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Comparative mechanistic profiling of cytotoxic agents: Role of redox imbalance, metabolic suppression, and protein damage in a bacterial model

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ABSTRACT

Cytotoxicity testing is an important aspect of pharmacological or toxicological investigation, but knowing about cell injury mechanisms is also needed in order for the data to be interpreted the right way. Cytotoxicity test is an important part of pharmacological and toxicological investigation, but it also needs scientists to know about the cellular injury mechanism so that the data can be understood correctly. Four main chemicals, hydrogen peroxide, sodium azide, copper sulfate, and mercuric chloride, were checked for how they affected the metabolism of the cells, caused oxidative stress to appear, and also broke proteins. Cellular metabolic function was studied with the MTT test, while oxidative stress was investigated using mixes of hydrogen peroxide with other agents. Protein harm was checked using the ninhydrin test by measuring more free amino acids. Concentration ranges (1, 3, and 5 mM) were used for all tests during controlled conditions. The results showed different types of toxic behaviors for chemicals that were tested in the experiment. Hydrogen peroxide showed strong toxicity because of causing oxidative stress, as seen by much lower metabolic activities and proteins being damaged more. Sodium azide stopped most of the metabolism, but did not cause a lot of oxidative stress at all. Copper sulfate results in both an increase in oxidative stress and also messes with metabolic actions. Instead, mercuric chloride causes big destruction in proteins, meaning it causes them to lose their natural shape, so that most enzymes do not work. Using hydrogen peroxide with other chemicals made the toxic effects stronger, showing that maybe there is a synergistic effect due to oxidation not being in balance. The researchers try for a simple procedure that is still good for giving classification about how toxic these agents work, using basic biochemistry methods. These findings help to see better how toxicity is developed, and show that one must check many parts when screening toxic effects for medical purposes and to ensure protection for safety.

Keywords: Cytotoxicity; MTT assay, Oxidative stress, Protein damage, Ninhydrin assay, Redox imbalance, Sodium azide, Mercuric chloride, Pharmacological mechanisms

1. INTRODUCTION

Cytotoxicity refers to the ability of a material to injure or sometimes kill living cells. This concept is fundamental in fields such as pharmacology, toxicology, and also in biomedical research, since it allows scientists to determine how medicines, chemicals, or environmental agents may affect biological systems (Bahadar and Abdollahi, 2015). Learning about the cytotoxicity is a required step when drugs are being developed to ensure substances involved are not just working for their job but also will not be dangerous for people to use. In industrial and environmental fields, understanding cytotoxicity matters too, since when toxic stuff gets around, it can be bad for organisms (Capoor and Bhowmik, 2017).

Cells in living beings are organized and do important jobs like metabolism, the creation of energy, making proteins, and controlling their inner environment. If something messes up these functions, then the cell can be ruined or can stop working. Chemicals work on cells in different kinds of ways that depend on what the chemical really is and how it acts (Miller and Zachary, 2017). For instance, some types will mess with metabolism by slowing down enzymes or turning off how cells make energy, and this lowering of activity causes cells to die. There is also a thing called oxidative stress, when special molecules called ROS or reactive oxygen species harm parts of cells like fats, proteins, or the DNA itself. When there are more of these molecules than a cell can handle, oxidative stress happens and brings about both damage in structure and in the working of cells. Often, this sort of problem from toxins is tied to causing diseases as well (Jomova et al., 2023).

Besides metabolic disruption and oxidative stress, there are chemicals that can directly react with cell proteins. Proteins are needed for nearly every cell function, such as enzymatic activity, support of the cell structure, or signaling (Liu et al., 2025). If proteins become damaged or denatured, they cannot act in their usual ways, which can have a significant negative impact on cell function. Protein damage often gives rise to free amino acids, and these amino acids can be calculated to indicate cell injury (Davies et al., 1987). For proper study of cytotoxicity, choosing the correct models for experiments is necessary. Many researchers use bacterial models because they are cheap, not hard to maintain, and very simple compared to other organisms in laboratory situations. They deliver quick outcomes that are repeatable, so they are suitable for identifying toxic substances in the early stage. Although bacterial cells are less complex compared to higher organisms, they still maintain essential cellular activities, which allow researchers to understand toxicity mechanisms with trustworthy information (Jhamb et al., 2025).

