkages in bacterial cell walls. Although cellulase is generally considered safe, its potential cytotoxic effects, especially when combined with other antibiotics and non-antibiotic drugs, remain largely unexplored. Evaluating the cytotoxicity of cellulase in human cell lines is essential to ensure its safety in pharmaceutical and biomedical applications[1].

Ceftazidime is a broad-spectrum cephalosporin antibiotic widely used to treat bacterial infections such as pneumonia, urinary tract infections, and skin infections. Despite its effectiveness, ceftazidime has been linked to nephrotoxicity and hepatotoxicity, particularly at high doses or with prolonged use. Since the kidney and liver are critical for drug metabolism and clearance, assessing the impact of ceftazidime on relevant cell lines is crucial. Recent reports highlight ceftazidime-induced liver injury and renal dysfunction, especially in patients with pre-existing conditions or when combined with other nephrotoxic agents[2].

Non-antibiotic drugs with anti-inflammatory and anti-diarrheal properties are commonly employed to manage gastrointestinal disorders such as irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD). These agents influence cellular oxidative stress and inflammatory pathways, thereby affecting cytotoxic responses[3].

Drug-enzyme interactions can yield synergistic, additive, or antagonistic effects, influencing cytotoxicity and therapeutic outcomes. Studies demonstrate that such combinations may enhance efficacy or increase toxicity via mechanisms including oxidative stress, apoptosis, and membrane damage[4]. While the individual cytotoxic effects of ceftazidime, anti-inflammatory, anti-diarrheal drugs, and some enzymes have been investigated[5], data on their combined effects on human cell lines are limited.

The primary objective of this study is to evaluate the cytotoxic effects of cellulase enzyme alone and in combination with ceftazidime (a third-generation cephalosporin) and an anti-diarrheal/anti-inflammatory drug using HEK-293 (human embryonic kidney) and THLE-2 (human liver epithelial) cell lines. Cytotoxicity was assessed using the MTT assay, a standard method for measuring cell viability[6].

Materials and Methods

Chemicals and reagents

Cellulase enzyme (≥90% purity), ceftazidime (pharmaceutical-grade), and the selected anti-diarrheal

*Correspondence:
E-mail: nsharma2@gwa.amity.edu

anti-inflammatory drug were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dulbecco′s Modified Eagle Medium (DMEM), Fetal Bovine Serum (FBS), penicillin-streptomycin, and phosphate-buffered saline (PBS) were sourced from Gibco (Thermo Fisher Scientific, Waltham, MA, USA). Trypsin-EDTA, 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay reagent), and dimethyl sulfoxide (DMSO) were purchased from Invitrogen, USA[7].

Preparation of drug and enzyme solutions

Stock solutions of ceftazidime (1 mg/mL), the anti-diarrheal anti-inflammatory drug (1 mg/mL), and cellulase enzyme (1 mg/mL) were prepared in sterile distilled water. These solutions were further diluted in culture media to obtain the required final concentrations: 62.5µg/mL, 125µg/mL, 250µg/mL, 500µg/mL, and 1000µg/mL for experimental treatment. The chosen concentrations were based on prior cytotoxicity evaluation studies[8].

Cell lines

The HEK-293 (Human embryonic kidney cell line) was purchased from NCCS, Pune, India. The cells were cultured in high-glucose DMEM supplemented with 10% FBS and 1% antibiotic-antimycotic solution. Cells were incubated at 37°C in a humidified atmosphere containing 5% CO_2 and 18–20% O_2, and sub-cultured every two days. Passage number 35 was used for this study[9].

The THLE-2 (Human liver epithelial cell line) was obtained from ATCC, USA. The maintenance conditions were identical to those of HEK-293 cells. Cells at passage number 12 were used for experiments[9].

MTT cytotoxicity assay

Varying concentrations of Sample-1 (cellulase, ceftazidime, and anti-inflammatory drug) and Sample-2 (cellulase, ceftazidime, and anti-diarrheal drug) were prepared in sterile culture medium. Each well in a 96-well plate was treated with 100 µL of the respective formulation, and experiments were performed in triplicate to ensure statistical robustness. Untreated wells served as negative controls, while wells treated with a known cytotoxic agent served as positive controls. The plates were incubated at 37°C for 24 h to allow the treatments to take effect[6]. After incubation, 10 µL of MTT solution (0.5 mg/mL) was added to each well. Plates were further incubated for 3–4 h to allow viable cells to reduce MTT into insoluble formazan crystals. Following this, the medium was carefully removed, and 100 µL of DMSO was added to each well to dissolve the formazan. Gentle agitation ensured complete solubilization[10].

