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# Evaluation of the Synergistic Cytotoxicity of Camptothecin with Silver Nanoparticles: Potential Anti-angiogenic, Anti-inflammatory, and Antioxidant Agents

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**Abstract**

**Background**: The high cytotoxicity of camptothecin is considered one of the greatest problems, aside from resistance within the course of cancer therapy. Conversely, silver nanoparticles (SNPs) provide great potential for increasing drug efficacy and reducing adverse effects. The purpose of the research was to explore the cytotoxic impact of camptothecin with SNPs on Various cancer cell lines and also to determine the level of several inflammatory, oxidative stress and angiogenesis biomarkers as possible targets influenced by these treatments.

**Methods**: The SNPs were produced by *Aspergillus flavus* and characterization was performed by scanning electron microscopy (SEM) and zetasizer. SNPs were tested separately and in combination with camptothecin against normal fibroblast cells and different cancer cell lines including A549, HT-29 MCF7 and PANC-1. In addition, several inflammatory, oxidative stress and angiogenesis biochemical markers were evaluated using Enzyme Linked Immuno Assay (ELISA).

**Results**: SNPs significantly increased the toxicity of camptothecin against A549, MCF7, and PANC-1 cells at 0.75 µg/ml, while having insignificant toxicity on normal fibroblast cells. In addition, SNPs caused a decrease in vascular endothelial growth factor (VEGF) production and changed the inflammatory marker and antioxidant enzyme profile; thus, it was evident that co-incubation of SNPs and camptothecin effectively interrupted the pathways important for cancer cell survivability.

**Conclusion**: Combined SNPs and camptothecin increase the potentiating effect of camptothecin cytotoxicity and affect several cancer-related processes such as oxidative stress, inflammation, and tumor vascularization. This approach offered a unique strategy against cancer treatment, particularly for cancers that do not respond to chemotherapeutic agents like pancreatic cancer. More research is required to establish this combination for its therapeutic application.

**Keywords**: inflammatory cytokines, antioxidant enzyme, angiogenesis, pancreatic cancer, Silver Nanoparticles (SNPs), Camptothecin.

## 1. Introduction

Cancer is now recognized as one of the most malign diseases of the twentieth century (Al-Rawashde *et al*., 2021, Hamlat *et al*., 2023). The number of cases of cancer in Jordan has increased, rising from 12.6 per 100,000 persons in 2005 to 17.2 per 100,000 in 2010, according to recent cancer statistics. With 11.3% of all new cases, cancer ranked as the second most frequent malignancy in both genders by 2012. In Jordan in 2013, malignancies of the colon, rectum, anus, and small intestine made up about 2% of all fatalities. The 5-year survival rate for individuals with cancer decreases dramatically with age, falling from 60.4% for those under 50 to 49.3% for those 70 years of age or over. As the illness worsens, survival rates similarly drop as follows: 72.1% for locally located cancer, 53.8% at regional stage, and 22.6% for distant metastases (Sharkas *et al*., 2017).

Natural phytochemicals provide effective alternatives in the treatment of cancers (Al-Rawashde *et al.*, 2023, Al-Rawashde *et al.*, 2022). Camptothecin, an alkaloid found in the bark of the *Camptotheca acuminata* plant, is a strong anticancer drug that causes apoptosis and DNA damage by blocking DNA topoisomerase I, which can increase DNA damage in the S-phase stage during the proliferation cycle, resulting in cell damage (Yakkala *et al.*, 2023, Kohorst and Kaufmann, 2023). However, clinical trials have not employed it because of its high toxicity, poor targeting, and solubility issues (Sun et al., 2021). Moreover, it has nonselective toxic effects that could affect all types of cells (healthy as well the malignant) and lead to undesired side effects. Furthermore, it could also influence the bone marrow cells, allowing them to become more susceptible to different diseases (Ghanbari-Movahed *et al*., 2021).