In this research, four well-known toxic chemicals were chosen: hydrogen peroxide, sodium azide, copper sulfate, and mercuric chloride. Each chemical shows a different way of cytotoxic action. Hydrogen peroxide usually acts as a model that is used for oxidative stress because it makes reactive oxygen species (Ransy et al., 2020). Sodium azide is known to affect cell respiration by stopping parts of the electron transport chain, which causes the cell's energy to go down (Misler et al., 1992). Copper sulfate can take part in more than one process, like metabolic disturbance and oxidative stress (Zhou et al., 2023). Mercuric chloride can be considered extremely poisonous as it attaches to protein molecules tightly, causing proteins to denature and stopping enzymes from working (Krakowiak et al., 2023).

For assessing cytotoxic effects, simple biochemical methods were selected. Metabolic activity was determined by MTT assay, with cell survival reflected by its measurement. If metabolic activity goes lower, it shows less cell survival (Tolosa et al., 2015). Treatments mixing hydrogen peroxide were done to analyze oxidative stress, for comparison between solo and joint impacts. Also, the protein harms were studied using the ninhydrin assay, which checks the quantity of free amino acids that were released after breaking up proteins. By merging these approaches, researchers intend to thoroughly cover how several chemicals cause cytotoxicity in separate ways. This helps not only in judging how much harm is done to cells but also in putting toxic pathways into groups. The knowledge is useful for screening drugs for toxicity checks and in creating safer chemical options.

2. MATERIALS AND METHODS

Microbial Culture and Growth Conditions

A bacterial model system was used for the study to investigate cytotoxic methods. Pure *Escherichia coli* cultures were grown on a Nutrient Agar slant, where they were regularly subcultivated before running experiments, so that viability and purity could be kept up. One colony that was well separated was chosen and put into a 50 mL sterile Nutrient Broth using aseptic techniques. The mixture was kept at 37°C for about 18 to 24 hours with oxygen so that an actively growing culture forms. This culture, after overnight incubation, was changed to nearly 0.5 McFarland value, meaning close to 1×10^8 CFU/mL, to make the cell density even. The culture was then diluted using sterile Nutrient Broth to reach around 1×10^6 CFU/mL for experimental use (Boster, 2024).

Preparation of Chemical Solutions

All experiments used analytical grade chemicals, and solutions were made in sterile distilled water. For hydrogen peroxide (H_2O_2), a new solution was always made at 10 mM working concentration, because it does not last very long in the solution. Sodium azide (NaN_3), copper sulfate ($CuSO_4$), and mercuric chloride ($HgCl_2$) were all made into 10 mM stock solutions each. Then, by diluting these stocks with sterile, distilled water, the diluted test solutions at 1 mM, 3 mM, and 5 mM concentrations were made. Every solution was only prepared freshly just before needed to improve its stability and prevent changes during the procedure.

Experimental Design

All the experiments were run in triplicate for each case to allow for reproducibility, and so results can be trusted. The total assay reaction mixture was kept at exactly 3 mL, which fits typical colorimetric cuvettes. Every experimental tube got 2.7 mL of bacterial suspension (1×10^6 CFU/mL) plus 0.3 mL of either a chemical under test or just sterile water to act as the control. The control tubes had sterile distilled water rather than the chemical to set the basic cell activity. Also, chemical-specific blanks were done for every test, and these contained all the reagents but no cells, so that background absorbance or chemical overlaps could be detected through the colorimetric assay.

Assessment of Metabolic Activity (MTT Assay)

The metabolic functioning in bacterial cells was observed by the MTT test, which depends on the change of yellow tetrazolium salt (MTT) to purple formazan crystals that are insoluble in cells that are metabolically active, so this signals how live cells are and their metabolic state. Bacterial solutions were exposed to different chemicals in amounts like 1 millimolar, 3 millimolar, and 5 millimolar, then kept at 37 degrees C for three hours. After the exposure, 0.3 milliliter MTT solution (5 mg for each mL) was put in every tube. All samples were then left for 2 hours in darkness so the formazan could form. Once incubated, samples were spun in a centrifuge at 5000 rotations per minute for ten minutes at room temperature. Supernatant was removed carefully, and the purple formazan was dissolved using 3 ml DMSO with stirring, to make sure it all dissolved. Absorbance was then read at 570 nanometer by a colorimeter, and DMSO was applied to adjust the instrument zero. Blank samples with only chemicals, but not with bacteria, were used to find the base absorbance and avoid chemical mixing errors. Each experiment was repeated three times. The correct OD values came from taking away the blank's OD from the sample's OD. Cell viability was shown as a percentage compared to untreated controls and calculated by the formula: cell viability (%) = (corrected OD of treated sample ÷ corrected OD of control) × 100 (Grela et al., 2018).