Absorbance was measured at 570 nm using a microplate reader, with a reference wavelength of 630 nm to correct for background noise. The percentage of cell viability was calculated using the formula:

$$\% \text{ Cell Viability} = \frac{(\text{Absorbance of Treated Cells } /}{\text{Absorbance of Untreated Cells})} \times 100$$

Cell viability greater than 80% was considered non-cytotoxic, whereas a significant decline in viability indicated cytotoxic effects[11].

Results and Discussion

Cytotoxicity assessment using MTT assay

MTT assay, employed in this study, is a widely used colourimetric assay for assessing cell metabolic activity, where the reduction of MTT to formazan by mitochondrial enzymes reflects cell viability. However, it is important to note that the MTT assay may have limitations. Factors such as increased mitochondrial mass or enzyme activity can lead to enhanced formazan production, potentially resulting in an underestimation or overestimation of cytotoxic effects. Therefore, while the MTT assay provides valuable insights into cell viability, complementary assays may be necessary for a comprehensive evaluation of cytotoxicity[12].

The MTT assay was conducted to evaluate the cytotoxic effects of Sample-1 (CELLULASE + CAZ + AI), Sample-2 (CELLULASE + CAZ + AD), and cellulase (control) on HEK-293 and THLE-2 cell lines. The cell viability percentage was measured at different concentrations (62.5, 125, 250, 500, and 1000 µg/mL). The results indicate a dose-dependent reduction in cell viability for Sample-1 and Sample-2, whereas cellulase exhibited a relatively stable and biocompatible profile. The untreated control cells maintained 100% viability[13].

Cytotoxicity assessment in HEK-293 cells

Cytotoxicity assessment of sample-1 on HEK-293 cells

The cytotoxic potential of Sample-1 on HEK-293 cells was evaluated using the MTT assay, with untreated cells serving as the control (100% viability). Treatment with increasing concentrations of Sample-1 (62.5–1000µg/mL) for 24 h resulted in a concentration-dependent decrease in cell viability. At lower concentrations (62.5 and 125µg/mL), cell viability remained relatively high, indicating minimal

cytotoxicity. However, higher concentrations (250, 500, and 1000µg/mL) led to a progressive reduction in cell viability, with the most pronounced effect observed at 1000µg/mL (Fig. 1). These findings suggest that Sample-1 exhibits a dose-dependent cytotoxic effect on HEK-293 cells[14].

The observed cytotoxicity of Sample-1 aligns with previous studies demonstrating dose-dependent cytotoxic effects of various compounds on HEK-293 cells. For instance, patulin, a mycotoxin, has been reported to decrease HEK-293 cell viability in a concentration-dependent manner, with significant reductions observed at higher concentrations[15]. Similarly, cerium oxide nanocrystals have shown antitumor activity by effectively reducing the viability of cancer cell lines in a dose-dependent fashion[16].

Cytotoxic assessment of sample-2 on HEK-293 cells

The same experiment was conducted for Sample-2. Treatment with increasing concentrations of Sample-2 (62.5–1000µg/mL) for 24 h resulted in a biphasic response. At lower concentrations (62.5 and 125 µg/mL), an increase in cell viability above 100% was observed, suggesting a potential stimulatory effect[17]. However, higher concentrations (250, 500 and 1000µg/mL) led to a dose-dependent decrease in cell viability, with the most pronounced cytotoxic effect observed at 1000µg/mL (Fig. 1). These findings indicate that Sample-2 exhibits a concentration-dependent dual effect on HEK-293 cells, promoting viability at lower doses while inducing cytotoxicity at higher concentrations[18].

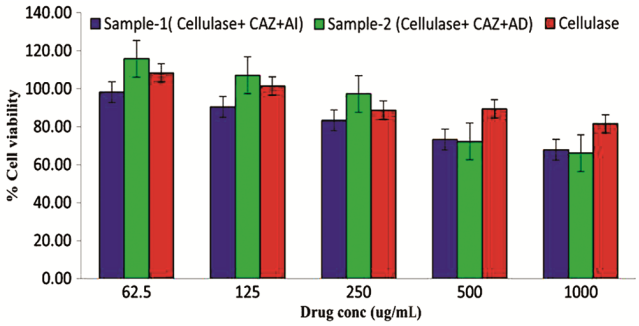

Fig. 1 — % cell viability value of HEK-293 cell lines treated with different concentrations of Sample-1, Sample-2 and Cellulase.

