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ORIGINAL RESEARCH ARTICLE

Investigation of the Effects of 5-Fluorouracil and Green Synthesized Silver Chloride Nanoparticles on Embryonic Kidney Cells (HEK293)

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Abstract

Background Despite its anticancer efficacy, 5-Fluorouracil exhibits toxicity toward normal cells. Similarly, silver chloride nanoparticles (AgCl NPs), although known for their anticancer potential, raise safety concerns. Due to limited data on the combined effects of these two agents on normal renal cells, this study aimed to evaluate the individual and combined cytotoxic effects of 5-Fluorouracil and AgCl NPs on HEK293 cells.

Methods HEK293 cells were treated with various concentrations of 5-Fluorouracil, AgCl NPs, and their combination. Cell viability was assessed using the MTT assay, and IC_{50} values were calculated. Statistical analysis was performed using one-way ANOVA and Tukey's post hoc test.

Results All treatments reduced cell viability in a dose-dependent manner. IC_{50} values were calculated as 125 µg/ml for AgCl NPs, 174 µg/ml for 5FU, and 181 µg/ml for the combination. The combined treatment exhibited a higher IC_{50} than either single agent, suggesting potential protective interactions.

Conclusion Co-treatment with 5-Fluorouracil and AgCl NPs may reduce cytotoxicity to normal cells while maintaining therapeutic efficacy. These findings highlight the need for further in vivo studies and mechanistic investigations to optimize safe and effective nanoformulation-based therapies.

Keywords 5-Fluorouracil, Silver chloride nanoparticles, HEK293 cells, MTT assay, Cytotoxicity

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1 Introduction

Chemotherapeutic agent, 5-Fluorouracil (5-FU) is a cytotoxic compound belonging to the family of pyrimidine antimetabolites, which has been used since 1962 for the treatment of cancers such as colorectal, gastric, pancreatic, and breast malignancies. This drug inhibits the enzyme thymidylate synthase, thereby blocking DNA synthesis and preventing the proliferation of cancer cells.[1,2] Intravenous administration of 5-FU may cause adverse effects such as mucositis, alopecia, and hematologic suppression, while topical application can lead to skin irritation.

Silver chloride nanoparticles (AgCl-NPs), owing to their antibacterial, anti-inflammatory, and anticancer properties, have attracted considerable attention in biomedical applications, including drug delivery and tissue engineering. These nanoparticles can be synthesized through chemical or green synthesis methods using plant extracts. Their biological activity is size- and dose-dependent, and they may induce cell death or cytotoxicity in certain normal or cancerous cells.[3-7]

Studies have shown that 5-FU may exert toxic effects on normal cells, particularly renal cells.[8] On the other hand, silver chloride nanoparticles (AgCl-NPs) have attracted considerable attention due to their anticancer properties. However, at concentrations above 20 µg/ml, these nanoparticles may cause damage to normal cells by inducing oxidative stress or activating apoptotic pathways.[9] Some studies have reported that the combination of 5-FU and silver nanoparticles produces greater cytotoxic effects than either agent alone. For example, in non-cancerous HT-29 cells, this combination has been shown to increase reactive oxygen species (ROS) generation, induce DNA damage, and promote cell death.[10,11] Nevertheless, several studies have reported conflicting findings. A 2022 study demonstrated that 5-FU at concentrations below 15.625 µg/ml did not produce a significant difference in cytotoxicity between HeLa cancer cells and normal HEK293 cells.[8] Moreover, another investigation reported that silver nanoparticles containing 5-FU exhibited minimal toxicity toward normal human endothelial cells, suggesting the potential selectivity of this nanoformulation for cancer cells.[12] Furthermore, the combined effects of drugs such as 5-FU with other agents may depend on factors including cell type, administration sequence, and the characteristics of the nanoformulation. In some cases, drug combinations have even demonstrated lower cytotoxicity compared with the individual drug alone.[13] Another study reported that a composite formulation of 5-FU loaded onto cellulose fibers exhibited no significant toxicity toward normal colorectal and nasopharyngeal cells, while exerting strong inhibitory effects on cancer cells.[14]

Despite numerous studies on 5-FU and silver nanoparticles, most research has focused on cancer cells, and limited information is available regarding the combined effects of these two agents on normal cells, particularly human embryonic kidney (HEK293) cells. Moreover, due to variations in experimental design, nanoparticle types, and cellular characteristics, existing results are often inconsistent and sometimes contradictory.