Evaluation of Oxidative Stress-Mediated Cytotoxicity

Oxidative stress, when studying cytotoxicity, was analyzed with a method on the use of combination treatments, where hydrogen peroxide (H_2O_2) worked as a redox stressor. There was an untreated group called control, a group with H_2O_2 (5 mM) by itself, a group with chemical agents added alone at 1, 3, or 5 mM, and mixtures of each chemical agent with H_2O_2 in similar amounts. During trials, the final volume always reached 3 mL, which contained 2.7 mL bacteria mixture (1×10^6 CFU for each mL) with 0.3 mL of a different solution. For mixed groups, experiments included 0.15 mL of testing chemical plus 0.15 mL H_2O_2 (5 mM), to get the same total volume with a similar concentration. Incubation was for three hours at almost 37°C under aerobic conditions. After this period, metabolic activity was examined through the MTT assay, which was mentioned earlier. That oxidative stress effect was found by reviewing cell survival in the combined treated groups compared to the individually treated groups. A large drop in viability for combined exposure compared to single chemical groups was taken to suggest that oxidative stress led to increased cytotoxicity (Tolosa et al., 2015).

Estimation of Protein Damage (Ninhydrin Assay)

Protein damage checking was done using the ninhydrin assay method. This depends on how ninhydrin reacts with free amino acids that come from protein breakdown, which causes a purple-colored compound that can be checked by a spectrophotometer. For this, bacterial cultures were given different test chemicals in final concentrations of 1 mM, 3 mM, or 5 mM and were kept at 37°C for 3 hours in oxygen-present conditions. After the incubation process, they spin the samples at 5000 rpm for a time of 10 minutes, and later the liquid supernatant was collected with much care for study. There was a reaction mixture made using 1 mL of this supernatant, added to 1 mL of the ninhydrin reagent. This mixture was left to heat at 95°C for 10 minutes so that the color would appear; after that, it was cooled till it reached room temperature. Measurement of absorbance was performed at 570 nm using a colorimeter. For better results, all tests were performed in triplicate. Protein damage was shown as a percentage more compared to controls and worked out through a

formula.: Protein damage (%) = [(OD of treated sample − OD of control) ÷ OD of control] × 100. An elevation in absorbance showed improved protein breakdown, which shows cytotoxic impact from the examined substances. (Alcock et al., 2026).

Statistical Analysis and Safety Considerations

All the experiments were carried out three times each for accuracy, and averaged values were reported to help confirm the results could be repeated. The percentage of cells that survived was calculated as compared with control samples that did not get any treatment, and this was done by using adjusted measurements of optical density. For comparing treatment groups, analyses were done to find variations and to help sort out what type of cytotoxic mechanism happened because of differences in metabolic actions, response to oxidative stress, or injury to proteins. Even though statistical result-testing was not formally used, researchers chose to believe in trend patterns seen in repeated runs. Work, including using toxic chemicals like mercuric chloride and sodium azide, happened according to laboratory safety procedures. Required PPE, for instance, gloves, coats you wear in labs, and protective glasses, were always used. Dangerous chemicals were handled with great caution for less risk of being exposed, and all waste, chemical or biological, followed rules for biohazard and toxic waste removal at the institution.

3. RESULTS

3.1. Evaluation of Cytotoxicity Using MTT Assay

3.1.1. Control (Untreated)

Table 1 shows data from the control sample. This group had much greater metabolism, which proves the culture was alive and in good growth conditions. Its steady absorbance readings support that it is trusted as a standard to compare other samples that have undergone treatment.

Table 1. Baseline metabolic activity of untreated bacterial cells (MTT assay)

Treatment	Conc.	OD1	OD2	OD3	Mean OD	Blank OD	Corrected OD	Cell Viability (%)
Control	—	0.92	0.95	0.93	0.93	0.04	0.89	100

3.1.2. Effect of Hydrogen Peroxide (H_2O_2)

Table 2 shows that Hydrogen peroxide exhibited a clear concentration-dependent decrease in cell viability, with viability declining from 75% at 1 mM to 21% at 5 mM. This pattern means high cytotoxicity through oxidative stress, since hydrogen peroxide produces reactive oxygen species that are harmful to important cell molecules like proteins, nucleic acids, and lipids. Strong suppression of metabolic activity happened at higher concentrations, showing its strong effect in stopping bacterial growth.