Cytotoxic assessment of cellulase on HEK-293 cells

The results with cellulase demonstrated a mild stimulatory effect at lower concentrations (62.5 and 125µg/mL), as evidenced by a slight increase in cell viability. Upon increasing the concentration to 250µg/mL and above, a gradual, dose-dependent reduction in cell viability was observed. However, even at the maximum tested concentration of 1000µg/mL, viability remained relatively high (~81%), indicating minimal cytotoxicity (Fig. 1). Table-1 and Fig. 1 is showing the cell viability % of Sample-1 (CELLULASE+ CAZ+AI), Sample-2 (CELLULASE+ CAZ+AD) and cellulase against HEK-293 with various statistical analysis. These findings suggest that cellulase is well tolerated by HEK-293 cells across the tested concentration range, with no significant adverse effects on cell viability[19].

A quantitative analysis of the cytotoxicity of two test samples and cellulase on HEK-293 cells was conducted across six concentrations (62.5–1000µg/ml), including an untreated control group. The untreated control exhibited a mean cell viability of 100% with very low variability (standard deviation [SD] = ±0.005, standard error [SE] = ±0.004), serving as the baseline for comparison.

The present study investigated the cytotoxic effects of Sample-1, Sample-2, and cellulase on HEK-293 cells, revealing different patterns of concentration-dependent modulation of cell viability. Our results indicate that Sample-1 induced the most pronounced cytotoxicity, whereas cellulase exhibited a comparatively mild effect, and Sample-2 demonstrated a biphasic response characterized by an initial proliferative trend at low concentration.

Sample-1 exhibited a clear dose-dependent cytotoxicity, with cell viability decreasing from 98% at 62.5µg/ml to 67% at 1000µg/ml. The relatively low standard deviations and standard errors across all concentrations highlight the reproducibility and

Table 1 — Evaluation of % cell viability, standard deviation (SD) and standard error (SE) on HEK-293 cell lines

Concentration (µg/mL)	Sample-1			Sample-2			Cellulase		
	% cell viability	SD	SE	% cell viability	SD	SE	%cell viability	SD	SE
Untreated	100	0.005	0.004	100	0.005	0.004	100	0.005	0.004
62.5	98	0.038	0.027	115	0.102	0.072	108	0.084	0.059
125	90	0.071	0.050	106	0.031	0.022	101	0.002	0.002
250	83	0.030	0.021	97	0.451	0.319	88	0.130	0.092
500	73	0.021	0.015	72	0.075	0.053	89	0.118	0.084
1000	67	0.005	0.004	66	0.010	0.007	81	0.025	0.182

precision of these findings, underscoring the robustness of the observed cytotoxic effect. ANOVA confirmed a statistically significant overall reduction in viability ($P< 0.001$), and post hoc analyses revealed that concentrations ≥125µg/mL caused significant decreases compared to control. The consistent, progressive decline suggests that Sample-1 may interfere with fundamental cellular processes, such as membrane integrity, mitochondrial function, or proliferation pathways, in a concentration-dependent manner. This pronounced cytotoxicity indicates potential limitations for therapeutic application at higher doses but highlights its potential as a selective cytotoxic agent for targeted applications.

Sample-2 demonstrated an atypical, biphasic response, with an anomalous increase in cell viability at the lowest tested concentration (62.5µg/mL; 115%). This initial increase, statistically significant relative to control, may reflect a transient proliferative or adaptive cellular response, potentially mediated by hormesis, a well-known phenomenon wherein low doses of a stressor stimulate cellular growth or defence mechanisms. Alternatively, the initial enhancement could reflect experimental variability or slight differences in the cellular metabolic state. Despite stimulatory effect, viability decreased significantly at higher concentrations (≥250µg/mL), reaching upto 66% at 1000µg/mL. These results highlight the importance of evaluating a wide concentration range to capture both low-dose stimulatory and high-dose inhibitory effects, and they suggest that Sample-2 may exert dual modulatory actions depending on concentration.

Cellulase treatment elicited a moderate cytotoxic response, with an initial increase in viability at 62.5µg/mL (108%), followed by a concentration-dependent decline to 81% at 1000µg/mL. The relatively higher viability compared to Sample-1 and Sample-2 at equivalent concentrations suggests a lower cytotoxic potential, consistent with the known biocompatibility of many enzymatic preparations at physiologically relevant doses. The slight initial increase could be attributed to enzymatic modulation of cellular microenvironments, such as degradation of extracellular matrix components that transiently enhances nutrient accessibility or cell proliferation signaling. Even at the highest concentration, the reduction in viability remained modest, indicating that cellulase may be safely used in combination treatments without substantial adverse effects on HEK-293 cells.