The aim of the present study is to systematically investigate the combined effects of 5-FU and silver chloride nanoparticles on human embryonic kidney cells. This investigation focuses on cell viability and the assessment of cytotoxicity intensity to identify differences between individual and combined treatments. The findings of this study may provide deeper insight into the mechanisms of toxicity in normal cells and contribute to the design of safer and more targeted nanoformulations for cancer therapy. Additionally, these results could guide strategies to minimize the adverse effects of chemotherapeutic drugs on healthy cells.

2 Methods

This analytical experimental study (case–control) was conducted following approval from the Ethics Committee for Biomedical Research of the International Association of Scientists (IAS), with the reference code Ref: IASONCOPROJ.24.005. Ethical principles were strictly observed throughout all stages of the research. These principles included maintaining the confidentiality of information, ensuring the health and safety of samples, and conducting experiments with precision. The study was performed experimentally under in vitro laboratory conditions.

Preparation and Storage of the Drug and Nanoparticles

High-purity 5-FU was obtained from BioBasic and diluted using a phosphate-buffered solution. The prepared solution was stored at 4°C.

Silver chloride nanoparticles were synthesized using a green synthesis method based on previous studies (3,5), employing an aqueous extract of milk thistle (Silybum marianum). First, fresh leaves of the plant were washed, dried, and ground into a fine powder. Ten grams of the powdered plant material were then boiled in 100 ml of distilled water, and after 30 minutes, the solution was filtered to obtain the aqueous plant extract.

For nanoparticle synthesis, a 1 mM aqueous solution of silver nitrate was prepared. The plant extract was then slowly added to the silver nitrate solution in a 1:1 (v/v) ratio, and the mixture was maintained at room temperature under continuous stirring for 1 to 2 hours. In this process, the bioactive compounds present in the extract, such as

flavonoids, phenolics, and antioxidants, acted as reducing and stabilizing agents, facilitating the reduction of silver ions (Ag^+) to silver chloride nanoparticles.

A color change of the solution to gray or light brown indicated the formation of nanoparticles. The resulting nanoparticles were then separated by centrifugation, washed with distilled water, and dried at room temperature. Subsequently, the nanoparticles were characterized and their nature confirmed using X-ray diffraction (XRD) analysis and scanning electron microscopy (SEM).

Cell Line Preparation and Maintenance

HEK293 cells were obtained from the cell bank of the Pasteur Institute of Iran. The cells were cultured in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin–streptomycin, and incubated at 37°C with 5% CO_2. During this period, the culture medium was replaced every 48 hours to maintain optimal conditions for cell growth.

Characterization of Silver Chloride Nanoparticles

SEM Analysis

For SEM imaging, an appropriate amount of the synthesized nanoparticles was placed on an aluminum stub. The sample surface was then coated with a thin layer of gold using a sputter coater to provide the necessary electrical conductivity. After preparation, the samples were placed in the chamber of a ZEISS Sigma 500 VP scanning electron microscope, and images were captured at various magnifications and suitable accelerating voltages. The images were digitally recorded to allow detailed examination of the surface and morphological characteristics of the nanoparticles.

XRD Analysis

For XRD analysis, the nanoparticle samples were first completely dried and then prepared as a homogeneous powder. A specific amount of the powder was placed on a dedicated sample holder of a PANalytical XRD instrument and inserted into the device. The analysis was performed within an appropriate angular range (2θ) with a defined scanning step. The intensity of the diffracted beams was recorded by the detector, and the data were stored as diffraction patterns for subsequent structural analysis.

Cell Treatment Method

To investigate the effects of silver chloride nanoparticles and 5-FU on HEK293 cells, the cells were first maintained in culture flasks containing DMEM supplemented with 10% FBS and 1% penicillin/streptomycin. Once the cells reached approximately 70–80% confluency, they were harvested and seeded into 96-well plates at a density of approximately 10^4 cells per well. The cells were incubated for 24 hours under standard conditions (37°C, 5% CO_2, and saturated humidity) to allow attachment to the substrate.