Therefore, combination therapy, which provides an alternative approach to treating cancer, has been given tremendous attention lately owing to its potential to decrease drug resistance in cancer cells by chemosensitizing tumor cells to chemotherapy drugs, as well as decreasing the doses of drugs and enhancing the selectivity (Abd El Latif *et al*., 2024, Wang *et al*., 2023), Moreover, a combination of biocompatible nanoparticles

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and anticancer drugs can overcome drug resistance development or impair it or, at least, lower the side effects to zero levels, reduce healthy cell damage and induce selective cytotoxicity only within cancer cells (Blagosklonny, 2023).

Furthermore, combinations of chemotherapeutic agents with silver nanoparticles (SNPs) have gained significant attention, since SNPs have emerged among several types of nanoparticles that possess antibacterial and anticancer capabilities. For example, when SNPs are taken by bacteria or living cells, they release and produce silver ions or reactive radical species, which causes essential cellular processes to be disrupted and ultimately leads to severe cellular damage and death. As a result, SNPs are now frequently found in food containers and antiseptic medical dressings (Talapko e*t al.*, 2020).

For that reason, this investigation aims to assess the cytotoxic effects of SNPs combined with camptothecin on healthy fibroblast cells and different cancer cell lines (A549, HT-29, MCF7, and PANC-1) and to evaluate the antiangiogenic, anti-inflammatory, and antioxidant effectiveness of these combinations.

## 2. Material and methods

### *2.1. Material*

Ten percent of fetal bovine serum, one percent of penicillin/streptomycin solution, and Dulbecco Modified Eagle Medium containing L-glutamine were acquired from EuroClone (UK). Additionally, we acquired trypsin and Phosphate Buffer Saline (PBS) from EuroClone (UK). Promega (USA) supplied MTT reagents, Trypan blue stain, stop solution, and Dimethyl Sulfoxide (DMSO), while TPP (Zollstraße, Switzerland) supplied cell culture plates.

### *2.2. Biosynthesis of SNPs*

*Aspergillus flavus* was selected to generate SNPs since it has been documented as a reliable source that creates silver particles with a small size (Gopa and Pullapukuri, 2023). In our previous work, the fungus was collected from the store buildings in Mutah University, Jordan. This was followed by identification analysis using a sequence similarity test and the accession number of MG973280.1 was acquired. Then, the *Aspergillus flavus* was used for the biogenesis of SNPs using XRD, ATR-FTIR, and UV-vis spectrum of absorption for characterization (Al-Soub *et al.*, 2022).

### *2.3. Morphology and Particle Size Analysis*

JEM-2010 microscope (JEOL, Tokyo, Japan) were used for scanning electron microscope (SEM) images. SEM images were obtained at pressures of 0.07 and 0.05 mbar and an acceleration voltage of 0.16 kV. Dynamic light scattering by Malvern Analytical in Malvern, United Kingdom, was used to determine the size distribution of the silver particles in liquid solutions with the Zetasizer Nano ZSP (Al-Limoun *et al.*, 2020, Abu Hajleh *et al.*, 2023).

### *2.4. Cancer cell lines culture*

Several human cancer cell lines were used including the lung cancer cell line (A549), the colorectal cancer cell line (HT-29), the breast cancer cell line (MCF7), and the pancreatic cancer cell line (PANC-1). A fibroblast cell line was also used for comparison purposes. The growing media for the cell lines have 15% fetal bovine serum, 100 µg/mL penicillin-streptomycin (Alqaraleh *et al.*, 2023, Al-Rawashde *et al.*, 2021, Alshaer *et al*., 2023). All the cell lines used in this study were identified and have no contamination according to the American Type Culture Collection (ATCC).