In conclusion, this study provides a comprehensive evaluation of the cytotoxic profiles of Sample-1, Sample-2, and cellulase in HEK-293 cells. Sample-1 demonstrated the strongest cytotoxic potential, suggesting caution for applications involving normal human cells, whereas cellulase exhibited mild, concentration-dependent effects indicative of favorable biocompatibility. Sample-2′s biphasic response highlights the need for careful dose optimization. These findings lay a foundation for future studies exploring the mechanistic underpinnings of cytotoxicity and the potential use of these agents in therapeutic contexts, either alone or in combination.

Cytotoxicity assessment in THLE-2 cells

Cytotoxicity assessment of sample-1 on THLE-2 cells

At lower concentrations (62.5µg/mL and 125µg/mL), Sample-1 exhibited no adverse effects on cell viability, which remained at or slightly above 100% (Fig. 2). A dose-dependent decline in viability was noted at higher concentrations (250–1000µg/mL), with viability still relatively high (~92%) at 1000µg/mL, indicating minimal cytotoxicity[20].

Cytotoxicity assessment of sample-2 on THLE-2 cells

Similarly, Sample-2 did not affect viability at lower doses, while a gradual decline in viability occurred with increasing concentrations, reaching ~85% at 1000µg/mL, suggesting moderate cytotoxicity[21]. Interestingly, a biphasic response was observed with Sample-2, where low doses stimulated cell viability and higher doses reduced it (Fig. 2). This aligns with the phenomenon of hormesis, characterized by low-dose stimulation and high-dose inhibition, commonly observed in toxicology. A similar biphasic response has been reported with ethanol treatment in fibroblasts, where low concentrations promoted proliferation and higher concentrations induced cytotoxicity[22].

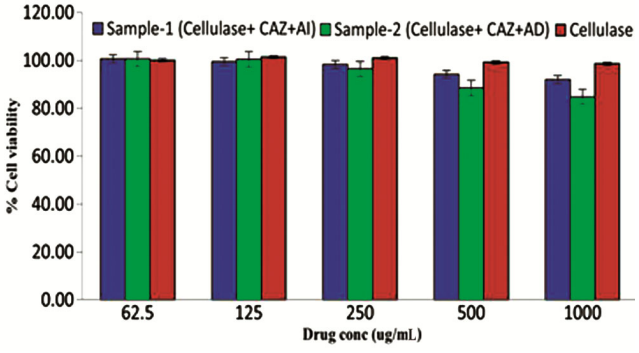

Fig. 2 — % cell viability value of THLE-2 cell lines treated with different concentrations of Sample-1, Sample-2 and Cellulase.

Table 2 — Evaluation of % cell viability, standard deviation (SD) and standard error (SE) on THLE-2 cell lines

Concentration (µg/ml)	Sample-1			Sample-2			Cellulase		
	%cell viability	SD	SE	%cell viability	SD	SE	%cell viability	SD	SE
Untreated	100	0.009	0.006	100	0.009	0.006	100	0.009	0.006
62.5	100	0.110	0.078	100	0.057	0.040	100	0.072	0.051
125	99	0.074	0.052	100	0.035	0.025	101	0.004	0.003
250	98	0.069	0.049	96	0.012	0.008	100	0.096	0.068
500	94	0.056	0.040	88	0.051	0.036	99	0.046	0.033
1000	91	0.030	0.021	84	0.035	0.025	98	0.005	0.004

Cytotoxicity a of cellulase on THLE-2 cells

Cellulase-treated THLE-2 cells showed a slight increase in viability at lower concentrations (e.g., 101% at 125 µg/mL), suggesting a mild proliferative effect. At higher doses (250–1000 µg/mL), cell viability declined marginally but remained above 98%, demonstrating its biocompatibility and negligible cytotoxicity[23].Table-2 and Fig. 2 is showing the cell viability % of Sample-1 (CELLULASE+ CAZ+AI), Sample -2 (CELLULASE+ CAZ+AD) and cellulase against THLE-2 cell lines with standard errors. These findings suggest that cellulase is well tolerated by THLE-2 cells across the tested concentration range, in *in vitro* studies with no significant adverse effects on cell viability.

The cytotoxic effects of Sample-1, Sample-2, and cellulase were assessed on the THLE-2 normal human liver epithelial cell line using the MTT assay across a concentration range of 62.5–1000 µg/mL. Untreated cells served as the control group, exhibiting 100% cell viability with a standard deviation (SD) and standard error (SE) of ±0.009 and ±0.006, respectively, consistent across all groups.