Following this, cells were treated with various concentrations of silver chloride nanoparticles and 5-FU. The selection of doses was based on previous laboratory experience and pilot study results to determine the optimal dose range for evaluating the cytotoxic and combined effects of 5-FU and silver chloride nanoparticles, ensuring accuracy and reproducibility. For all experiments, third-passage HEK293 cells were used to maintain cellular stability and characteristics, providing more reliable results.

Cells were divided into different groups: one treated with nanoparticles only, one with 5-FU only, one with a simultaneous combination of both agents at a 1:1 concentration ratio, and a control group that received fresh culture medium only. Treatments at various concentrations were applied individually and in combination, and cells were incubated under standard conditions for 24 hours.

MTT Assay

At the end of the treatment period, the culture medium was carefully removed, and 5 mg/ml of MTT solution was added to each well. The cells were then incubated for 4 hours at 37°C, allowing viable cells to reduce the yellow tetrazolium salt to purple formazan crystals. Subsequently, the MTT solution was discarded, and the formed crystals were dissolved using dimethyl sulfoxide (DMSO). The absorbance of the resulting solution was measured at 570 nm using a BioTek microplate reader. The obtained data were used to generate dose–response curves and to determine the half-maximal inhibitory concentration (IC_{50}).

Statistical Analysis

Data were analyzed using SPSS software, version 20. The normality of the data distribution was assessed using the Kolmogorov–Smirnov test. One-way analysis of variance (ANOVA) was then performed to compare differences between groups, and, in cases where significant differences were detected, Tukey's post hoc test was applied for further pairwise comparisons. A significance level of less than 0.05 was considered statistically significant.

3 Results

Morphological Evaluation of Green-Synthesized Silver Nanoparticles

The morphology of silver nanoparticles synthesized via a biocompatible green method was assessed using SEM to determine their shape, size, and surface characteristics. Figure 1 presents a high-resolution image of the

nanoparticles, providing detailed structural information. Point-by-point analysis revealed that the nanoparticles ranged in size from 50 to 70 nm. These size variations may be attributed to several factors, including reaction conditions, the composition of the plant extract used, and the kinetic characteristics of the synthesis process. SEM images also indicated that the nanoparticles were spherical or nearly spherical in shape with a uniform distribution and minimal aggregation, demonstrating the successful application of the green synthesis method for producing silver nanoparticles.

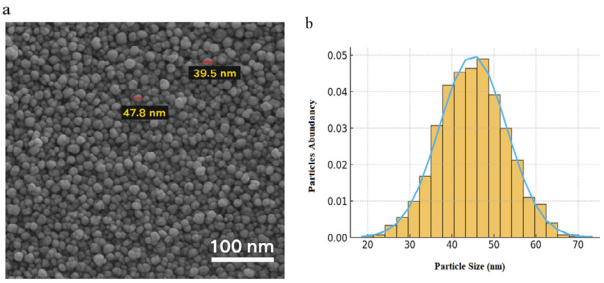

Figure 1 (a) SEM image of the nanoparticles with an average size of approximately 45 nm and a scale bar of 100 nm; **(b)** the corresponding particle size distribution histogram, showing a relatively normal distribution around the mean size

Crystalline Structure and Purity Assessment of Nanoparticles Using XRD

As shown in Figure 2, XRD analysis was employed to investigate the crystalline structure and evaluate the purity of the silver nanoparticles.[10–13] The obtained diffraction pattern showed distinct peaks at 2θ angles of 38.4°, 44.5°, and 64.8°, which correspond to the (111), (200), and (220) planes of a face-centered cubic (FCC) silver structure, respectively. These peaks were identified based on the standard file No. 04-0783 from the Joint Committee on Powder Diffraction Standards (JCPDS). The absence of additional peaks in the pattern indicates that no impurities were present in the samples, confirming the high purity of the nanoparticles and demonstrating the successful synthesis of silver nanoparticles.