### *2.5. Colorimetric MTT assay*

Microculture tetrazolium test using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide dye (MTT) to determine the cell viability. 5 * 10^3 of cultured A549, HT-29, MCF7, PANC-1, and A fibroblast cells were seeded in each well of the ninety-six well-plates (Al-Tawarah *et al.*, 2020). After 24 h, the cultured cells were exposed to camptothecin with 0. 75 to 200 µM to the cells and SNPs of 0. 75 to 200 µg/ mL. Further, cells were treated with a medium containing 0. 75 µg/ ml of SNPs and with camptothecin at ( 0. 75 to 200 µM) for 72 hours. Afterwards, 15 µL of the dye was added to each well in the plate, followed by incubation for Four hours. Consequently, 100 µL of organic solvent was added to each well. The cell viability was determined using Microplate Reader BiotechTM ELx800TM (Bio-Tek Instrument, USA) with absorbance at 590 /630 nm.

### *2.6. Angiogenesis, Inflammation and Antioxidant Assays*

The PANC-1 cell line was seeded at 500,000 cells to each well, and the following day the cells were cultured with 0.75 µg/ mL of SNPs, and a combination of 0.75 µM of camptothecin followed by incubation for 72 hours at 37 °C. The markers' concentrations such as interleukin 1 beta (IL-1 beta), tumor necrosis factor-alpha (TNF alpha), Catalase (CAT), Glutathione peroxidase (GPX), matrix metalloprotease 9 (MMP9) and VEGF were determined by following the manufacturer's ELISA instructions.

### *2.7. Statistical analysis*

The results were revealed as the average ± SD based on three to four separate experiments. ANOVA was used to evaluate the statistical differences between the treatment and control groups. Dunnett's post hoc test was then used for additional analysis using GraphPad Prism version 10. P-value <0.0001 (****), P-value <0.001 (***) and P-value <0.01 (**); all of these P-value were viewed as highly significant statistical differences, while p-values < 0.05 (*) were regarded as statistically significant.

## 3. Results

### *3.1. Morphology and Particle Size results*

The charge, size, and polydispersity index (PDI) variations before and after lyophilization are depicted in Figure 1. The average size and average charge before lyophilization were 150.455 nm and -25 mV, respectively, with a standard variation of 10.415 nm and 2. The PDI was 0.27. However, following lyophilization, the size became 277.5 nm, the PDI was 0.29, and the average charge changed to -14 mV. The particles after lyophilization are made up of many smaller particles, all less than 0.3 µm, according to SEM images Figure 2.

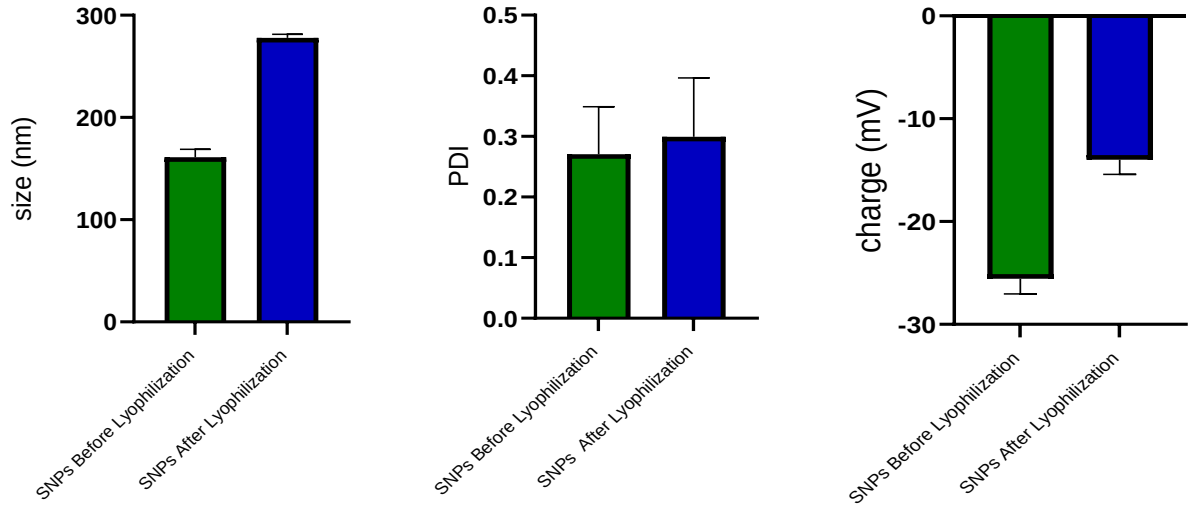


**Figure 1.** SNPs size, Zeta potential distribution, and polydispersity index were measured before and following lyophilization.