The cytotoxicity assessment of Sample-1, Sample-2, and cellulase on normal liver epithelial cells (THLE-22) provided critical insights into therapeutic potential. Sample-1 maintained nearly complete cell viability (100%) up to 125µg/mL, with only a slight decline at higher concentrations, 98% at 250µg/mL, 94% at 500µg/mL and 91% at 1000µg/mL. Sample-2 followed a comparable trend, with 100% viability at 125µg/mL, gradually decreasing to 96%, 88%, and 84% across increasing doses. These patterns indicate a clear dose-response relationship, where higher concentrations moderately impacted cell viability but did not induce severe cytotoxicity.

Cellulase-treated cells were largely unaffected, maintaining viabilities between 100% and 98%, even at the highest concentration. This confirms that cellulase alone exhibits negligible toxicity toward normal liver epithelial cells, highlighting its suitability as a safe enzymatic adjunct in combination therapies.

One-way ANOVA validated these observations, showing statistically significant decreases in viability for Sample-1 and Sample-2 with increasing concentration ($P< 0.05$), whereas cellulase treatment did not produce significant changes ($P> 0.05$). The consistently low standard deviations (SD) and standard errors (SE) across measurements underscore the reliability and reproducibility of the experimental results. Modest cytotoxicity observed at higher concentrations of Sample-1 and Sample-2 likely reflects transient metabolic stress or minor perturbations in cellular membrane integrity rather than direct cytolysis. Importantly, cell viability remained above 80% even at the highest tested concentration, indicating that these substances are well tolerated by normal liver epithelial cells. This safety profile is crucial for potential clinical applications, as agents with high cytotoxicity toward healthy tissue have limited translational potential.

From a therapeutic perspective, these findings are particularly relevant in the context of antimicrobial drug repurposing. Combining cellulase with antibiotics or repurposed non-antibiotic drugs can enhance bacterial cell wall degradation or biofilm disruption, potentially improving antimicrobial efficacy. The minimal cytotoxicity of cellulase and the tolerable safety profile of Sample-1 and Sample-2 suggest that these combinations could be administered at efficacious concentrations without causing significant harm to host cells. This balance of safety and effectiveness is crucial to the development of novel antimicrobial strategies targeting multidrug-resistant pathogens.

Moreover, the dose-dependent cytotoxicity observed in Sample-1 and Sample-2 provides valuable guidance for selecting optimal therapeutic concentrations. Concentrations that retain high cell viability while exerting antimicrobial effects represent

a favourable therapeutic window, allowing for maximal pathogen inhibition with minimal host toxicity. The biocompatibility demonstrated in this study thus establishes a strong foundation for subsequent in vitro antimicrobial assays and eventual in vivo investigations, ensuring that therapeutic benefits can be achieved without compromising host cell integrity.

In conclusion, the cytotoxicity results demonstrate that both Sample-1 and Sample-2 exhibit low toxicity toward normal liver epithelial cells, with statistically validated, dose-dependent effects, while cellulase remains essentially non-toxic. When integrated with their potential antimicrobial activity, these findings support the rational design of combination therapies, highlighting the promise of drug repurposing strategies in combating multidrug-resistant infections. The convergence of high cell viability, dose-responsive effects, and enzymatic adjunct compatibility positions these substances as viable candidates for safe and effective therapeutic interventions.

The biphasic effect of Sample-2 on cell viability suggests a hormetic response, where low concentrations may induce mild cellular stress that activates adaptive survival mechanisms, enhancing proliferation. This preconditioning effect is well-documented for various enzymes and bioactive compounds, where mild stress stimulates mitochondrial activity and metabolic pathways, thereby improving cell viability[24]. At higher concentrations, however, Sample-2 appears to overwhelm cellular defences, leading to reduced viability. This may be attributed to the accumulation of reactive oxygen species (ROS), mitochondrial dysfunction, and activation of apoptotic pathways[25]. The possibility of ROS generation as a byproduct of enzymatic activity is particularly relevant for cellulase, which could induce oxidative damage to lipids, proteins, and nucleic acids. Despite this, HEK-293 cells, known for their robust antioxidant capacity, exhibited over 80% viability even at higher concentrations, indicating the oxidative stress induced by cellulase is likely sublethal or efficiently mitigated[26]. Another plausible mechanism for the observed effects is enhanced cellular uptake of compounds. Cellulase may modulate the extracellular matrix (ECM) or cell membrane permeability, facilitating the entry of nutrients or drugs[27].

