Gamma and UV Radiation-Based Advanced Oxidation Processes for Degradation of Anti-Cancer Drug Daunorubicin: Environmental Toxicity and Wastewater Treatment Implications

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Summary: Pharmaceutical contamination poses a significant threat to ecosystems and advanced oxidation processes (AOPs) offer promising solutions. This study investigates the innovative degradation of daunorubicin, an anticancer drug, utilizing a novel combination of UV and gamma (γ) radiation with H_2O_2 (1 µmol L^{-1}). This strategy significantly enhances degradation efficiency compared to conventional advanced oxidation processes (AOPs). The results demonstrated that while UV/H_2O_2 achieved 89.89% degradation of a 5 mg L^{-1} daunorubicin solution in 90 minutes, the gamma/H_2O_2 system achieved complete degradation at a lower treatment time and a gamma-absorbed dose of 4 kGy. This unique synergy combines the rapid generation of reactive oxygen species under UV radiation with the deeper penetration and oxidative capabilities of gamma radiation, offering unparalleled degradation efficiency. Chemical oxygen demand (COD) reduction of 56% for UV/H_2O_2 and 83% for gamma/H_2O_2 further emphasizes the environmental benefit of this approach. Kinetic parameters (G-Value, dose constant, $D_{0.50}$, $D_{0.90}$, $D_{0.99}$) were also evaluated for gamma-treated samples, demonstrating a statistically significant performance improvement. Ecotoxicological assessments, including the *Allium cepa* bioassay and Ames test, revealed up to 96.21% and 95.83% reductions in mutagenicity (for TA100 and TA98, respectively) with gamma/H_2O_2, highlighting the detoxification potential. Analytical techniques such as HPLC, GC-MS and FTIR confirmed the successful breakdown of daunorubicin by-products. The study's methodology was optimized using response surface methodology (RSM), ensuring robust and reproducible results. This research underscores the superiority of integrating UV and gamma radiation with H_2O_2, presenting a transformative approach to pharmaceutical wastewater treatment.

Keywords: Daunorubicin; Advanced Oxidation Process; Cytotoxicity; Mutagenicity.

Introduction

The discharge of untreated pharmaceutical waste into the environment poses a significant threat to ecosystems and human health [1]. Particularly, hospital waste effluent containing anti-cancer drugs adversely affects the aquatic biota by inhibiting normal cellular processes [2-4] [5, 6]. Daunorubicin, an anti-cancer drug, has been identified as a persistent pollutant due to its complex structure, consisting of a tetracycline quinoid aglycon linked to an amino sugar-daunosamine. The increasing demand for pharmaceuticals has led to their widespread environmental prevalence, raising urgent concerns [7]. Researchers have explored various routine methods, including filtration, adsorption and reverse osmosis, for removing pharmaceuticals from wastewater. The conventional methods are deficient, cause incomplete removal and may transform the primary pollutants into secondary pollutants rather than eliminate them [8-10]. Advanced oxidation processes (AOPs) have emerged as a promising alternative offering a more effective way to degrade organic pollutants like

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daunorubicin [11]. The advanced oxidation processes (AOPs) generate hydroxyl radicals (•OH), which not only neutralize harmful species like •H and e^-_{aq} but also help produce more hydroxyl radicals. These radicals break down complex organic compounds into simpler substances, such as carbon dioxide (CO_2) and water (H_2O) [12, 13]. The AOPs, such as photolysis, UV/O_3, gamma/H_2O_2, UV/Fe^{2+}/H_2O_2, UV/TiO_2, etc., are believed to be synergetic techniques to degrade the pharmaceutical waste effluent [14-17]. The combination of UV and gamma radiation with H_2O_2 offers several advantages over traditional AOPs like Fenton reactions and sonolysis. Fenton reactions require acidic conditions, which can lead to secondary pollutants and sludge, while sonolysis is energy-intensive and less effective at low contaminant concentrations. In contrast, the UV-gamma-H_2O_2 method is more energy-efficient, works well under neutral to slightly alkaline conditions and produces hydroxyl radicals more efficiently, enabling faster and more effective degradation of contaminants, even at low concentrations. This makes it a more sustainable and scalable option for wastewater treatment. UV irradiation in conjunction with H_2O_2-based AOP generates (•OH) and can efficiently degrade the drug according to the following equations 1 and 2 [18-22].

$$H_2O_2 + UV \rightarrow 2^{\bullet}OH \quad (1)$$

$$^{\bullet}OH + \text{Daunorubicin} \rightarrow \text{Further Oxidation} \quad (2)$$

The γ/H_2O_2 process is also vital in breaking down pharmaceutical compounds. The hydroxyl radicals (•OH) generated during water radiolysis can neutralize reducing species like e^-_{aq} and •H, which in turn boosts the production of more •OH, speeding up the degradation process, as shown in equations 3-5 [23-25].

$$H_2O + \text{ϒ-radiations} \rightarrow {}^{\bullet}OH + {}^{\bullet}H + e^-_{aq} + H_2 + H_2O_2 + H_3O^+ \quad (3)$$

$$H_2O_2 + e_{aq}^- \rightarrow {}^{\bullet}OH + OH^- \quad (4)$$

$$H_2O_2 + {}^{\bullet}H \rightarrow {}^{\bullet}OH + H_2O \quad (5)$$

While AOPs have demonstrated several potential benefits, certain challenges remain unaddressed. For instance, previous studies have highlighted issues such as incomplete mineralization of pharmaceuticals, the formation of potentially toxic by-products and high operational costs. Additionally, some AOP techniques, such as UV/H_2O_2 or γ/H_2O_2, require specific conditions (e.g., UV light intensity, pH adjustments, or chemical dosages) to achieve optimal degradation efficiency, which can complicate their practical application. Despite the ability of AOPs to degrade organic pollutants, many studies have not thoroughly assessed the toxicity of the resulting by-products, which could pose further environmental and health risks [26-30].

This study aims to fill these gaps by exploring the combined use of UV and γ-radiation with H_2O_2 to break down daunorubicin. Unlike previous studies that often overlook toxicity concerns, we incorporate toxicity evaluation techniques such as the Ames test and *Allium cepa* bioassays to ensure that the degradation products are less toxic and environmentally safe. Using techniques like Fourier transform infrared spectroscopy (FTIR), high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), this study also identifies the by-products formed during degradation. It offers a thorough analysis of both the effectiveness and safety of AOPs in pharmaceutical degradation. The application of response surface methodology (RSM) further allows for a robust statistical assessment of the process, addressing previous limitations in optimization and scalability.

Experimental

A commercial drug, daunorubicin (Fig 1), used as a model compound, was provided by The Pharmedic Laboratories (Pvt.) Limited, Lahore, Pakistan and used before purification. The other chemicals of analytical grade were taken from Sigma-Aldrich. To ensure the accuracy and reproducibility of the measurements, a calibration curve was prepared using standard solutions of daunorubicin in the concentration range of 1–20 mg/L. The calibration curve exhibited excellent linearity with an R^2 value of 0.998. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were determined to be 0.5 mg/L and 1.0 mg/L, respectively. All the experiments were performed at room temperature (25 ^{0}C) and the pH of the reaction systems was maintained at approximately 6.5, which is the natural pH of the aqueous drug solutions. No pH adjustment was made during the degradation process.

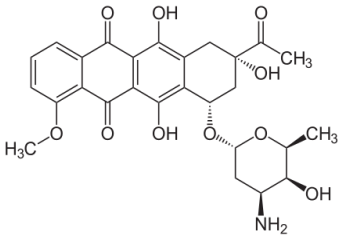


Fig 1: Structural formula of daunorubicin (Molecular formula $C_{27}H_{29}NO_{10}$, molecular mass 527.52 (g/mol), λ_{max} 480 nm).