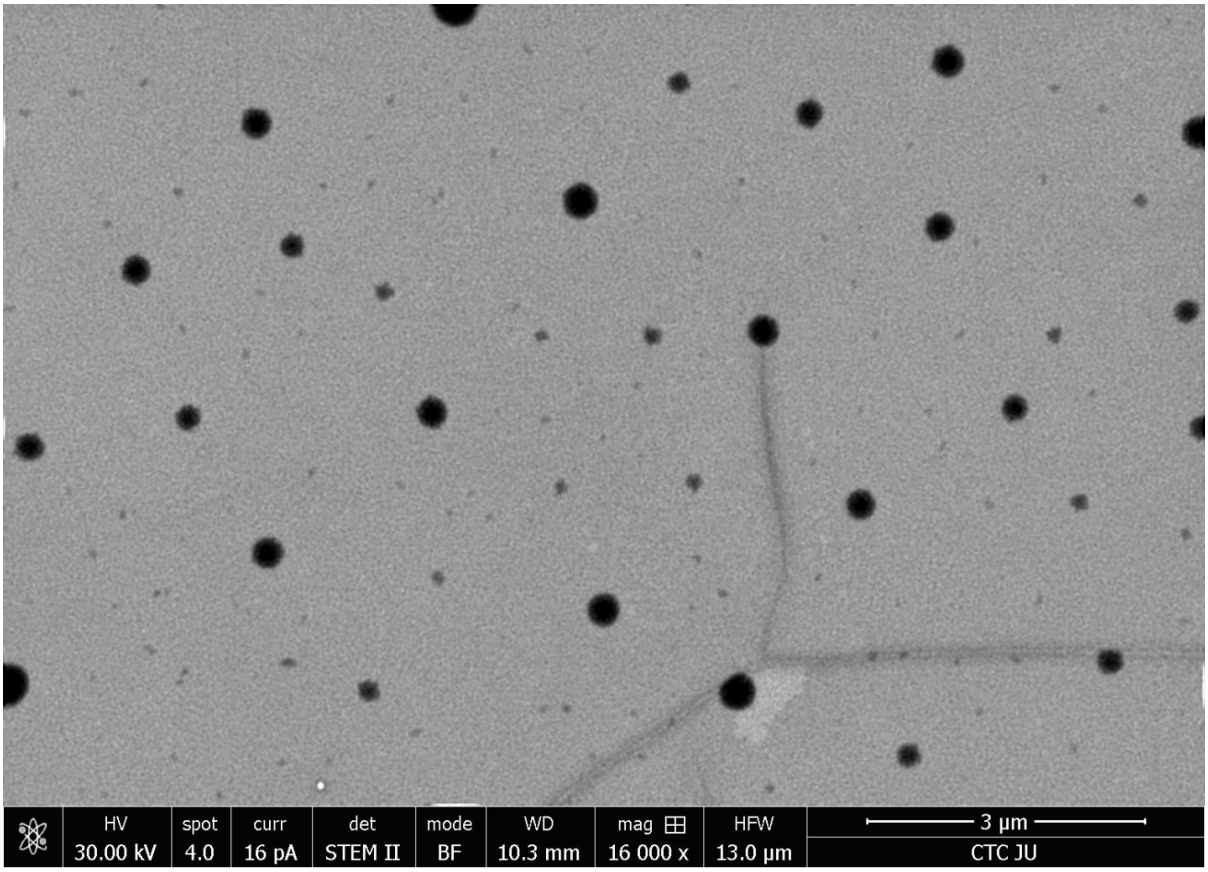


**Figure 2**. SEM image of SNPs produced by reacting 1.0 mM silver nitrate with *Aspergillus flavus*

### *3.2. Cytotoxicity results*

The harmful impact of SNPs on the fibroblast cell line is displayed in Figure 3A to evaluate selective cytotoxicity. At concentrations from 1.5 to 200 µg/ ml, significant cell death was seen. A concentration of 0.75 µg/ ml of SNPs was chosen for additional cytotoxicity testing to reduce toxicity to the normal fibroblast cell line and increase cytotoxicity when combined with camptothecin against different cancer cell lines. The cytotoxicity of camptothecin both by itself and in conjunction with 0.75 µg/ml of SNPs is depicted in Figure 3B. The results show that when camptothecin and SNPs are used together, the combined effect is significantly less cytotoxic than when they are used independently.

The toxic influences of SNPs on the A549 cell line are illustrated in Figure 4A at concentrations from 0.75 to 200 µg/ ml. Notably, cell death is significantly observed at concentrations between 1.5 and 200 µg/ ml. The effects of camptothecin both by itself and in conjunction with 0.75 µg/ ml of SNPs are shown in Figure 4B. The findings show that when camptothecin and SNPs are combined, the cytotoxicity increases significantly in comparison to when camptothecin is used alone at 0.75 µm, 1.5 µm and 3 µm.

The HT-29 cell line is used to demonstrate the cytotoxicity of SNPs in Figure 5A, where doses from 1.5