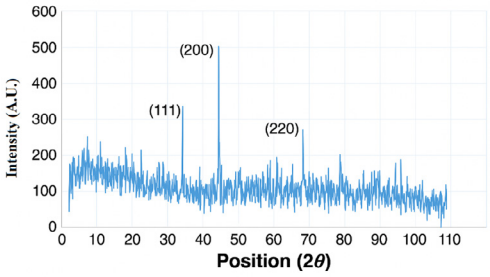

Figure 2 XRD pattern of silver nanoparticles synthesized using plant extract, indicating the characteristic crystalline structure of silver

Assessment of HEK293 Cell Growth in Culture

To evaluate the health and stability of HEK293 cells in the laboratory culture environment and to provide optimal conditions for subsequent drug treatments, cells were cultured under standard conditions, and microscopy images were captured at different time points.

At the initial stage of culture (Figure 3a), cells appeared spherical, single, and suspended in the culture medium. This condition indicates entry into the lag phase, showing that cells had not yet adhered to the surface and that attachment and proliferation processes had not started. Over time, with optimal physiological conditions provided, including 37°C temperature, appropriate humidity, and nutrient-rich medium, the cells entered the logarithmic growth phase (Figure 3b). During this phase, cells began adhering to the surface, changing shape, elongating, and actively undergoing cell division, gradually covering the intercellular spaces.

Finally, in the late culture stage, the cell population reached a high density with an estimated confluency of 80–90% (Figure 3c). At this stage, cells were fully attached, uniform, and compact, ready for drug treatments. These three consecutive images of cell growth clearly demonstrate the health, stability, and proper proliferation of HEK293 cells in the laboratory culture, confirming that an appropriate platform was established for further experimental procedures.

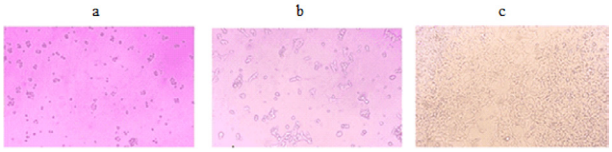

Figure 3 HEK293 cells observed at three stages of culture: **(a)** initial stage, where cells are spherical, single, and suspended, corresponding to the lag phase; **(b)** intermediate stage, where under optimal conditions, cells adhere to the surface, change shape, and enter the logarithmic growth phase; and **(c)** final stage, where increased cell density and approximately 80–90% confluency result in a uniform coverage of the surface, preparing the cells for experimental treatments.

Effect of 5-FU on HEK293 Cells

After stabilizing growth conditions and confirming the health of HEK293 cells, the cells were treated with various concentrations of the chemotherapeutic drug 5-FU. The results of the cell viability assay (MTT) shown in Figure 4 indicate that 5-FU reduced HEK293 cell viability in a dose-dependent manner. This reduction in cell survival was statistically significant at concentrations above 31.25 µg/ml. According to the data presented in Figure 4, cell viability decreased to approximately 55%, 45%, 30%, 20%, and 15% at concentrations of 31.25, 62.5, 125, 250, and 500 µg/ml, respectively. These findings demonstrate a pronounced and reliable effect of 5-FU in reducing cell viability at higher concentrations.

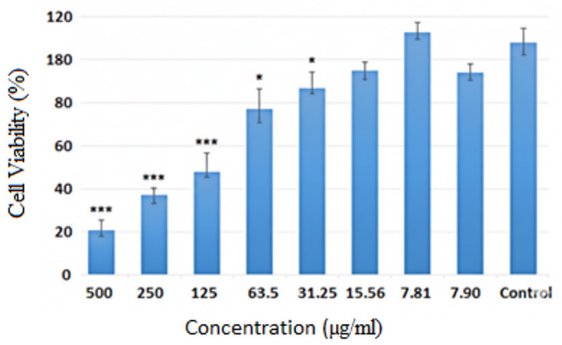

Figure 4 Cell viability reduction in response to various concentrations of 5-FU. Asterisks indicate statistically significant differences compared with the control group: ***p < 0.001, **p < 0.01, and *p < 0.05.