Radiation treatment

The 20 mL of drug aqueous solutions having concentrations of 5, 10 and 15 mg L^{-1} were treated with UV radiation alone and then with H_2O_2 (µmole L^{-1}) under the radiation exposure time of 15, 30, 45, 60, 75 and 90 minutes. The reactions were performed in a batch system, using a laboratory-scale UV reactor equipped with UV lamps emitting at 254 nm, with a light intensity of 144 W at the Chemistry Department, Government College University (GCU), Faisalabad, Pakistan. The gamma (γ) irradiation treatment of daunorubicin for the aforementioned concentrations was performed using a Cs-137 γ-rays source, installed at the Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad, Pakistan. The calibration was performed with the help of Mohr's salt using a Fricke dosimeter and Eq. (6) was utilized for the measurement of the dose rate [31, 32], which was calculated at 1.25 kGy h^{-1} at irradiation time.

$$D = [N*\Delta A*100/\varepsilon*\rho*G\ (Fe\ (III))] \quad (6)$$

where D represents the absorbed dose, ε molar extinction coefficient of ferric ion (Fe^{+3}) (0.2205 M^{-1} cm^{-1}) at 304 and 25 ^{0}C, A is difference in absorbance of irradiated and un-irradiated samples, N is Avogadro's number, while ρ is the density of Fricke solution (1.024 g/cm^3) for 0.4 M H_2SO_4 and G (Fe^{+3}) is the number of Fe^{+3} ions formed/100 eV of absorbed energy. The percentage degradation was carried out by using Equation 7.

$$\text{Degradation (\%)} = [(A_i - A_f)/A_i] \times 100 \quad (7)$$

Where,
A_i = absorbance of drug solutions before treatment
A_f = absorbance of drug solution after treatment

Analytical Techniques

The drug concentrations were measured using a double-beam UV/Vis Spectrophotometer (U-2800, Hitachi, Japan) at a wavelength of 480 nm (λ_{max}). Degradation by-products were analyzed with a Thermo Scientific GC-MS, featuring a DB-5MS column (30 m × 0.25 mm × 0.25 µm). The ionization was performed using Electron Impact (EI) and the temperature program began at 50°C (held for 2 minutes) and increased at 10°C/min to 300°C. For further confirmation of degradation, an Agilent 1200 HPLC system was employed, with chromatographic separation achieved on a C18 reverse-phase column (4.6 mm × 150 mm). The mobile phase consisted of a 50:50 (v/v) methanol: water mixture, with a flow rate of 1 mL/min.

Measurement of chemical oxygen demand (COD)

The COD reduction for the untreated and treated samples was determined by using the method given in [33]. After shaking and cooling the 50 mL samples and 1 g $HgSO_4$ mixture, 25 mL of 0.042 M $K_2Cr_2O_7$ was added. After refluxing the ingredients for two hours, a few drops of ferroin indicator were added and the mixture was titrated against Mohr's salt. The color transformed from bluish-green to reddish-brown, indicating the endpoint. A similar procedure was carried out using 50 mL of water as a blank to obtain the measurements for the blank. To calculate the COD, the following equation 8 was utilized.

$$COD = (A-B) * M * 800 / mL\ of\ sample \quad (8)$$

where,

A is the ferrous ammonium sulfate volume (cm^3) for the blank. M is the ferrous ammonium sulfate's molarity and B is the sample's ferrous ammonium sulfate volume (cm^3).

Measurement of G, $D_{0.50}$, $D_{0.90}$ and $D_{0.99}$ values

The G-value of the drug aqueous solution under the gamma radiation absorbed dose of 0.3 to 4 kGy was determined using the following Eq. (9) [34].

$$G = [R]\ N_A / D\ (6.24 \times 10^{17}) \quad (9)$$

Where D is the absorbed dose in kGy, 6.24 x 10^{17} is the conversion factor from 100 eV/L, NA is Avogadro's number and [R] is the drug solution concentration in mmol/L at a particular dose. The K is the constant of the slope plotted [R] versus dose (kGy), $D_{0.50}$ (dose required for 50% degradation), $D_{0.90}$ (90% degradation) and $D_{0.99}$ (99% degradation) values were calculated by using equations (10-12)[35].

$$D_{0.50} = \ln 2/k \quad (10)$$

$$D_{0.90} = \ln 10/k \quad (11)$$

$$D_{0.99} = \ln 100/k \quad (12)$$

Cytotoxicity evaluation

A small amount of MnO_2 was added to the irradiated samples to extract the H_2O_2 to prevent its toxic effect during the cytotoxicity evaluation by the *Allium Cepa* and the Ames test [24, 36].

The onions were germinated in tap water for 72 hours during the *Allium Cepa* test, after which the outer loose scales were scraped off and the dry bases were removed. Root length growth was followed by exposure to dye samples (both treated and untreated). After the treatment of dye samples for the recommended time, the onion was dipped in an acetone: alcohol (1:3) mixture and then kept in 1 N HCl at 60 °C to hydrolyze until it became softened before measuring the root lengths (RL) and root count (RC) and slides were organized for microanalysis for mitotic index (MI) measurement[26]. Methyl methanesulfonate (MMS) and pure water were used as positive and negative controls, respectively [37]. The analysis that was conducted to determine the sample size for the *Allium Cepa test* indicated that a minimum of 10 bulbs per treatment group would be required to achieve adequate power. Therefore, we used 12 bulbs per treatment group, resulting in 48 bulbs across four groups. The mutagenicity experiment was conducted through the Ames test using TA98 and TA100 bacterial strains [38], considered a reliable bioassay. The $K_2Cr_2O_7$ (0.01 g l^{-1}) and NaN_3 (0.5 µg/100 µl) were taken as positive controls for TA98 and TA100, respectively, while distilled water was used as a negative control. The mixture (200 µL) was transferred into 96-well plates. After incubation for 3 days at 37 °C, the well plates were sealed in plastic bags. A positive test indicates the presence of carcinogenic chemicals. This test is an easy-to-use and effective method to determine if a material is carcinogenic [39]. The power analysis calculations suggested that a minimum of 5 replicates per concentration group would be necessary to assess mutagenicity confidently. We ultimately included 6 replicates per group, allowing for a thorough examination of the mutagenic potential of the degradation products, totaling 18 replicates across three concentration groups.

Statistical analysis

The response surface methodology (RSM) was applied using the statistical software "Design Expert" version 11. The independent variables, such as initial drug concentration, gamma dose/UV exposure time and H_2O_2 concentration, were analyzed in relation to dependent variables like percentage degradation and COD reduction. From the RSM analysis, both the highest and lowest response values were identified. The experimental design and the suitability of the model were assessed using an analysis of variance (ANOVA), which tested the statistical accuracy and significance of the developed model [40].

Results and Discussion

UV and UV/H_2O_2 degradation results

Aqueous solutions of the drug (5–15 mg L^{-1}) were exposed to UV radiation for 15, 30, 45, 60, 75 and 90 minutes. The highest degradation rates were 86.52%, 55.06% and 68.60% for the 5, 10 and 15 mg L^{-1} solutions, respectively, at 90-minute UV exposure (Fig 2A). When the solutions were treated with UV radiation in the presence of H_2O_2, the degradation improved to 89.90%, 87.40% and 84.30% for these concentrations (Fig 2B). The significance of the factors and their interactions was determined using ANOVA. The results in ST1 show that contact time had a very significant effect ($p < 0.002$, $F = 52.32$), followed by the initial drug concentration ($p = 0.02$, $F = 15.51$) and H_2O_2 dose ($p = 0.08$, $F = 4.93$). Higher degradation percentages were observed at lower drug concentrations, as UV light can break down more daunorubicin molecules at these levels, leading to a higher degradation rate. At higher concentrations, degradation was lower due to a decrease in the generation of hydroxyl radicals (•OH). The degradation rate increased with longer UV exposure and higher H_2O_2 doses, but no significant improvement was seen beyond optimal levels. This could be due to competition between intermediate species •OH and the hydroperoxyl radical (HO_2•), which scavenges •OH and reduces the overall degradation rate [41]. The results align with studies such as Jung et al., which demonstrated that UV radiation effectively degrades pharmaceutical compounds by initiating advanced oxidation reactions. Furthermore, the selected dosage corresponds to established parameters in UV-based advanced oxidation processes [11], ensuring reliability and practical applicability. No systematic patterns were observed in the residuals despite the slightly significant lack-of-fit value ($p = 0.039$), which supports the model's reliability. The response surface plots effectively demonstrate the experimental trends, confirming the predictive strength of the model. Additionally, the observed degradation behavior aligns with findings from prior studies (e.g., Iqbal et al., 2017; Jung et al., 2012), further emphasizing the model's applicability in optimizing parameters for daunorubicin degradation.