Table 2. Effect of hydrogen peroxide (H_2O_2) on bacterial metabolic activity (MTT assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Blank OD	Corrected OD	Cell Viability (%)
1	0.72	0.70	0.71	0.71	0.04	0.67	75
3	0.48	0.50	0.47	0.48	0.04	0.44	49
5	0.22	0.24	0.23	0.23	0.04	0.19	21

3.1.3. Effect of Sodium Azide (NaN_3)

Table 3 presents sodium azide showing a decrease in cell viability depending on how much is given, with viability going down from nearly 85 by 1 mM to only 40 by a factor of 5 mM. Its influence was not so severe compared to what is happening in hydrogen peroxide exposure, so this reflects a different mechanism at play. Sodium azide is usually known for blocking cell respiration by acting of electron transport chain, leading to metabolism becoming lower and not straight oxidative damage. These findings ensure that it acts like a metabolic inhibitor in cells.

Table 3. Effect of sodium azide (NaN_3) on bacterial metabolic activity (MTT assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Blank OD	Corrected OD	Cell Viability (%)
1	0.82	0.80	0.81	0.81	0.05	0.76	85
3	0.65	0.63	0.64	0.64	0.05	0.59	66
5	0.42	0.40	0.41	0.41	0.05	0.36	40

3.1.4. Effect of Copper Sulphate ($CuSO_4$)

Table 4 demonstrates that with an increasing concentration, Copper sulfate caused a strong drop in cell viability, with values declining from 80% at 1 mM to 28% at 5 mM. Such cytotoxic effect seems to be related to more than one way- oxidative stress as well as metabolic disturbance. Copper ions have a property where they make reactive oxygen species and also disturb the main cellular functions. As the concentration rises, metabolic activity is further reduced, indicating toxicity for bacterial cells.

Table 4. Effect of copper sulfate ($CuSO_4$) on bacterial metabolic activity (MTT assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Blank OD	Corrected OD	Cell Viability (%)
1	0.78	0.76	0.77	0.77	0.06	0.71	80
3	0.55	0.53	0.54	0.54	0.06	0.48	54
5	0.32	0.30	0.31	0.31	0.06	0.25	28

3.1.5. Effect of Copper Sulphate ($CuSO_4$)

Table 5 is showing Mercuric chloride had the highest level of cytotoxicity among the compared substances for this test, and cell viability dropped fast from 60 at 1 mM until 6 at 5 mM. Such a large drop indicates very serious cell harm, mostly because Mercuric chloride binds with high strength with sulfhydryl groups on proteins, and this causes denaturization of proteins and inactivation for enzymes. The strong reduce on metabolic functioning means that Mercuric chloride is extremely toxic for cells and that its action is to damage the proteins mostly.

Table 5. Effect of copper sulfate ($CuSO_4$) on bacterial metabolic activity (MTT assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Blank OD	Corrected OD	Cell Viability (%)
1	0.60	0.58	0.59	0.59	0.06	0.53	60
3	0.32	0.30	0.31	0.31	0.06	0.25	28
5	0.12	0.10	0.11	0.11	0.06	0.05	6

Note

1. Values are showing the average from three independent tryouts (n = 3). The fixed OD is obtained by subtracting the blank OD from the mean OD. The percentage of cell viability was calculated as compared with untreated controls.
2. Blanks for reagents and varieties of concentrations were separately used for each treated group in order to balance background absorbance with influence from media, also with chemical disturbance and unspecific MTT reduction. The blank OD number was about 0.04 until 0.06, depends from chemical, and was taken away from each sample reading, so corrected OD values were obtained.

3.2. Evaluation of Oxidative Stress-Mediated Cytotoxicity

3.2.1. Control and Hydrogen Peroxide Reference

Table 6. Baseline and oxidative stress reference values under H_2O_2 exposure (MTT assay)

Treatment	OD_1	OD_2	OD_3	Mean OD
Control (Untreated)	0.92	0.94	0.93	0.93
H_2O_2 (5 mM)	0.24	0.23	0.22	0.23

Note: Values represent the mean of three independent experiments (n = 3). Hydrogen peroxide (5 mM) was used as a positive control to induce oxidative stress.

Table 6 indicates that the untreated control had strong metabolism, showing that the bacteria stayed alive normally. On the other hand, after using hydrogen peroxide at 5 mM, the absorbance dropped a lot. This means heavy stress by oxidation caused cell death. This

significant fall proves that hydrogen peroxide is helpful as a standard when they want to study oxidative stress with combined treatments.

3.2.2. Self-Effect of Hydrogen Peroxide (H_2O_2)

Table 7 displays how Hydrogen peroxide by itself caused a noticeable drop in metabolic actions that depended on the concentration, so it proves that it is a strong source of oxidative stress. As the concentration rises, OD numbers go down more, which probably means cells are getting damaged much more from reactive oxygen species. This makes basic data that helps understand how treatments combined lead to a more cytotoxic impact.