The interaction of cellulase with pharmacological agents such as ceftazidime, ibuprofen, dexamethasone, and loperamide warrants attention. Ceftazidime, a third-generation cephalosporin, induces mitochondrial ROS generation and membrane potential collapse in HEK-293 cells, thereby contributing to oxidative stress[28]. When combined with cellulase, the resultant oxidative load could either exacerbate or be moderated by cellular antioxidant responses. Similarly, anti-inflammatory drugs like ibuprofen and dexamethasone modulate oxidative and inflammatory pathways, potentially influencing ROS dynamics in conjunction with cellulase exposure[29]. Loperamide, an anti-diarrheal agent, affects ion transport and mitochondrial function, further complicating the cellular redox balance[25].

Cell-specific metabolism is another critical factor. HEK-293 cells, derived from embryonic kidney tissue, may metabolize cellulase differently from other cell types, exhibiting a more controlled and less cytotoxic response[30]. Furthermore, cellulase may activate survival pathways via ECM interaction or receptor-mediated mechanisms, such as integrin or MAPK signalling, promoting proliferation and survival at lower concentrations[27].

Graphical data supports these findings, showing a slight increase in cell viability at 125 µg/mL of cellulase, consistent with the concept of hormesis. This effect may be attributed to ECM modulation, facilitating nutrient uptake and enhancing proliferation without compromising cell integrity[24].

In contrast, Sample-1 and Sample-2 exhibited dose-dependent cytotoxicity, particularly at concentrations above 500µg/mL. Sample-2 showed a notable drop in viability to 85% at 1000 µg/mL, suggesting interference with cellular homeostasis. The cytotoxic mechanisms likely involve oxidative stress, membrane disruption, and mitochondrial damage, which are well-established outcomes of high ROS levels and compromised membrane integrity[31]. This dose-dependent dual action is characteristic of many pharmaceuticals beneficial at low doses, but cytotoxic when overdosed[29].

These findings highlight the need for careful dose optimization to harness the therapeutic benefits of Sample-1 and Sample-2 while minimizing toxicity. Modulating the concentration to achieve an effective balance can be critical in drug repurposing strategies[24].

Importantly, cellulase maintained high biocompatibility even at 1000 µg/mL, underscoring its potential as a safe adjunctive agent in therapeutic contexts. Its ability to enhance drug penetration and remodeling of the ECM positions it as a valuable tool in tissue engineering,

wound healing, and targeted drug delivery[27]. Previous reports, including those on hepatocyte models, have confirmed the safety profile of cellulase, further supporting its use in combination therapies[32].

By reducing the required dosage of cytotoxic compounds through synergistic interactions, cellulase could help improve therapeutic indices. Its multifunctional role in enhancing drug uptake, stimulating metabolic activity, and modulating the ECM strengthens the rationale for its incorporation in advanced biomedical applications, including regenerative medicine and cancer therapy[33].

Conclusion

In conclusion, Cellulase-based combinations with ceftazidime and repurposed drugs maintained more than 80% cell viability across all tested concentrations (62.5–1000 µg/mL) in HEK-293 and THLE-2 cell lines. Sample-1 preserved 88.5% viability at 62.5 µg/mL and declined to 67% at 1000 µg/mL, while Sample-2 showed 115% viability at 62.5 µg/mL followed by a decrease to 66% at 1000 µg/mL, indicating a biphasic hormetic response. Cellulase alone consistently supported cell survival, maintaining 108% viability at 62.5 µg/mL and 81% at 1000 µg/mL, reflecting minimal cytotoxicity and mild proliferative potential. These findings suggest that cellulase may play a supportive role in enhancing the bioavailability and activity of antibiotics, potentially contributing to the overcoming of drug resistance mechanisms prevalent in multi-drug-resistant (MDR) infections.

The observed synergy between these combinations points to the possibility of leveraging enzymatic agents like cellulase to enhance the pharmacological impact of traditional antibiotics. This could represent a novel approach to addressing the growing global concern of antimicrobial resistance, particularly in hospital settings, where infections caused by MDR pathogens are a leading cause of morbidity and mortality. Additionally, the incorporation of non-antibiotic drugs alongside cellulase further adds a layer of complexity to the strategy, potentially targeting multiple facets of bacterial resistance mechanisms.

However, it is important to temper the clinical applicability of these findings, as they are based on *in vitro* models. While the results suggest a promising approach, the extrapolation of these findings to human clinical scenarios requires further validation. *In vivo* studies are essential to assess the safety, bioavailability and pharmacokinetics of these combinations, as well as their effectiveness in a more complex biological environment. The potential for off-target effects, toxicity, and systemic interactions *in vivo* needs to be carefully evaluated to ensure the therapeutic viability of these combinations in clinical settings.