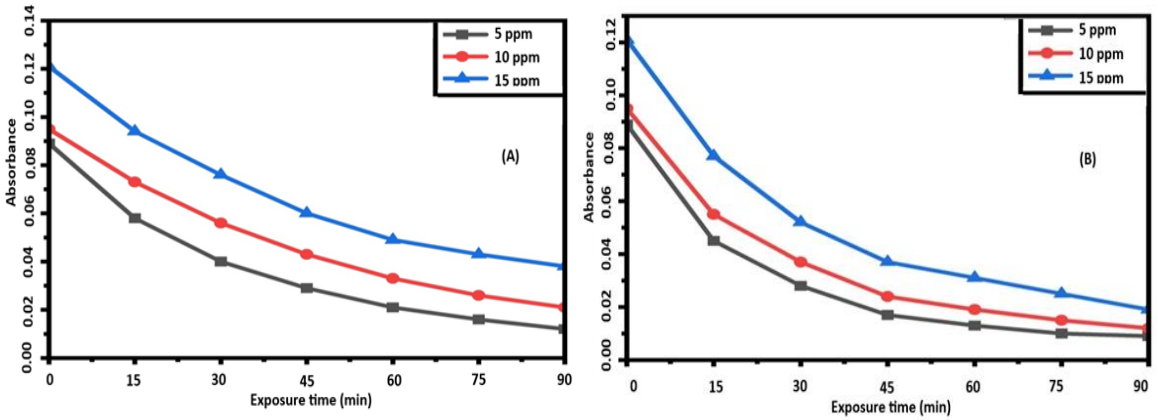


Fig 2: Degradation of daunorubicin under (A) UV and (B) UV/H_2O_2 treatment, monitored via absorbance measurements.

Gamma and gamma/H_2O_2 degradation results

The degradation of drug aqueous solutions with concentrations of 5-15 mg L^{-1} was also studied using gamma radiation alone and in combination with H_2O_2. When exposed to a gamma radiation dose of 4 kGy, the degradation was 97.75% for the 5-mg L^{-1} solution, gradually decreasing to 89.55% and 81.44% for the 10-mg L^{-1} and 15-mg L^{-1} concentrations (Fig 3A), respectively. However, when gamma radiation was combined with H_2O_2, the degradation increased to 100% for the 5-mg L^{-1} solution, 94.74% for the 10-mg L^{-1} solution and 90.91% for the 15-mg L^{-1} solution, as shown in Fig 3B. The gamma/H_2O_2 combination generates hydroxyl radicals ($^{\bullet}OH$), which not only neutralize e^-_{aq} and $H^{\bullet}$ species but also enhance the production of more $^{\bullet}OH$ (as shown in Equations 3-5), leading to a significant increase in drug degradation [24]. Preliminary findings indicate that neither H_2O_2 nor gamma radiation alone is effective in degrading daunorubicin at lower doses. However, at higher absorbed doses, gamma radiation shows improved degradation. To enhance the removal efficiency and address the limitations, a combined treatment using H_2O_2 and gamma radiation was applied. The addition of H_2O_2 not only boosts the degradation efficiency but also reduces the required radiation dose and exposure time. Increasing the concentration of H_2O_2 further accelerates the removal process. Studies such as Iqbal et al. support the use of gamma radiation, demonstrating their ability to degrade persistent organic pollutants with minimal secondary contamination [41]. Additionally, the chosen dosage aligns with reported values in advanced oxidation process research by Muneer et al., confirming its suitability for organic pollutant degradation [19].

Our results linearly correlate with the findings of Iqbal et al., who studied wastewater treatment by advanced oxidation and obtained a similar trend in their results. Fig 4 and S1 present response surface plots of daunorubicin degradation efficiency as a function of the initial drug concentration, UV exposure time, gamma absorbed dose and H_2O_2 concentration. Response Surface analysis revealed that a 4 kGy absorbed dose of gamma radiation in combination with H_2O_2 was the optimal condition for maximum Daunorubicin degradation as compared with UV radiation.

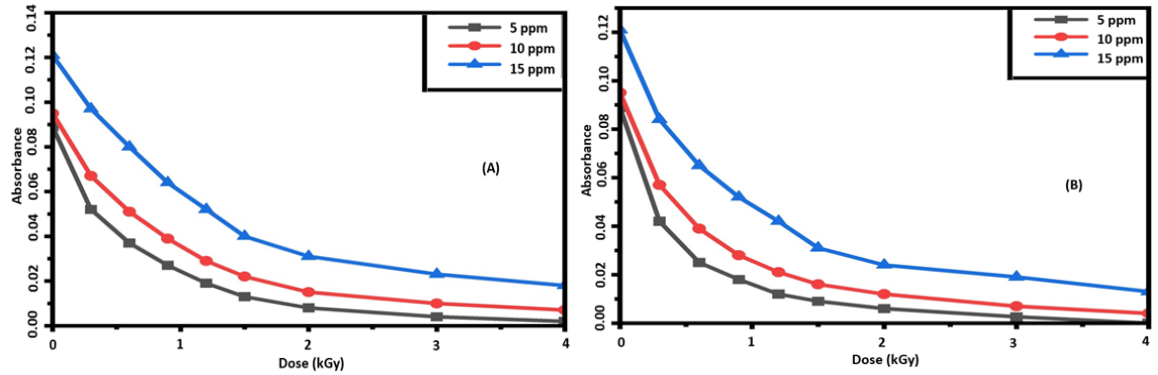


Fig. 3: Degradation of daunorubicin under (A) gamma and (B) gamma/H_2O_2 treatment, monitored via absorbance measurements