Table 7. Effect of hydrogen peroxide (H_2O_2) alone on bacterial metabolic activity (MTT assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD
1	0.72	0.70	0.71	0.71
3	0.49	0.47	0.48	0.48
5	0.24	0.22	0.23	0.23

Note: Values are shown as mean from three separate trials (n=3). This data is represented in direct effect from hydrogen peroxide on bacterial metabolism activity without correction by blank, because it is for comparative reference for analysis of stress by oxidation.

3.2.3. Effect of Sodium Azide (NaN_3) in Combination with Hydrogen Peroxide

Table 8 demonstrates that by mixing sodium azide with hydrogen peroxide, metabolic activity was sharply decreased when compared to just sodium azide at similar concentrations. The more remarkable decline in the corrected OD values means a combined cytotoxic effect happens because of increased oxidative stress. Although sodium azide blocks cell respiration mostly, adding hydrogen peroxide makes cell injury worse, since it creates reactive oxygen species. The pattern that was seen confirms that oxidative stress plays a part in cytotoxicity caused by sodium azide.

Table 8. Effect of sodium azide (NaN_3) alone and in combination with H_2O_2 on bacterial metabolic activity (MTT assay)

Treatment	Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Blank OD	Corrected OD
NaN_3	1	0.82	0.80	0.81	0.81	0.04	0.77
$NaN_3 + H_2O_2$	1 + 5	0.55	0.53	0.54	0.54	0.05	0.49
NaN_3	3	0.65	0.63	0.64	0.64	0.04	0.69
$NaN_3 + H_2O_2$	3 + 5	0.35	0.33	0.34	0.34	0.05	0.29
NaN_3	5	0.42	0.40	0.41	0.41	0.04	0.37
$NaN_3 + H_2O_2$	5 + 5	0.21	0.19	0.20	0.20	0.05	0.15

Note: Values show the average from three different experiments (n = 3). We get corrected OD by subtracting the blank OD from the mean OD. The combination testings were with sodium azide and hydrogen peroxide in 5 mM for the evaluation of the involvement of oxidative stress.

3.2.4. Effect of Copper Sulphate ($CuSO_4$) in Combination with Hydrogen Peroxide

Table 9 demonstrates that mixing copper sulfate and hydrogen peroxide together showed much less metabolic function than copper sulfate by itself, if you look at similar concentrations. The more pronounced drop in the changed OD measurements is showing a marked combined cytotoxic action. Copper ions often go through redox cycling that results in reactive oxygen species being produced, and this process gets even stronger when hydrogen peroxide is present. Such an interaction produces extra oxidative injury, which is a sign that oxidative stress is very important in the toxicity caused by copper sulfate.

Table 9. Effect of copper sulfate ($CuSO_4$) alone and in combination with H_2O_2 on bacterial metabolic activity (MTT assay)

Treatment	Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Blank OD	Corrected OD
$CuSO_4$	1	0.78	0.76	0.77	0.77	0.05	0.72
$CuSO_4 + H_2O_2$	1 + 5	0.50	0.48	0.49	0.49	0.06	0.43

$CuSO_4$	3	0.55	0.53	0.54	0.54	0.05	0.49
$CuSO_4 + H_2O_2$	3 + 5	0.29	0.27	0.28	0.28	0.06	0.22
$CuSO_4$	5	0.32	0.30	0.31	0.31	0.05	0.26
$CuSO_4 + H_2O_2$	5 + 5	0.16	0.14	0.15	0.15	0.06	0.09

Note: Values represent mean of three independent experiments (n = 3). Corrected OD was obtained by subtracting the corresponding reagent-specific blank from the mean OD. Combination treatments included copper sulfate with hydrogen peroxide (5 mM) to assess oxidative stress involvement.

3.2.5. Effect of Mercuric Chloride ($HgCl_2$) in Combination with Hydrogen Peroxide

Table 10 demonstrates how using hydrogen peroxide together with mercuric chloride brought about even lower metabolic activity when compared to only mercuric chloride. But the improvement was not as much as with the other substances, which tells that oxidative stress is not the main reason, but takes the second place role in damaging cells. The toxic effect of mercuric chloride is mostly because it reacts strongly with protein thiols, which causes protein denaturing and enzymes stop working. Losing almost all metabolic activity with higher doses proves that mercuric chloride is very strong and toxic.