Based on the data presented in Figure 5, the IC_{50} of 5-FU was determined to be 174 µg/ml. This value represents the concentration at which 50% of the cell population is inhibited and serves as an indicator of the cytotoxic potency of the drug. The plotted curve clearly demonstrates the dose-dependent cytotoxicity of 5-FU. At concentrations below 15.6 µg/ml, minimal changes in cell viability were observed, whereas starting from approximately 31.25 µg/ml, the curve shows a marked downward trend. This decline becomes progressively steeper at higher concentrations, leading to a significant reduction in cell survival.

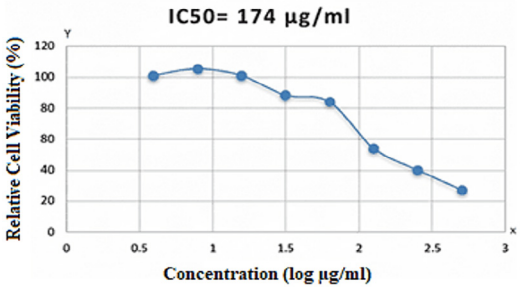

Figure 5 Dose–response curve of 5-FU in the studied cells, from which the IC_{50} was determined to be 174 µg/ml, illustrating the concentration-dependent decrease in cell viability. The horizontal axis is presented on a logarithmic scale to allow more precise determination of the IC_{50} value.

Effect of Silver Chloride Nanoparticles on HEK293 Cells

Treatment of HEK293 cells with silver chloride (AgCl) nanoparticles demonstrated that these nanoparticles exhibit strong, dose-dependent cytotoxic effects (Figure 6). As shown in Figure 6, compared with 5-FU, AgCl nanoparticles caused significant reductions in cell viability at lower concentrations. The IC_{50} value for AgCl nanoparticles was lower than that of 5-FU, indicating higher toxicity toward normal cells. These findings suggest that despite their anticancer potential, the clinical application of AgCl nanoparticles may pose safety challenges. The cell viability data following treatment with AgCl nanoparticles further revealed a clear dose-dependent decrease. At concentrations of 500 and 250 µg/ml, cell viability decreased to less than 20% and approximately 25%, respectively. At 125 µg/ml, viability remained around 35%, and at 62.5 µg/ml, it was approximately 50%. These reductions in cell viability were statistically significant, with p-values of <0.001, <0.001, <0.01, and <0.05 for the respective concentrations.

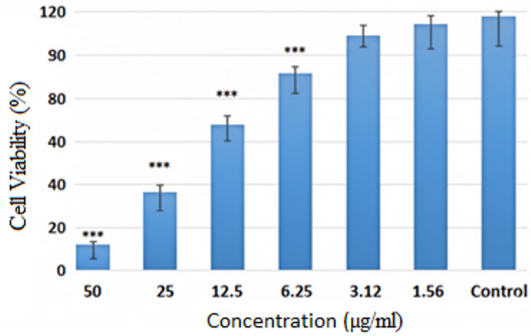

Figure 6 Cell viability reduction in response to various concentrations of silver chloride nanoparticles. Asterisks indicate statistically significant differences compared with the control group: ***p < 0.001, **p < 0.01, and *p < 0.05.

In the dose–response curve for silver chloride nanoparticles (Figure 7), the IC_{50}, representing the concentration required to reduce 50% of cell viability, was determined to be 125 µg/ml. The plotted curve exhibited a sigmoidal pattern with a steep slope in the mid-range, indicating a rapid onset of cytotoxicity even at relatively low concentrations.

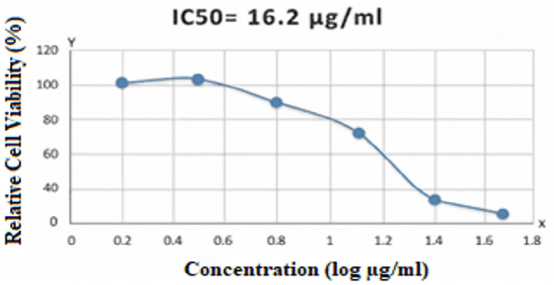

Figure 7 Dose–response curve for determining the IC_{50} of silver chloride nanoparticles on HEK293 cells. The horizontal axis represents nanoparticle concentration (logarithmic scale), and the vertical axis indicates the percentage of cell viability relative to the control group. The curve clearly illustrates the gradual decrease in cell viability with increasing nanoparticle dose. The horizontal axis is presented on a logarithmic scale to allow more precise determination of the IC_{50}.