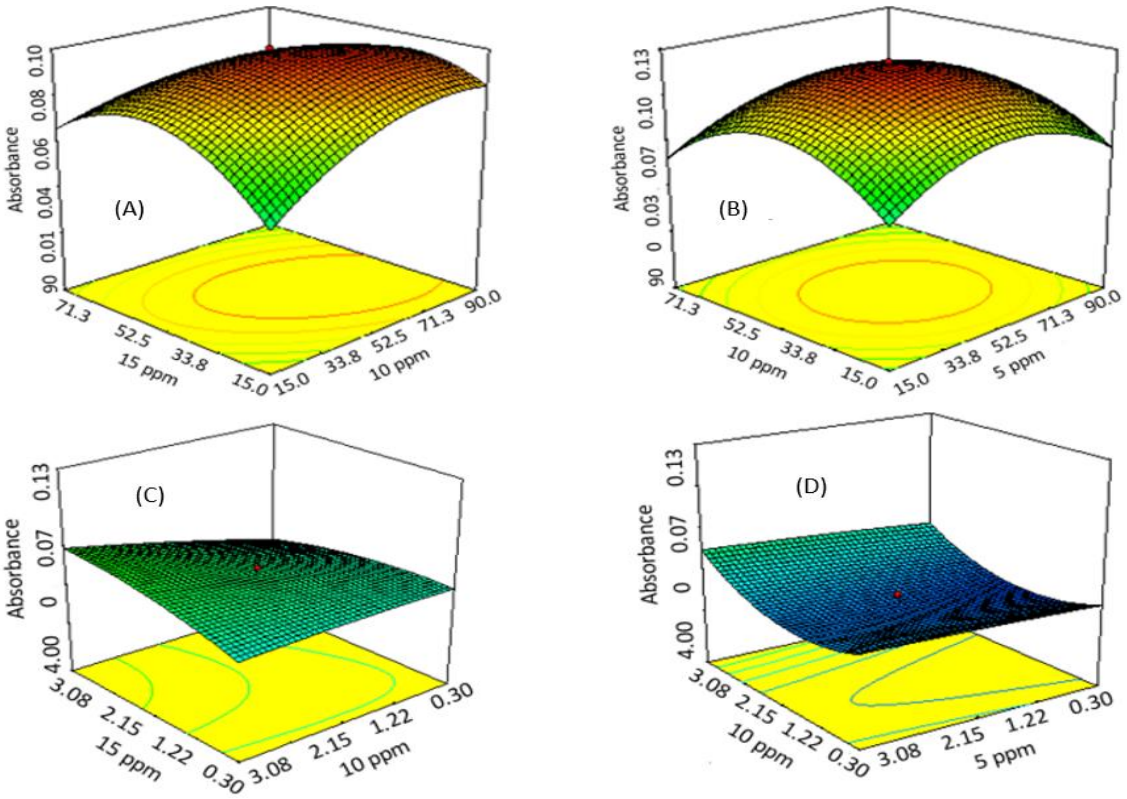


Fig. 4: Response surface plots demonstrate the impact of (A) UV, (B) UV/H_2O_2, (C) gamma and (D) gamma/H_2O_2 on the absorbance of daunorubicin.

UV and UV/H_2O_2 COD results

The COD reduction significantly increased to 56, 50 and 43 for the mentioned concentrations when the samples were treated with UV radiation along with H_2O_2. Based on these findings, the rate of COD reduction rises with longer UV radiation exposure and decreases as the initial drug concentration increases (Fig 5A). UV radiation, combined with H_2O_2, generates hydroxyl radicals (•OH), which in turn accelerate the drug degradation rate (Fig 5B). Our results align with the study by Kanjal et al. [14], who degraded methotrexate (an anti-rheumatic drug) in an aqueous solution using UV and gamma radiation with H_2O_2 as part of an advanced oxidation process.

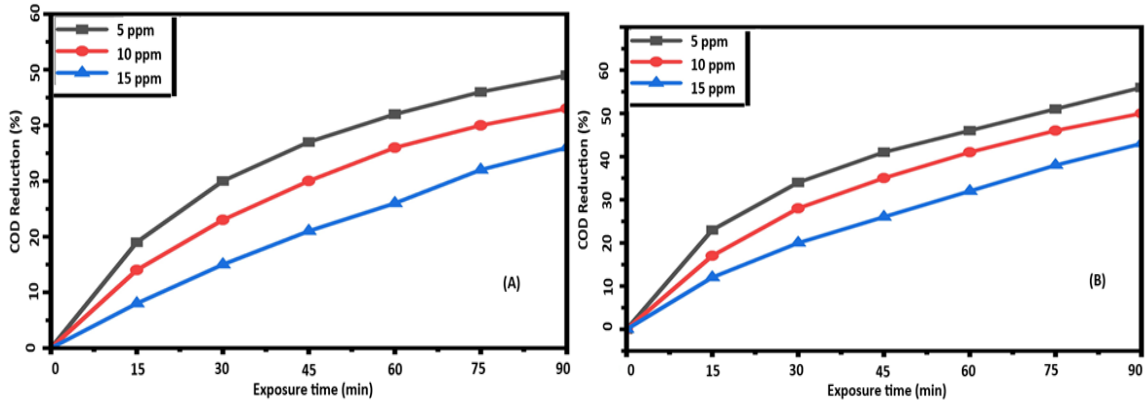


Fig. 5: A reduction in COD of daunorubicin by (A) UV alone, (B) UV in the presence of H_2O_2.

Gamma and gamma/H_2O_2 COD results

The COD reduction of daunorubicin aqueous solutions was also assessed using gamma radiation alone and in combination with H_2O_2. A 78% COD reduction was achieved for the 5-mg L^{-1} solution, 71% for the 10-mg L^{-1} solution and 63% for the 15-mg L^{-1} solution when exposed to gamma radiation at a dose of 4 kGy (Fig 6A). When the samples were treated with gamma radiation and H_2O_2, the COD reduction increased significantly to 83%, 76% and 69% for the respective concentrations (Fig 6B). These results highlight that gamma radiation is more effective than UV radiation in reducing COD, with an even stronger effect when combined with H_2O_2. Gamma radiation, being high-energy, breaks down complex organic compounds into simpler molecules, lowering COD, which measures the oxygen demand required to degrade organic matter. However, as the concentration of H_2O_2 increases beyond the optimal level, the hydroxyl radicals (•OH) produced can interact with and scavenge reducing species, potentially decreasing COD reduction. Our findings align with the study by Iqbal et al. [42], who observed a reduction in COD through photocatalysis in industrial wastewater, indicating a reduction in organic content. The RSM plots show a significant improvement in COD reduction when the samples were treated with UV radiation and H_2O_2 together, compared to UV radiation alone (Fig 7 and S2). The response surface methodology further confirmed that gamma radiation was more effective than UV radiation in reducing COD when combined with H_2O_2.

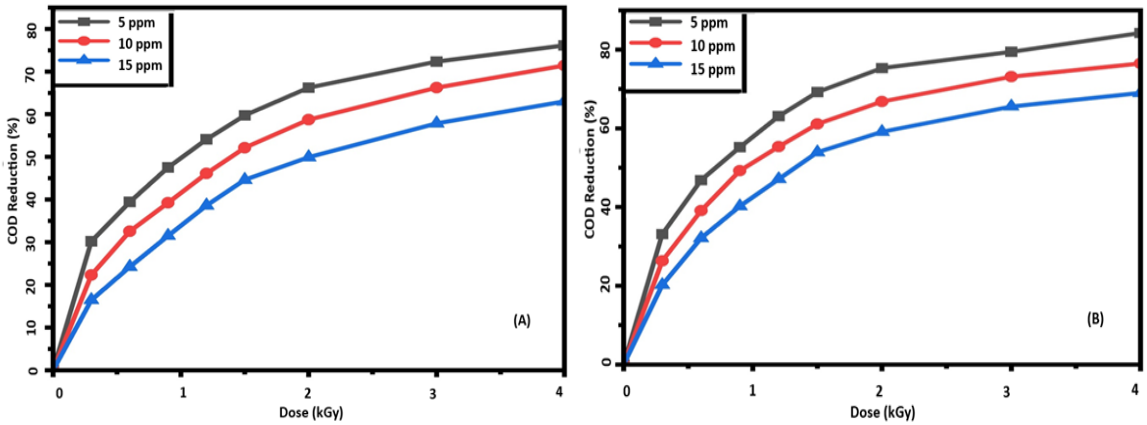
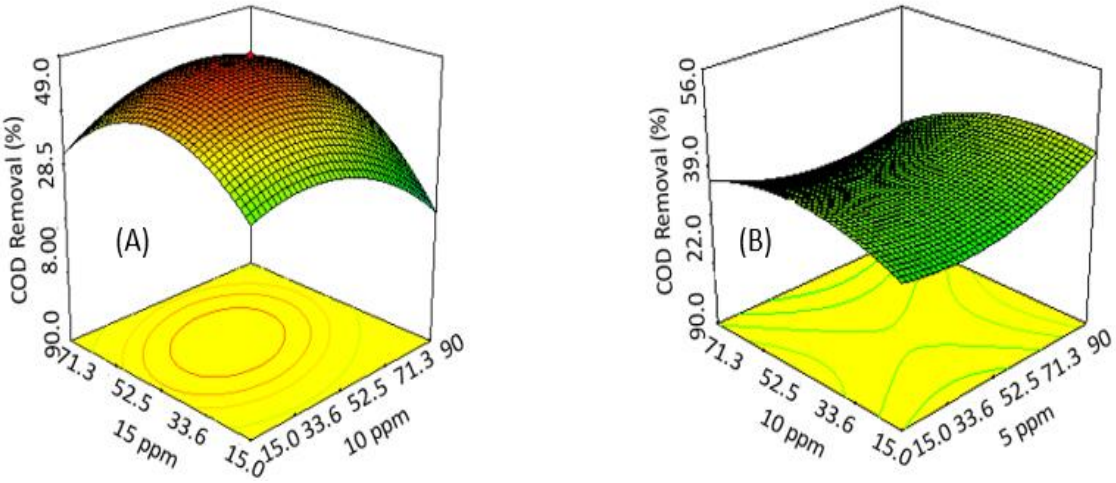


Fig. 6: A reduction in COD of daunorubicin by (A) Gamma alone, (B) Gamma in the presence of H_2O_2.