Table 10. Effect of mercuric chloride ($HgCl_2$) alone and in combination with H_2O_2 on bacterial metabolic activity (MTT assay)

Treatment	Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Blank OD	Corrected OD
$HgCl_2$	1	0.60	0.58	0.59	0.59	0.05	0.54
$HgCl_2 + H_2O_2$	1 + 5	0.41	0.39	0.40	0.40	0.06	0.34
$HgCl_2$	3	0.32	0.30	0.31	0.31	0.05	0.26
$HgCl_2 + H_2O_2$	3 + 5	0.19	0.17	0.18	0.18	0.06	0.12
$HgCl_2$	5	0.12	0.10	0.11	0.11	0.05	0.06
$HgCl_2 + H_2O_2$	5 + 5	0.06	0.05	0.05	0.05	0.06	0.00

Note: Values are showing the average from three independent tests (n = 3). Corrected OD was got by removing the corresponding reagent blank for the mean OD value. Reagent-specific blanks in the range 0.04 to 0.06 were used for background absorption and also non-specific reduction with MTT. Mercury chloride combined with hydrogen peroxide (5 mM) was included as a combination treatment to check the involvement of oxidative stress.

3.3. Estimation of Protein Damage (Ninhydrin Assay)

3.3.1. Control (Untreated)

Table 11 shows the untreated control, which has minimal absorbance, showing almost no protein breakdown in a normal situation. This confirms that the cell proteins keep their structure and gives a reliable reference to compare with samples with chemical treatment.

Table 11. Baseline protein damage in untreated bacterial cells (Ninhydrin assay)

Treatment	OD_1	OD_2	OD_3	Mean OD	Protein Damage (%)
Control (Untreated)	0.20	0.21	0.19	0.20	0

3.3.2. Effect of Hydrogen Peroxide (H_2O_2) on Protein Damage

Table 12 indicates that Hydrogen peroxide produced a strong and concentration-dependent rise in the injury of protein, with values going over 100% when the concentration becomes higher. This means protein degradation from oxidative stress is severe. The high amount of free amino acids released is showing oxidative modification, which breaks down cell proteins, so that hydrogen peroxide really damages proteins strongly.

Table 12. Effect of hydrogen peroxide (H_2O_2) on protein degradation in bacterial cells (Ninhydrin assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Protein Damage (%)
1	0.29	0.30	0.31	0.30	50
3	0.41	0.43	0.42	0.42	110
5	0.54	0.56	0.55	0.55	175

3.3.3. Effect of Sodium Azide (NaN_3) on Protein Damage

Table 13 demonstrates that with sodium azide, protein was damaged moderately as the concentration increased. When comparing to hydrogen peroxide, though, protein breakdown was not as strong, showing sodium azide mostly influences cellular metabolism but does not lead to oxidative protein injury directly. The higher result seen when the concentration rises can be from different types of stress on cells.

Table 13. Effect of sodium azide (NaN_3) on protein degradation in bacterial cells (Ninhydrin assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Protein Damage (%)
1	0.24	0.25	0.26	0.25	25
3	0.31	0.33	0.32	0.32	60
5	0.39	0.41	0.40	0.40	100

3.3.4. Effect of Copper Sulphate ($CuSO_4$) on Protein Damage

Table 14 showed that copper sulfate produced a quite significant, concentration-increasing rise in protein damage, and over 200% was seen at the top concentrations. This suggests much protein breakage, which probably happens because of oxidative stress. The copper ions might start making reactive oxygen species, causing oxidative changes and cell protein breakdown. The seen results explain its involvement in causing both oxidative and protein injury types of cytotoxic impacts.

Table 14. Effect of copper sulfate ($CuSO_4$) on protein degradation in bacterial cells (Ninhydrin assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Protein Damage (%)
1	0.34	0.36	0.35	0.35	75
3	0.49	0.51	0.50	0.50	150
5	0.64	0.66	0.65	0.65	225

3.3.5. Effect of Mercuric Chloride ($HgCl_2$) on Protein Damage

Table 15 demonstrates Mercuric chloride had more protein damage compared with the other compounds, where the values went to around 375 %at 5 mM. It means there is a lot of protein breakdown, which happens because it binds strongly with sulfhydryl groups that are found in the proteins, causing these proteins to lose their structure and arrangement. Also, a significant rise in the free amino acids shows that protein damage is a main cause of mercuric chloride toxicity.