Combined Effect of 5-FU and Silver Chloride Nanoparticles on HEK293 Cells

Simultaneous treatment of HEK293 cells with 5-FU and silver chloride nanoparticles resulted in a significant reduction in cell viability (Figure 8). Although this combination exhibited stronger cytotoxic effects at certain doses compared with either treatment alone, the IC_{50} for the combined treatment was calculated to be 181 µg/ml, which is higher than the IC_{50} values of silver chloride nanoparticles (125 µg/ml) and 5-FU (174 µg/ml). This surprising finding suggests that, despite its stronger inhibitory effect at specific concentrations, the combination may induce less toxicity in normal cells. This phenomenon could be attributed to drug interactions such as reduced cellular uptake, competition within molecular pathways, or partial neutralization of the toxic effects of one agent by the other. A detailed examination of the data presented in Figure 8, which illustrates the response of HEK293 cells to various concentrations of the combined 5-FU and silver chloride nanoparticles, reveals a pronounced dose-dependent decline in cell viability. With increasing concentrations of the combination from 0.5 to 3 µg/ml (logarithmic scale), a gradual reduction in cell survival was observed, and at concentrations above 2 µg/ml, viability decreased to less than 40%. This pattern indicates a strong yet gradual effect of the combination on normal cells.

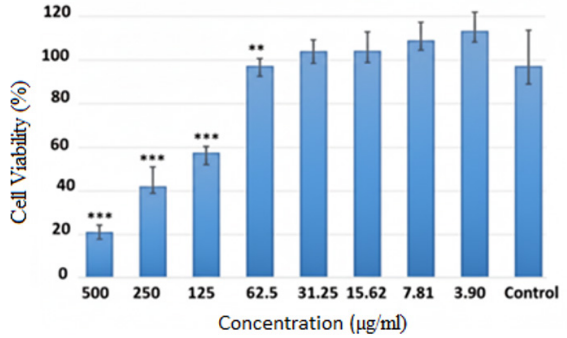

Figure 8 Cell viability reduction in response to various concentrations of the combination of 5-FU with silver chloride nanoparticles on HEK293 cells. Asterisks indicate statistically significant differences compared with the control group: ***$p < 0.001$, **$p < 0.01$, and *$p < 0.05$.

The IC_{50} curve shown in Figure 9 indicates that the IC_{50} for the combined treatment is 181 µg/ml, which is higher than the IC_{50} values obtained for the individual treatments. This finding suggests that the combined treatment may exert lower toxicity on HEK293 cells compared with silver chloride nanoparticles and even 5-FU, despite greater reductions in cell viability at certain doses. This unexpected result underscores the importance of carefully evaluating potential synergistic effects and drug interactions in combination therapies.

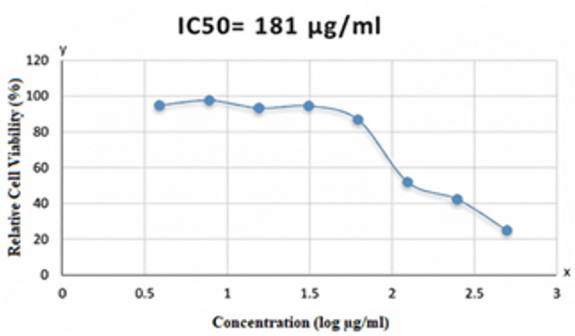

Figure 9 Dose–response curve for determining the IC_{50} of the combined 5-FU and silver chloride nanoparticles on HEK293 cells. The IC_{50} of the combination was determined to be 181 µg/ml. The horizontal axis represents the concentration of the combination (logarithmic scale), and the vertical axis indicates the percentage of cell viability relative to the control group. The curve illustrates the onset of cytotoxic effects at low concentrations and a gradual decrease in cell viability with increasing doses of the combination. The horizontal axis is presented on a logarithmic scale to allow more precise determination of the IC_{50}.