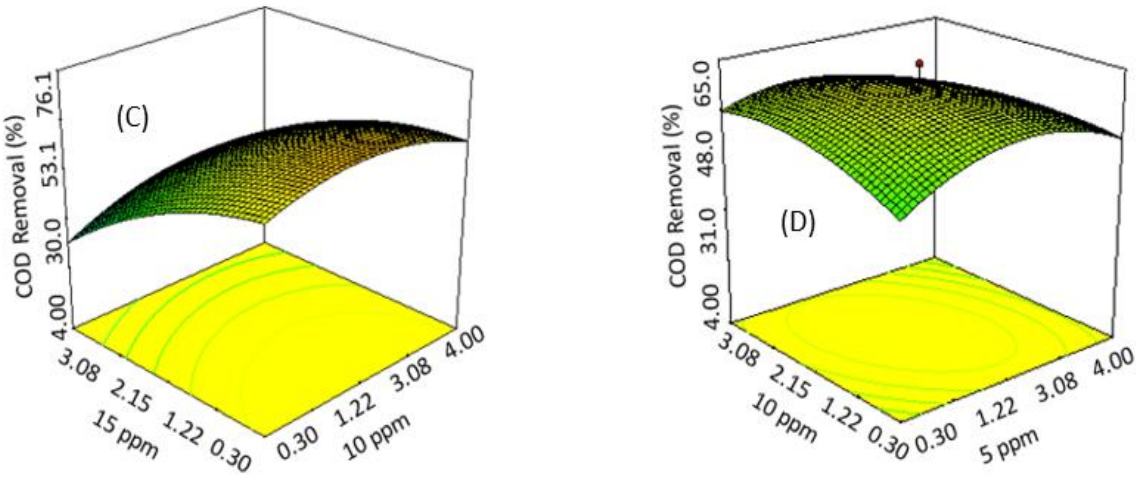


Fig. 7: Response surface plots demonstrate the impact of (A) UV, (B) UV/H_2O_2, (C) gamma and (D) gamma/H_2O_2 on the COD of daunorubicin.

Table-1: Removal efficiency (G-value), dose constant (k), $D_{0.50}$, $D_{0.90}$ and $D_{0.99}$ values of daunorubicin.

Parameters							
Treatment	**[Drug] (mg L^{-1})**	**Gamma dosage (kGy)**	**G-value (µmol/J)**	**Dose Constant (µM/kGy)**	**$D_{0.50}$ (kGy)**	**$D_{0.90}$ (kGy)**	**$D_{0.99}$ (kGy)**
Gamma	**5**	**0.3-4**	**0.1269-0.0008**	**0.916**	**0.765**	**2.511**	**5.022**
	10	**0.3-4**	**0.1604-0.003**	**0.557**	**1.242**	**4.127**	**8.254**
	15	**0.3-4**	**0.2269-0.0023**	**0.526**	**1.317**	**4.375**	**8.751**
Gamma/H_2O_2	**5**	**0.3-4**	**0.1610-0.0005**	**1.389**	**0.498**	**1.656**	**3.313**
	10	**0.3-4**	**0.2696-0.0016**	**0.758**	**0.914**	**3.037**	**6.074**
	15	**0.3-4**	**0.3477-0.0030**	**0.567**	**1.221**	**4.057**	**8.114**

Gamma radiation treatment efficiency

Table 1 presents the kinetic parameters for 5, 10 and 15 mg L^{-1} daunorubicin aqueous solutions, including dose constants (k), $D_{0.50}$, $D_{0.90}$ and $D_{0.99}$, for gamma radiation-treated samples both with and without H_2O_2. The results show that as the drug concentration increases from 5 to 15 mg L^{-1}, the values for $D_{0.50}$, $D_{0.90}$ and $D_{0.99}$ also increase. Meanwhile, the G-value decreases with higher concentrations, suggesting that a greater amount of gamma radiation is needed to achieve 50%, 90% and 99% degradation at higher concentrations. This is likely due to competition between reaction intermediates and reactive species in the parent molecules, meaning that more gamma radiation is required to break down higher concentrations of the drug [43].

Cytotoxicity and Mutagenicity findings

The cytotoxicity of daunorubicin was evaluated both before and after UV and gamma radiation, as well as in the presence of H_2O_2. The data from the UV and UV/H_2O_2 treatments of drug aqueous solutions using the Allium cepa test are presented in ST 2, while the gamma and gamma/H_2O_2 results are shown in ST 3. A 41.17%, 35.44% and 43.75% increase in root count (RC), root length (RL) and mitotic index (MI) was observed with UV radiation alone. When the samples were treated with UV radiation and H_2O_2, the increases were 47.36%, 38.55% and 52.63% for RC, RL and MI, respectively, after 90 minutes of UV exposure (Fig 8A). Similarly, using gamma radiation alone, RC, RL and MI increased by 52.38%, 42.04% and 57.14%, respectively. When gamma radiation was combined with H_2O_2, the increases were 56.52%, 43.68% and 60.86% for RC, RL and MI, respectively, at a gamma-absorbed dose of 4 kGy (Fig 8B). These results align with the findings of Jadhav et al., who reported a mitotic index of 11.68% before radiation treatment, which increased to 14.25% after radiation therapy [37]. Recently, Iqbal and Nisar performed an *Allium cepa* test and observed an increase in root count of 41.17% and in root length of 41.12% after the gamma/H_2O_2 treatment [44].

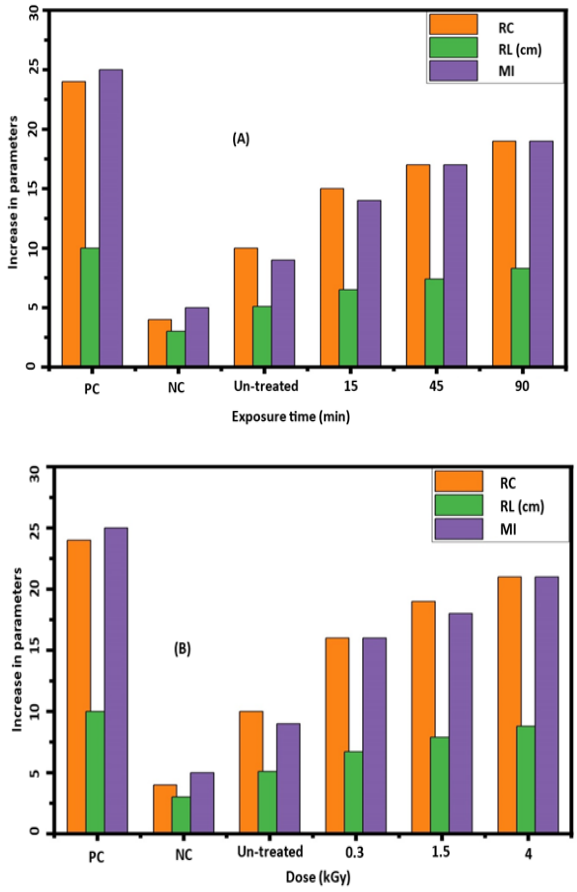


Fig. 8: The Allium cepa test showing the effect of radiation on the toxicity (A) UV/H_2O_2 (B) Gamma/H_2O_2.