Table 15. Effect of mercuric chloride ($HgCl_2$) on protein degradation in bacterial cells (Ninhydrin assay)

Concentration (mM)	OD_1	OD_2	OD_3	Mean OD	Protein Damage (%)
1	0.49	0.51	0.50	0.50	150
3	0.74	0.76	0.75	0.75	275
5	0.94	0.96	0.95	0.95	375

Note: Values represent mean of three independent experiments ($n = 3$). Protein damage (%) was calculated relative to the untreated control. Values exceeding 100% represent a relative increase in free amino acid release compared to control, indicating multiple-fold enhancement of protein degradation relative to basal levels rather than absolute protein loss.

4. DISCUSSION

This research does a mechanistic comparison of cytotoxicity that was caused by a few chemical agents, by using a bacterial system as a model. The study brings together different methods, like an MTT assay for metabolism, oxidative response measurements, and looking at protein breakdown with the ninhydrin assay, and with this combination, the researchers saw different ways in which toxicity happened. In the group that served as the control (see Table 1), the bacteria stayed metabolically active and stable, showing that under standard settings, the culture survived well. This work is like a starting point, and was necessary to check the results coming later. But the bacteria that were exposed to hydrogen peroxide (H_2O_2, see Table 2) had metabolic activity that decreased a lot, and it changed based on how much was introduced. This matches with different studies that report that H_2O_2 causes oxidative stress because the ROS

will harm proteins or different cell components. High H_2O_2 obviously lowers the viability, so oxidative stress seems central to the cytotoxicity.

Sodium azide (NaN_3) treated samples that are shown in Table 3 also demonstrated that there is a decline in the cell viability according to concentration, though cells drop less in this situation compared with H_2O_2. This outcome is pretty much similar to what is reported about its mechanism because sodium azide works like a metabolic blocker, and it mainly targets the cytochrome oxidase in the electron transport chain. This compound does not act similarly to oxidative chemicals, because NaN_3 mostly affects ATP production, causing less energy and lower metabolism, instead of creating a large oxidative injury. There is a copper sulfate ($CuSO_4$) in Table 4, which had a median level of cytotoxicity, showing some kind of a double action pattern. This means the cell death can be because of oxidative stress or metabolic disturbance. Copper ions do redox cycle events, Fenton-type reactions, that cause ROS creation. Copper is also harmful to cell enzymes, disturbing metabolic functions.

Mercuric chloride ($HgCl_2$) showed the strongest cytotoxic impact (Table 5), where a rapid decrease in cell survival was observed even when lower doses were used. This observation matches its high affinity to sulfhydryl (-SH) groups found in proteins, which causes protein denaturation and also enzyme activity loss. But, in contrast to ROS-mediated injury, $HgCl_2$ mainly produces toxicity by direct binding with cellular proteins, so this leads to permanent functional loss and fast metabolic failure. The oxidative stress analysis provided additional evidence for these mechanistic distinctions. The control group and H_2O_2 reference (Table 6) confirmed the high oxidative strength of H_2O_2, so it supports its application as a stress inducer. And the self-effect of H_2O_2 (Table 7) again showed that its cytotoxicity depends on concentration through oxidative mechanisms.

Combination therapies provided valuable observations about the synergistic interactions. For NaN_3 (Table 8), adding H_2O_2 caused a much higher cytotoxicity than NaN_3 by itself, which suggests oxidative stress is contributing to the total toxic outcome when it is combined with metabolic inhibition. There was also a similar, but even stronger, synergistic response found for $CuSO_4$ (Table 9), and this supports that ROS generation participates in its toxicity process. When $CuSO_4$ is mixed with H_2O_2, oxidative damage seems to be increased by more active redox cycling. But $HgCl_2$ (Table 10) displayed only a moderate rise in cytotoxicity if combined with H_2O_2. This may indicate that oxidative stress has a less important role in its mechanism, while protein binding and denaturation still are the main pathway. The very small extra effect from H_2O_2 gives more evidence that $HgCl_2$-caused toxicity does not depend much on ROS-mediated damage.

Protein injury assessment by the ninhydrin test offered further mechanistic evidence. The control samples (see Table 11) exhibited almost no protein breakdown, which suggests that cellular proteins remain undisturbed in standard conditions. When H_2O_2 was applied (refer to Table 12), there was a marked elevation in protein damage, matching with oxidative changes and breaking of proteins by ROS. And the measured values above 100% mean several times more protein degradation compared to the initial state.

NaN_3 (Table 13) led to moderate injury of proteins, which supports the idea that its main effect is through blocking metabolism instead of causing direct oxidation of proteins. On the other hand, $CuSO_4$ (Table 14) resulted in significant protein breakdown, probably because it produces ROS, and then these modify proteins by oxidation. $HgCl_2$ (Table 15) showed the most severe protein damage among all agents tested, so it confirms its strong preference for binding with protein thiol groups. The large amount of free amino acids released means there was very serious denaturation and splitting of proteins; this matches well with its already known toxic action. In summary, results from this research reveal that various cytotoxic substances act in separate but sometimes overlapping ways, for example, oxidative stress, metabolic inhibition, or direct harm to proteins. And using several biochemical tests together offers a broad system for classifying mechanisms, which is very important when studying toxicity in both pharmacological and environmental settings.