to 200 µg/ ml led to substantial cell death. The cytotoxic effects of camptothecin both by itself and in combination with 0.75 µg/ml of SNPs are displayed in Figure 5B. The results show that, when compared to camptothecin alone, the addition of SNPs to camptothecin does not significantly increase cytotoxicity.

Comparably, Figure 6A shows how SNPs cytotoxicity affects the MCF7 cell line, with doses between 1.5 and 200 µg/ml causing notable cell loss. The cytotoxicity of camptothecin alone and in combination with 0.75 µg/ml of SNPs is displayed in Figure 6B. Compared to the cytotoxicity of camptothecin alone, Figure 6B shows a considerable increase in the cytotoxicity of camptothecin and SNPs combination, especially at doses between 1.5 µm and 0.75 µm.

Furthermore, Figure 7A demonstrates the toxic effects of SNPs on the PANC-1 cell line, with doses from 1.5 to 200 µg/ ml showing significant cell loss. The cytotoxicity of camptothecin, both by itself and in combination with 0.75 µg/ ml of SNPs on the PANC-1 cell line is illustrated in Figure 7B. When camptothecin is mixed with SNPs, the results show a significant increase in cytotoxicity compared to camptothecin alone, with effective cell death occurring at all concentrations that have been tested.

In conclusion, results indicate that the combination of silver particles and camptothecin is considered a viable combination for improving cancer treatment, particularly for pancreatic cancer; therefore, the PANC-1 cell line was used for subsequent investigations in this study.