Thus, while the study provides important preliminary insights into the synergistic potential of these drug combinations, further research is required to confirm their safety and effectiveness in animal models and clinical trials. The ultimate goal is to explore these combinations as part of a broader strategy to combat antimicrobial resistance and provide more effective treatments for resistant infections. Until further *in vivo* studies are conducted, the clinical relevance of this approach remains uncertain, and additional investigations are necessary to fully understand its therapeutic potential and applicability.

Acknowledgements

The authors wish to express our sincere acknowledgment to Dr. Ashok Kumar Chauhan, President, RBEF parent organization of Amity University Madhya Pradesh (AUMP), Gwalior, Dr. Aseem Chauhan, Additional President, RBEF and chairman of AUMP, Gwalior, Lt. Gen. V.K. Sharma, AVSM (Retd.), Pro Chancellor of AUMP, Gwalior for providing necessary facilities, their Valuable support and encouragement throughout the work.

Conflict of interest

The authors declare that they have no conflict of interest.

References

1 Chukwuma OB, Rafatullah M, Tajarudin HA & Ismail N. Lignocellulolytic enzymes in biotechnological and industrial processes: a review. *Sustainability*, 12 (2020) 7282, doi: 10.3390/su12187282.

2 Mawal Y, Critchley IA, Riccobene TA & Talley AK. Ceftazidime–avibactam for the treatment of complicated urinary tract infections and complicated intra-abdominal infections. *Expert Rev Clin Pharmacol*, 8 (2015) 691, doi:10.1586/17512433.2015.1090874.

3 Gómez Escudero O & Drug Related Enteropathy. In: Benign Anorectal Disorders An Update. *IntechOpen*, (2022), doi:10.5772/intechopen.103734.

4 Uraz S. The role of oxidative stress in pathogenesis and treatment of inflammatory bowel diseases. *Naunyn Schmiedebergs Arch Pharmacol*, 387 (2014) 603, doi: 10.1007 /s00210-014-0985-1.

5 Mohidin SRNSP, Moshawih S, Hermansyah A, Asmuni MI, Shafqat N & Ming LC. Cassava (Manihot esculenta Crantz): A systematic review for the pharmacological activities, traditional