The Ames test was conducted using two mutagenic bacterial strains, TA98 and TA100 (*Salmonella typhimurium*). The untreated drug solutions showed mutagenic effects against both the TA98 and TA100 strains [45]. The drug solutions treated with UV radiation alone showed non-mutagenicity of 18.75, 15.63 and 12.5%, whereas 16.67, 12.5 and 10.42% were observed using UV/H_2O_2 for the TA98 bacterial strain. While 16.67%, 13.54% and 10.42% used UV radiation alone and 14.58%, 12.5% and 8.33% along with UV/H_2O_2 for TA100 under the UV exposure time of 15, 45 and 90 minutes, respectively, as shown in Fig 9. A significant reduction in mutagenicity was observed when the solutions were treated with gamma radiation alone and further when combined with H_2O_2. The mutagenicity decreased up to 10.42, 9.38 and 6.25 % by using gamma radiation alone whereas 9.92, 6.24 and 4.17 % by using gamma/H_2O_2 for TA98 bacterial strain while, 14.58, 10.43 and 6.30% using gamma radiation alone and 12.5, 8.33 and 5.17% along with gamma/H_2O_2 for TA100 at gamma radiation absorbed doses of 0.3, 1.2 and 4 kGy respectively as shown in Fig 10. It was observed that UV and gamma-irradiated daunorubicin solutions, when treated with H_2O_2, exhibited a significantly reduced mutagenic effect on the TA98 and TA100 strains, making them safer for potential environmental release without posing hazards (ST 4 & ST 5). This is because the anti-cancer compounds in daunorubicin are metabolized in living cells, where they are transformed into chemical products that interact with cellular macromolecules and participate in oxidation-reduction reactions [46, 47]. The outcomes of the cytotoxicity and mutagenicity evaluations in this study assure that the advanced oxidation processes employed not only degrade daunorubicin but also result in degradation products that are unlikely to pose significant environmental or health risks. The UV/gamma/H_2O_2 process is highly scalable for wastewater treatment, requiring moderate energy inputs and generating hydroxyl radicals efficiently. It integrates seamlessly with existing systems through minor upgrades, such as UV reactors and H_2O_2 dosing units. Standard safety measures ensure safe operation and its ability to function under neutral to alkaline conditions minimizes the risk of secondary pollutants, making it ideal for large-scale applications, particularly in industries handling pharmaceutical effluents.

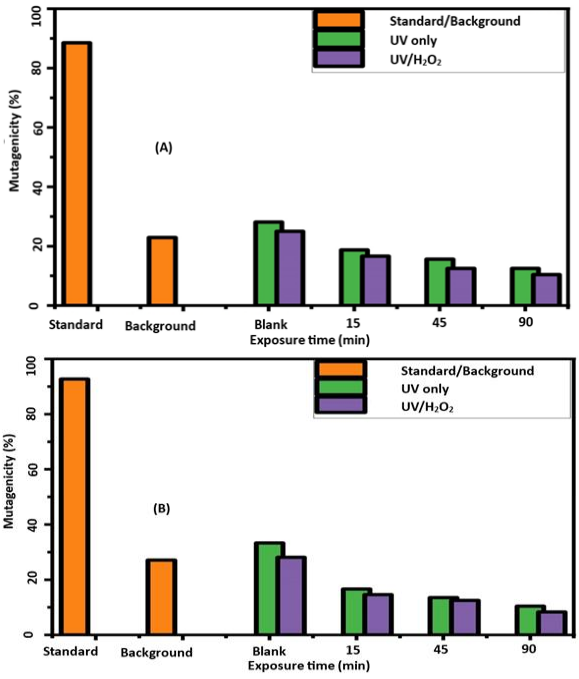


Fig. 9: The Ames test illustrating the effect of UV radiation alone and UV/H_2O_2 on the mutagenicity of daunorubicin using bacterial strains (A) TA98 and (B) TA100.

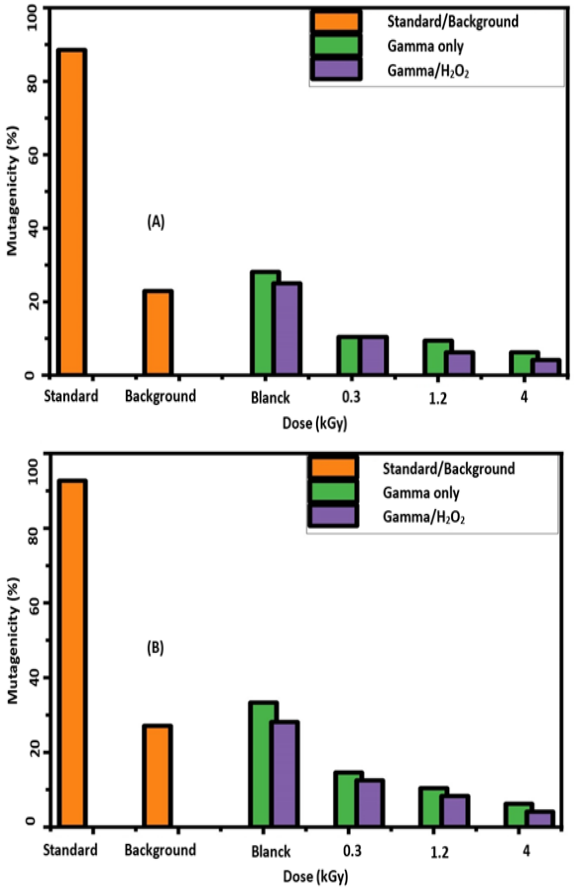


Fig. 10: The Ames test demonstrating the effect of gamma radiation alone and gamma/H_2O_2 on the mutagenicity of daunorubicin using bacterial strains (A) TA98 and (B) TA100.

Analytical (FTIR, GC-MS, HPLC)

The FT-IR spectra of daunorubicin (Fig 11) provided valuable insights into its structural composition and purity. FTIR analysis provides insights into functional group changes, which support the hypothesis of degradation, but it cannot confirm the molecular identities of the degradation products. The spectrum exhibited two prominent absorption bands at 1616 cm^{-1} and 1579 cm^{-1}. The band at 1616 cm^{-1} corresponds to the stretching vibration of quinone carbonyl groups, a characteristic feature of daunorubicin's molecular structure. The peak at 1579 cm^{-1} is attributed to the stretching vibration of C=C bonds within the aromatic rings. Additionally, a broad band at 3390 cm^{-1} was observed, corresponding to O-H stretching, which is consistent with the hydroxyl groups present in the drug molecule. These characteristic peaks confirm the integrity and purity of daunorubicin in the untreated sample. Upon treatment of daunorubicin with radiation, significant changes were noted in the FT-IR spectra. No distinct peaks corresponding to the parent molecular structure were identified after exposure, indicating the breakdown of the original drug molecule. The disappearance of key peaks, particularly those associated with the aromatic rings and quinone carbonyl groups, suggests that the drug underwent extensive degradation. This supports the hypothesis that hydroxyl radicals (˙OH), generated during the radiation process, initiated a chain oxidation reaction that led to the cleavage of the aromatic rings and the breakdown of the complex drug molecules into simpler compounds. Further evidence of degradation was provided by gas chromatographic-mass spectrometric (GC-MS) analysis, which monitored the breakdown products of daunorubicin in the treated samples (Fig 12). The GC-MS results showed a marked reduction in both the intensity and number of peaks, particularly following UV exposure, with an even more substantial decrease after treatment with gamma radiation at an absorbed dose of 4 kGy. Notably, no characteristic peaks associated with daunorubicin or its complex degradation intermediates were observed after treatment with gamma radiation and hydrogen peroxide (H_2O_2), suggesting complete degradation and mineralization of the drug. This was further supported by the absence of molecular fragments linked to daunorubicin, indicating full decomposition into smaller, more environmentally benign compounds. While several degradation products were identified, their precise structural identification remains provisional. GC-MS provides valuable insights into the degradation process, but definitive molecular structures can only be confirmed through advanced techniques like MS/MS or HRMS, which are essential for accurate identification and structural verification of the transformation products. The degradation process was further evaluated using high-performance liquid chromatography (HPLC). The HPLC data (Fig 13) showed a substantial decrease in the peak area corresponding to daunorubicin in both UV and gamma radiation-treated samples. Specifically, when the drug solution was exposed to UV radiation alone, an 86.52% degradation was observed, while gamma radiation treatment resulted in 97.75% degradation. The presence of H_2O_2 during the radiation treatment significantly enhanced the degradation process. When UV radiation was combined with H_2O_2, 89.89% degradation was achieved. In the case of gamma radiation with H_2O_2, complete degradation (100%) was observed. This result indicates that H_2O_2 acts synergistically with gamma radiation, promoting the formation of reactive oxidative species like hydroxyl radicals, which are highly effective in breaking down daunorubicin into lower-mass species. FTIR, HPLC and GC-MS