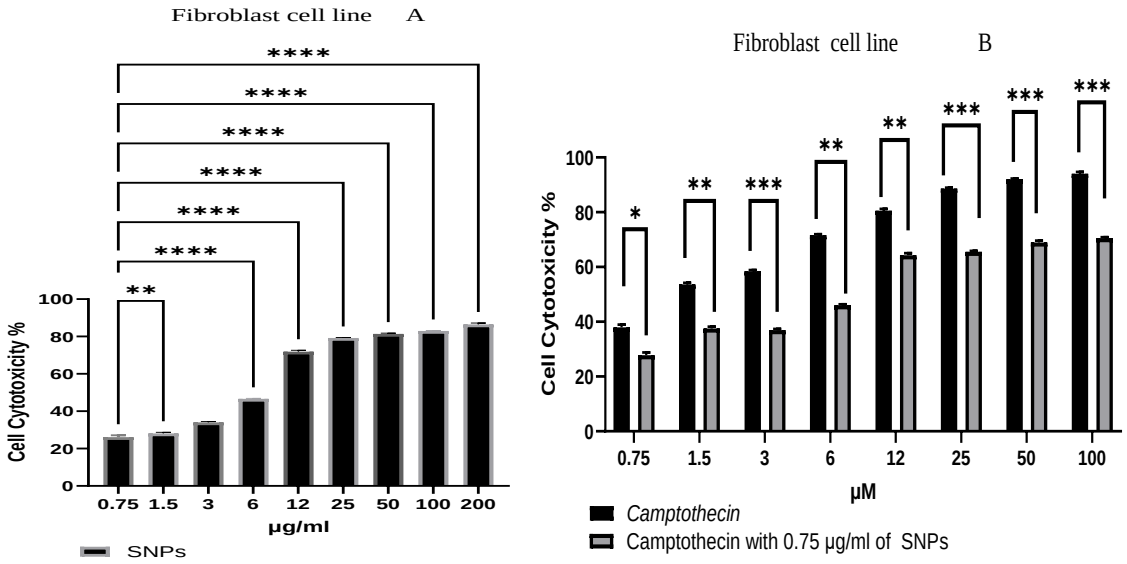


**Figure 3**. cytotoxicity effect of A- SNPs, B- Camptothecin and Camptothecin with 0.75 µg/ml of SNPs on fibroblast cell line. The averages of the three measurements ± standard deviation are used to describe the results (n = 3–4 independent repetitions).

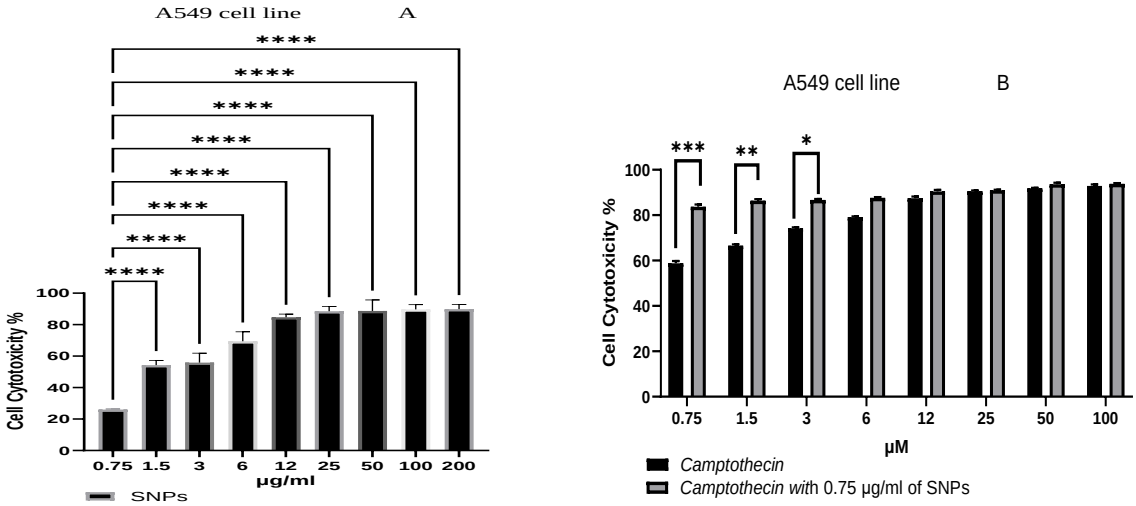


**Figure 4**. cytotoxicity effect of A- SNPs, B- Camptothecin and Camptothecin with 0.75 µg/ml of SNPs on A549 cell line. The averages of the three measurements ± standard deviation are used to describe the results (n = 3–4 independent repetitions.