Overall Comparison of the Three Treatments on HEK293 Cell Cytotoxicity

Analysis and comparison of the effects of individual and combined treatments with 5-FU and silver chloride nanoparticles on HEK293 cells revealed that all three treatments induced a dose-dependent decrease in cell viability, although the magnitude and pattern of the response differed among them. Among the three treatments, silver chloride nanoparticles alone exhibited the highest toxicity toward normal HEK293 cells, with an IC_{50} of 125 µg/ml, the lowest value observed among the treatments. Moderate toxicity was demonstrated by 5-FU, with an IC_{50} of 174 µg/ml, and showed notable cytotoxic effects at higher concentrations.

For the combined treatment of 5-FU and silver chloride nanoparticles, despite the dose–response curve indicating a more pronounced reduction in cell viability at certain concentrations, the calculated IC_{50} was 181 µg/ml, which is higher than that of either individual treatment. This unexpected finding may suggest a type of interactive effect between the two compounds that results in an overall reduction of toxicity toward normal cells. Such an effect could be due to factors including competition for cellular uptake, interference with cell death pathways, or partial neutralization of the toxic effects of one agent by the other.

Overall, this comparison indicates that silver chloride nanoparticles alone exert the most pronounced cytotoxic effect on HEK293 cells, whereas the combined treatment, despite causing substantial reductions in cell viability at specific concentrations, may be overall safer than the individual treatments in terms of general cytotoxicity.

4 Discussion

In this study, the chemotherapeutic agent 5-FU exhibited a significant dose-dependent cytotoxic effect on HEK293 cells, with an IC_{50} value calculated at 174 µg/ml. These results are fully consistent with previous studies. For instance, Abd Elaty et al. reported that treatment with 5-FU caused histopathological and biochemical damage in the liver and kidneys of rats, indicating the systemic toxicity of the drug.[15] Additionally, Lamberti et al. demonstrated that 5-FU can induce apoptosis via caspase-dependent pathways, a process that is modulated by autophagy regulation and may have specific clinical and workplace implications .[16]

Existing scientific evidence indicates that the effects of 5-FU on normal cells depend on cell type, drug dose, and exposure duration; in some cell types, particularly at low doses, effects such as increased proliferation have also been observed.[17-19] Focaccetti et al. reported that 5-FU induces morphological alterations, inhibits proliferation, and triggers apoptosis and autophagy in endothelial cells and cardiomyocytes, while simultaneously increasing ROS production.[18] The primary mechanisms of 5-FU cytotoxicity include inhibition of thymidylate synthase, which disrupts DNA and RNA synthesis, leading to cell cycle arrest and cell death. Additionally, caspase-dependent apoptotic pathways and ROS generation are critical contributors to cell death. In this context, Arabnezhad et al. demonstrated that the cytotoxicity of this drug in HEK293 and HeLa cells is strongly dependent on apoptosis induction.[8]

This study further showed that, compared to 5-FU, silver chloride nanoparticles exerted stronger cytotoxic effects on HEK293 cells at lower doses. This finding aligns with the results of Jiang et al., who reported that silver nanoparticles induce cytotoxicity via DNA damage, apoptosis induction, ROS generation, and alterations in the biomechanical properties of cells.[20] Moreover, characteristics such as nanoparticle size, surface coating (e.g., with biocompatible materials such as chitosan or albumin), and the presence of chloride ions play a crucial role in nanoparticle toxicity, as confirmed by studies by Ardalani et al. and Jafari.[21,22]

On the other hand, biologically synthesized silver nanoparticles not only exhibit cytotoxicity against cancer cells but also possess antioxidant properties, which may help prevent excessive damage to healthy cells. Additionally, commercially produced silver nanoparticles generally display higher toxicity compared to biogenic nanoparticles. Particle size influences the production and distribution of ROS, with smaller particles inducing greater cytotoxicity. The primary mechanisms underlying silver nanoparticle toxicity include oxidative stress, DNA damage, and inflammation. Moreover, silver nanoparticles have the potential to cause genotoxic effects, highlighting the need for further investigation.[23-26]