analyses collectively indicate that daunorubicin's toxicity is reduced after degradation. FTIR shows the breakdown of key functional groups, suggesting the formation of less harmful by-products. HPLC confirms a decrease in the parent compound concentration, while GC-MS reveals the presence of smaller, less toxic fragments. These chemical changes are consistent with the observed reduction in cytotoxicity and mutagenicity, reflected in lower IC50 values and a decreased mutagenic response in the Ames test, highlighting the successful reduction in toxicity. The obtained results demonstrate that daunorubicin undergoes substantial degradation when exposed to UV and gamma radiation, with H_2O_2 further enhancing the degradation process. The possible fragmentation of daunorubicin is shown in Scheme 1.

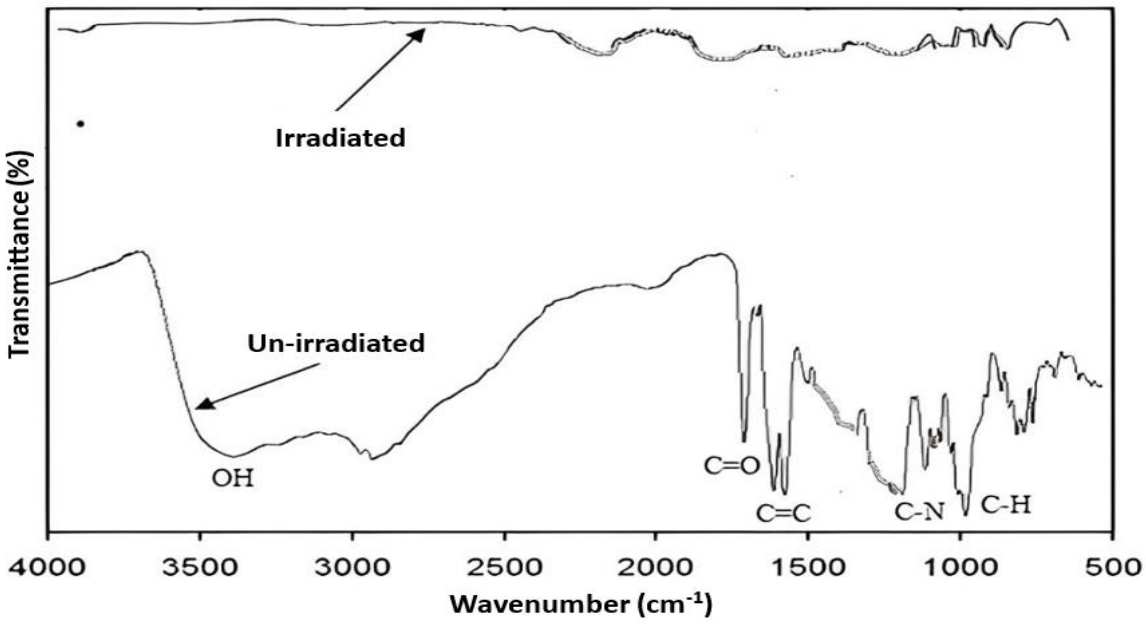


Fig. 11: FT-IR profile of unirradiated and irradiated daunorubicin.

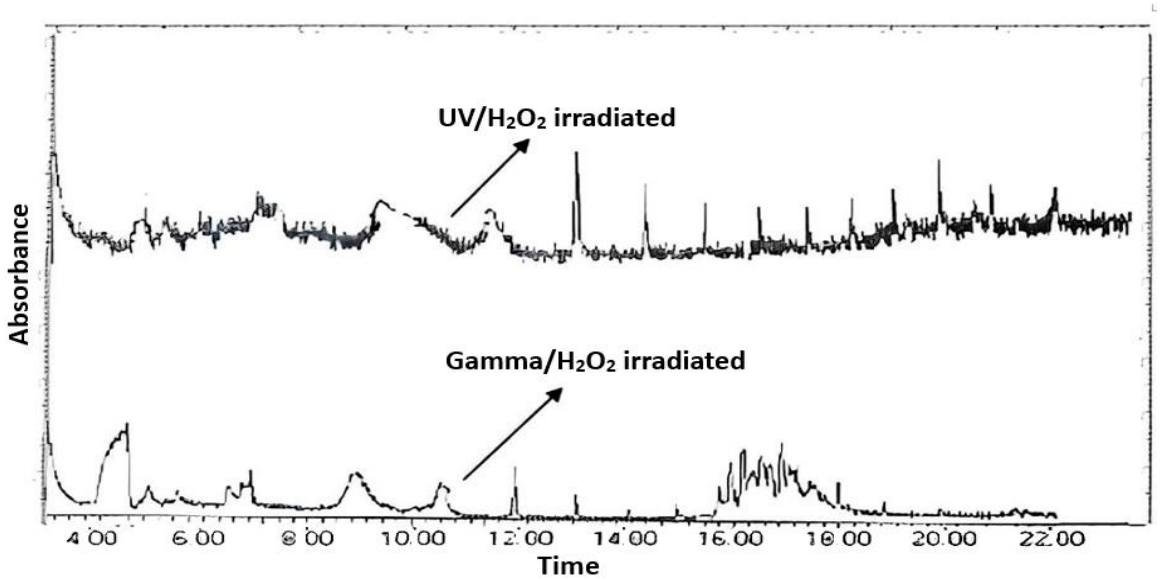


Fig. 12: GC-MS profile of daunorubicin irradiated with UV/H_2O_2 and gamma/H_2O_2.