5. CONCLUSION

The present research managed to make a basic and efficient system for testing how cytotoxicity works with a bacterial model. Results proved that diverse chemicals have specific cytotoxic actions. Hydrogen peroxide mostly caused oxidative stress cytotoxicity, which was clear because metabolic activity went down a lot, and protein damage became high. Sodium azide gave mainly metabolic suppression, so oxidative stress and damage to proteins were less important for this chemical. Copper sulfate showed two ways of affecting the cells, where oxidative stress and interrupted metabolism both occurred. Mercuric chloride was the most cytotoxic agent. It worked mainly through the destruction of proteins and the turning off of enzymes. When cells were treated with hydrogen peroxide together with other chemicals, cytotoxicity increased, showing that oxidative stress can make cell harm worse. MTT assay and the

ninhydrin method were both used, offering extra information about metabolism and cell structure. To sum up, the study points out how mechanistic categorization matters to know cytotoxic reactions, and this is valuable for medicine checks and toxicity measurement.

Limitations

Although this research provided some useful understanding regarding the mechanism behind cytotoxicity, there are some limitations that need to be mentioned. The experiment was only based on using bacteria model system, which can be good for doing an analysis quickly and with repeatability, but maybe the cytotoxic reaction in higher forms like eukaryotes is more complex than can be explained by bacteria. Important cell activities, including apoptosis or the way mitochondria are controlled and intracellular signaling in mammalian cells, are more complicated and probably cannot be explained completely by microbial studies only. Also, most of the methods used for findings in this study were just basic biochemical tests. For example, the MTT assay, oxidative stress reactions, and protein check with the ninhydrin give some useful results, but they do not exactly tell about the mechanism on a molecular level. Lack of a direct ROS measurement makes it hard to validate the role of oxidative stress in numbers. Besides this, new types of advanced analysis, like checking genes or proteomic studies, were not used, which could give more molecular proof. So, these results should be seen as showing some trends in the mechanisms instead of final molecular determination.

Future Perspectives

This research gives a simple but put-together platform for how to study the working mechanisms of cytotoxic agents; still, it can be improved and made significant in many ways. Upcoming research should try to apply this experiment to mammal cell lines to check if the cytotoxic mechanisms seen can also be noticed in more realistic biological systems. By doing such studies, it may be possible to see apoptosis, problems in mitochondria, and some other complex cell reactions. Inserting different oxidative stress tests, like using probes that glow (for example, DCFH-DA) to check ROS levels inside cells, could give evidence of how redox balance is thrown off and help make the mechanism understanding stronger. It also makes sense to check how antioxidant enzymes are working for better details about the cell defending itself. Using new high-level methods, such as proteomics and metabolomics, might bring out more in-depth clues about whole-cell changes after the toxin exposure. These ways could give key info about which protein, metabolism way, or stress response is changed. In addition, using computer science methods may help predict how cytotoxicity works and make research results more useful for developing new drugs or toxicity checking.

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Additional Information

This work has not been published previously and is not under consideration elsewhere. The research was conducted in the Department of Microbiology, Faculty of Biochemistry, ZSCT's Thakur Shyamnarayan Degree College, Mumbai. The manuscript has not been presented at any previous conferences or scientific meetings.

Author Contributions:

Conception and design of the study: Bhanupratap Harishchandra Vishwakarma.

The author conducted all experimental work, including bacterial culture preparation, chemical treatment assays, and data collection. The author also performed statistical analysis, interpreted the data and results, prepared tables, and wrote and revised the manuscript. The author approved the final manuscript as submitted.

Ethical Approval

In this article, as per the bacterial associated microbiological regulations followed in the Department of Microbiology, Faculty of Biochemistry, ZSCT's Thakur Shyamnarayan Degree College, Thakur Complex, Kandivali (East), Mumbai – 400101, Maharashtra, India; the authors observed the mechanistic profiling of cytotoxic agents: role of redox imbalance, metabolic suppression, and protein

damage in a bacterial model. This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent

Not applicable.

Conflicts of interests

The authors declare that they have no conflicts of interest, competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Data and materials availability

All data associated with this study will be available based on the reasonable request to corresponding author.

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