Interestingly, in the present study, the combined treatment of 5-FU and silver chloride nanoparticles, despite causing a marked reduction in cell viability at certain doses, resulted in an increased IC_{50} of 181 µg/mL. This increase may indicate the presence of protective interactions between the two agents, which could occur through modulation of silver ion release, reduced cellular uptake, or regulatory effects on cellular signaling pathways. Previous studies have shown that coating nanoparticles with materials such as chitosan or albumin can reduce their combined toxicity with chemotherapeutic drugs.[3,27] However, some studies have reported that combining silver nanoparticles with drugs may enhance toxicity, which depends on the nature of the nanoparticles, dosage, cell type, and experimental conditions.[28,29] Therefore, differences in the physicochemical properties of nanoparticles and experimental methodologies play a crucial role in the outcomes. Some research has also indicated the selective activity of silver chloride nanoparticles toward cancer cells and the antioxidant effects of these particles in normal cells.[30,31] This supports the findings of the present study, which suggest relatively lower toxicity of the combined 5-FU and nanoparticle treatment in normal cells.

A major limitation of this study is that the experiments were conducted in vitro using the HEK293 cell line. In vivo, drug metabolism, interactions with the immune system, absorption and distribution across tissues, and other complex biological parameters can significantly alter cellular responses to treatment. Therefore, extrapolation of these results to real-world or clinical settings requires more comprehensive studies in animal and human models.

The precise molecular mechanisms underlying the interactions between 5-FU and silver chloride nanoparticles remain incompletely understood, and further mechanistic and molecular investigations are needed to elucidate these processes. Additionally, the cytotoxic effects of nanoparticles at low concentrations raise safety concerns, which should be addressed through extensive preclinical safety evaluations to determine potential therapeutic applications with minimal adverse effects.

Finally, investigating cellular resistance to 5-FU and the impact of its combination with silver chloride nanoparticles on drug resistance represents an important avenue for future research. Drug resistance is a major challenge in cancer therapy, and understanding these mechanisms could contribute to the development of more effective treatment strategies.

5 Conclusion

The findings of this study demonstrate that both 5-FU and silver chloride nanoparticles exert dose-dependent cytotoxic effects on human embryonic kidney (HEK293) cells when applied individually. Notably, silver chloride nanoparticles exhibited higher cytotoxicity, as indicated by a lower IC_{50} compared to 5-FU. Interestingly, the simultaneous combination of these two agents, despite causing a more pronounced reduction in cell viability at certain doses, resulted in a higher IC_{50}, suggesting a relative decrease in overall toxicity toward normal cells. These results indicate that the combined use of 5-FU and silver chloride nanoparticles may mitigate cytotoxic effects through interactions in the underlying mechanisms of toxicity, potentially via reduced silver ion release, modulation of ROS production, or improved targeted drug delivery. Such findings highlight the potential of this combination for the development of targeted nanomedicine strategies with enhanced efficacy and reduced side effects, providing a promising avenue for designing safer therapeutic regimens for cancer treatment. However, further mechanistic studies and in vivo investigations are necessary to confirm and validate these advantages.

Declarations

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Artificial Intelligence Disclosure
Artificial intelligence tools were not used for generating or interpreting the data presented in this study. AI assistance (ChatGPT, OpenAI) was utilized solely for language editing, grammar checking, and minor revisions, all performed under the direct supervision and critical judgment of the authors.

Authors' Contributions
Study Design: Parand Torabi, Hadis Rostami Motamed; Experiment Execution: Parand Torabi; Data Analysis: All authors; Manuscript Writing and Editing: All authors.

Availability of Data and Materials
Data will be made available upon request.

Conflict of Interest
The authors declare that they have no conflicts of interest related to this study.

Consent for Publication
Not applicable.

Ethical Considerations
This study was conducted following approval from the Ethics Committee of the International Association of Scientists (IAS) with the reference number Ref: IASONCOPROJ.24.005.

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