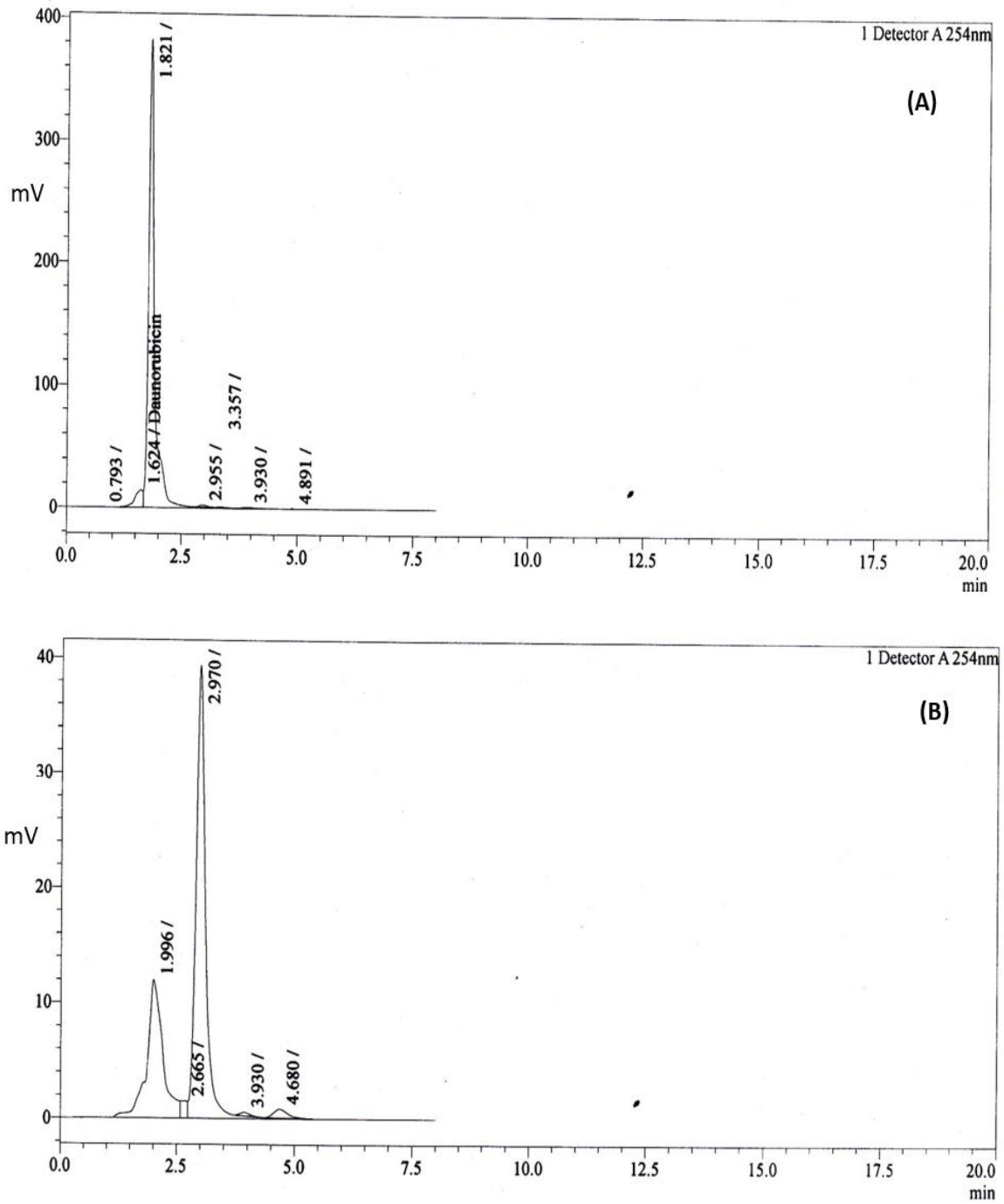
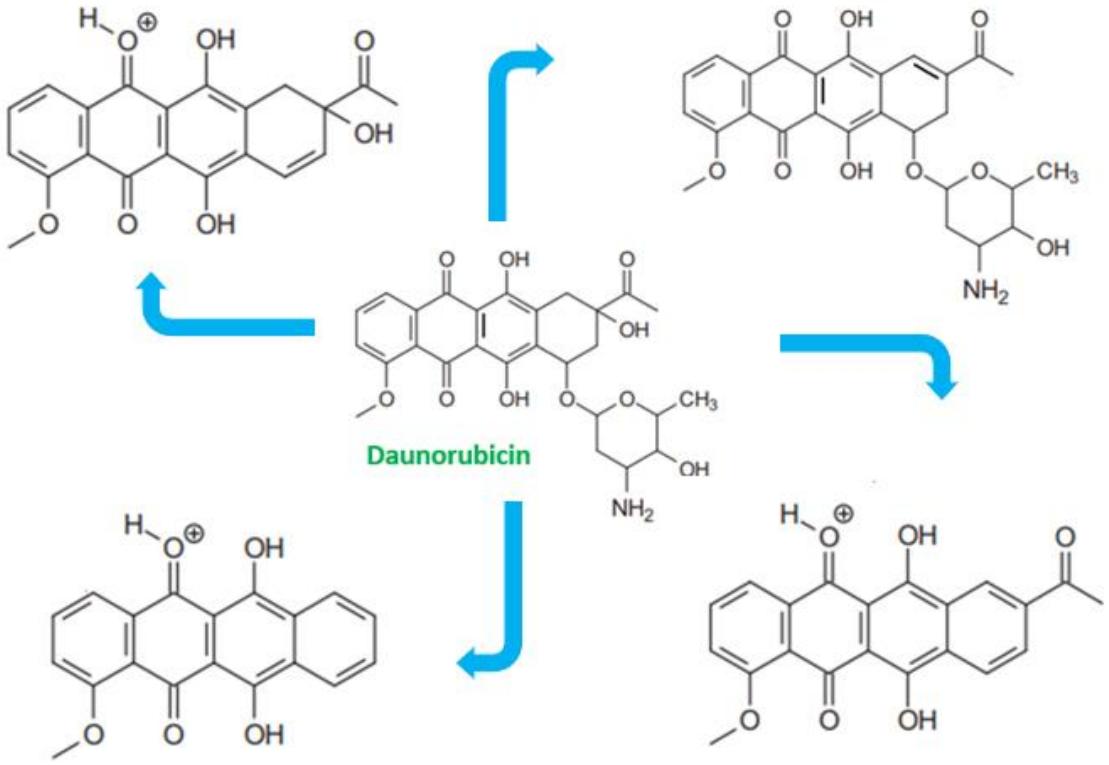


Fig. 13: HPLC Chromatogram of daunorubicin treated with (A) UV/H_2O_2, (B) gamma/H_2O_2.



Scheme-1: Possible fragments of daunorubicin.

The results demonstrate that the synergistic combination of multiple oxidation processes substantially improves degradation performance while reducing the toxicity of the treated solution. This integrated approach represents a promising, environmentally benign, and highly efficient strategy for advanced water and wastewater treatment applications [48-49]. Furthermore, these findings are consistent with previous reports demonstrating that AOPs are highly effective for the degradation of a wide range of toxic pollutants while minimizing the formation of secondary pollutants, making them a superior alternative to many conventional wastewater treatment technologies [50-52].

Conclusions

The results indicate that advanced oxidation processes (AOPs) using UV/H_2O_2 and gamma/H_2O_2 were highly efficient in degrading daunorubicin. Specifically, the UV/H_2O_2 system achieved 89.89% degradation after 90 minutes of UV exposure, while the gamma/H_2O_2 system achieved complete degradation (100%) at a 4 kGy gamma absorbed dose. Correspondingly, chemical oxygen demand (COD) reductions of 56% and 83%, respectively, were observed, suggesting that AOPs can effectively mitigate the cytotoxicity of toxic compounds like daunorubicin. The Allium cepa and Ames tests proved effective for cytotoxicity and mutagenicity assessments, further supporting the potential of these processes for reducing toxicity. HPLC, GC-MS and FTIR analyses revealed no significant peaks after treatment; however, more advanced spectroscopic techniques are needed to fully identify and quantify the radiolytic and degradation products across all treated levels. Future studies should focus on comprehensive toxicity evaluations of treated wastewater over extended exposure periods to ensure long-term environmental safety. This study underscores the effectiveness of combining radiation with H_2O_2 for daunorubicin degradation, offering valuable insights for enhancing wastewater treatment strategies. To further clarify the role of H_2O_2 in these processes, future research should incorporate control experiments with H_2O_2 alone, enabling a better understanding of its contribution and confirming whether the observed effects stem from H_2O_2 alone or its interaction with radiation. These additional experiments would help pinpoint the specific roles of each component and strengthen the evidence for their synergistic effects.

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