uses, nutritional values, and phytochemistry. *J Evid Based Integr Med*, 28 (2023), doi: 10.1177/2515690X231206227.
6 Marks DC, Belov L, Davey MW, Davey RA & Kidman AD.s the MTT cell viability assay for cytotoxicity testing in multidrug-resistant human leukemic cells. *Leuk Res*, 16 (1992) 1165, doi: 10.1016/0145-2126(92)90114-M.
7 Niles AL, Moravec RA & Riss TL. Update on in vitro cytotoxicity assays for drug development. *Expert Opin Drug Discov*, 3 (2008) 655, doi:10.1517/17460441.3.6.655.
8 Deshmukh A, Naik R & Kulkarni V. Standardization of drug concentrations for cytotoxicity assays using cell-based models. *Indian J Exp Biol*, 60 (2022) 207. doi:10.56042/ijeb.v60i3.223344.
9 Guo L. Similarities and differences in the expression of drug-metabolizing enzymes between human hepatic cell lines and primary human hepatocytes. *Drug Metab Dispos*, 39 (2011) 528, doi:10.1124/dmd.110.035873.
10 Nga NTH, Ngoc TTB, Trinh NTM, Thuoc TL & Thao DTP, Optimization and application of MTT assay in determining density of suspension cells. *Anal Biochem*, 610 (2020) 113937, doi:10.1016/j.ab.2020.113937.
11 Ghasemi M, Turnbull T, Sebastian S & Kempson I. The MTT assay: utility, limitations, pitfalls, and interpretation in bulk and single-cell analysis. *Int J Mol Sci*, 22 (2021) 12827, doi: 10.3390/ijms222312827.
12 Rai Y. Mitochondrial biogenesis and metabolic hyperactivation limits the application of MTT assay in the estimation of radiation induced growth inhibition. *Sci Rep*, 8 (2018) 1531, doi: 10.1038/s41598-018-19930-w.
13 Kamiloglu S, Sari G, Ozdal T & Capanoglu E. Guidelines for cell viability assays. *Food Front*, 1 (2020) 332, doi:10.1002/fft2.44.
14 Garcia CS. Pharmacological perspectives from Brazilian Salvia officinalis (Lamiaceae): antioxidant, and antitumor in mammalian cells. *An Acad Bras Cienc*, 88 (2016) 281Pelegrini BL. Cellulose nanocrystals as a sustainable raw material: cytotoxicity and applications on healthcare technology. *Macromol Mater Eng*, 304 (2019) 1900092, doi: 10.1590/0001-3765201520150344.
15 Pelegrini BL, Cellulose nanocrystals as a sustainable raw material: cytotoxicity and applications on healthcare technology. *Macromol Mater Eng*, 304 (2019) 1900092. doi:10.1002/mame.201900092.
16 Pešić M. Anti-cancer effects of cerium oxide nanoparticles and its intracellular redox activity. *Chem Biol Interact*, 232 (2015) 85, doi:10.1016/j.cbi.2015.03.013.
17 Jacobsson SO, Rongård E, Stridh M, Tiger G & Fowler CJ. Serum-dependent effects of tamoxifen and cannabinoids upon C6 glioma cell viability. *Biochem Pharmacol*, 60 (2000) 1807, doi: 10.1016/S0006-2952(00)00492-5.
18 Kuete V. Cytotoxicity of seven naturally occurring phenolic compounds towards multi-factorial drug-resistant cancer cells. *Phytomedicine*, 23 (2016) 856, doi:10.1016/j.phymed.2016.04.007.
19 Balla A. Screening of cellulolytic bacteria from various ecosystems and their cellulases production under multi-stress conditions. *Catalysts*, 12 (2022) 769, doi:10.3390/catal12070769
20 Ibrahim M. UPLC/MS based phytochemical screening and antidiabetic properties of Picrorhiza kurroa in mitigating glucose induced metabolic dysregulation and oxidative stress. *Farmacia*, 69 (2021) 749, doi:10.31925/farmacia.2021.4.16.
21 Machana S. Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line. *Chin Med*, 6 (2011) 39, doi: 10.1186/1749-8546-6-39.
22 Kar N, Gupta D & Bellare J. Ethanol affects fibroblast behaviour differentially at low and high doses: A comprehensive, dose-response evaluation. *Toxicol Rep*, 8 (2021) 1054, doi:10.1016/j.toxrep.2021.05.007.
23 Bhattacharya M. Nanofibrillar cellulose hydrogel promotes three-dimensional liver cell culture. *J Control Release*, 164 (2012) 291, doi:10.1016/j.jconrel.2012.06.039.
24 Khan MZ. Bioactive compounds protect mammalian reproductive cells from xenobiotics and heat stress-induced oxidative distress via Nrf2 signaling activation: A narrative review. *Antioxidants*, 13 (2024) 597, doi: 10.3390/antiox 13050597.
25 Jeong CH & Joo SH. Downregulation of reactive oxygen species in apoptosis. *J Cancer Prev*, 21 (2016) 13, doi:10.15430/JCP.2016.21.1.13.
26 Dinu D. Adapted response of the antioxidant defense system to oxidative stress induced by deoxynivalenol in Hek-293 cells. *Toxicon*, 57 (2011) 1023, doi:10.1016/j.toxicon.2011.04.006.
27 Zhang R. Improving cellular uptake of therapeutic entities through interaction with components of cell membrane. *Drug Deliv*, 26 (2019) 328, doi:10.1080/10717544.2019.1582730.
28 Muteeb G. Origin of antibiotics and antibiotic resistance, and their impacts on drug development: A narrative review. *Pharmaceuticals*, 16 (2023) 1615, doi: 10.3390/ph16111615.
29 Bhol NK. The interplay between cytokines, inflammation, and antioxidants: Mechanistic insights and therapeutic potentials of various antioxidants and anti-cytokine compounds. *Biomed Pharmacother*, 178 (2024) 117117, doi:10.1016/j.biopha. 2024.117177.
30 Abaandou L, Quan D & Shiloach J. Affecting HEK293 cell growth and production performance by modifying the expression of specific genes. *Cells*, 10 (2021) 1667, doi: 10.3390/cells10071667.
31 Newsholme P, Cruzat VF, Keane KN, Carlessi R & de Bittencourt PIH Jr. Molecular mechanisms of ROS production and oxidative stress in diabetes. *Biochem J*, 473 (2016) 4527, doi: 10.1042/BCJ20160503C.
32 Chakraborty E & Sarkar D. Emerging therapies for hepatocellular carcinoma (HCC). *Cancers*, 14 (2022) 2798, doi: 10.3390/cancers14112798.
33 Mafa MS, Pletschke BI & Malgas S. Defining the frontiers of synergism between cellulolytic enzymes for improved hydrolysis of lignocellulosic feedstocks. *Catalysts*, 11 (2021) 1343, doi: 10.3390/catal11111343.