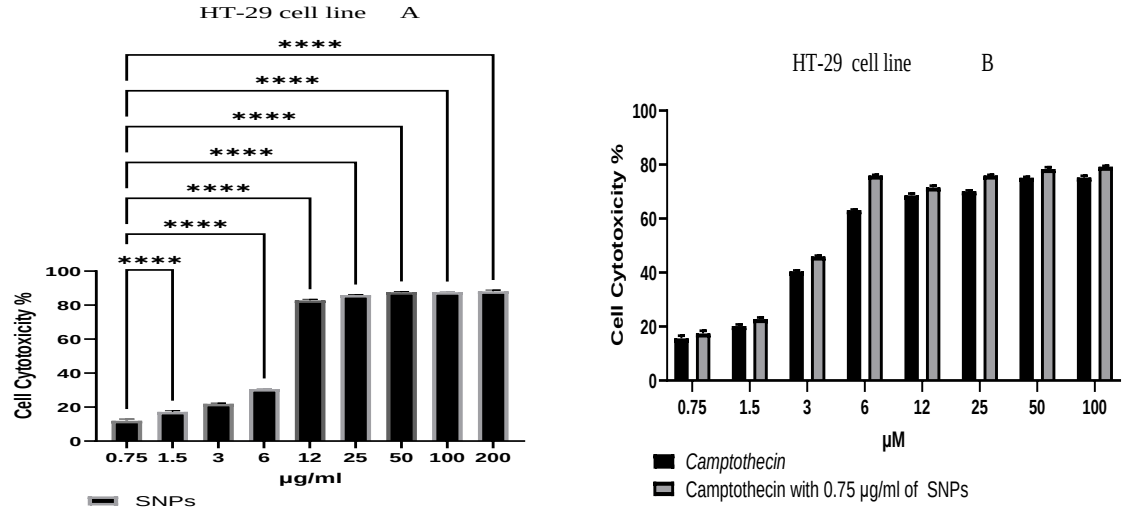


**Figure 5.** cytotoxicity effect of A- SNPs, B- Camptothecin and Camptothecin with 0.75 µg/ml of SNPs on HT-29 cell line. The averages of the three measurements ± standard deviation are used to describe the results (n = 3–4 independent repetitions).

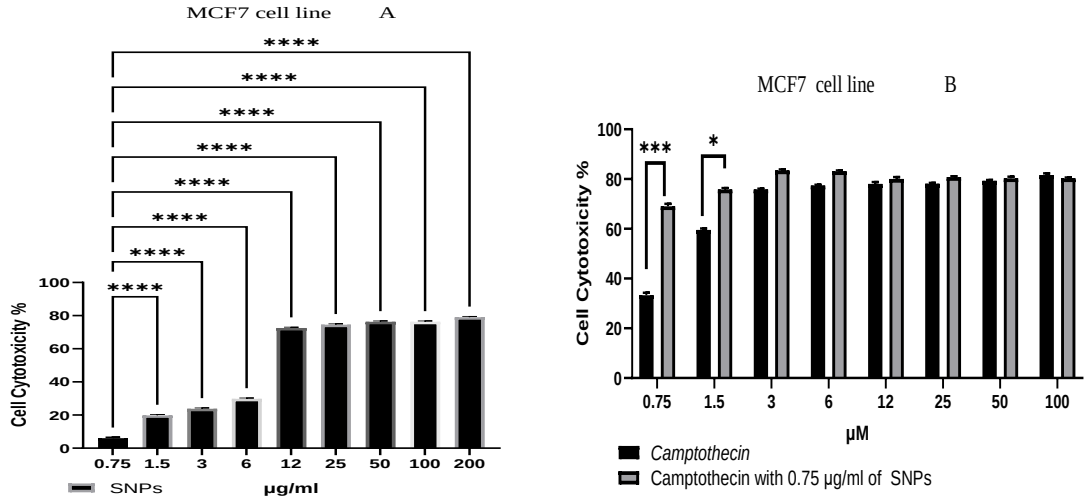


**Figure 6**. cytotoxicity effect of A- SNPs, B- Camptothecin and Camptothecin with 0.75 µg/ml of SNPs on MCF7 cell line. The averages of the three measurements ± standard deviation are used to describe the results (n = 3–4 independent repetitions).

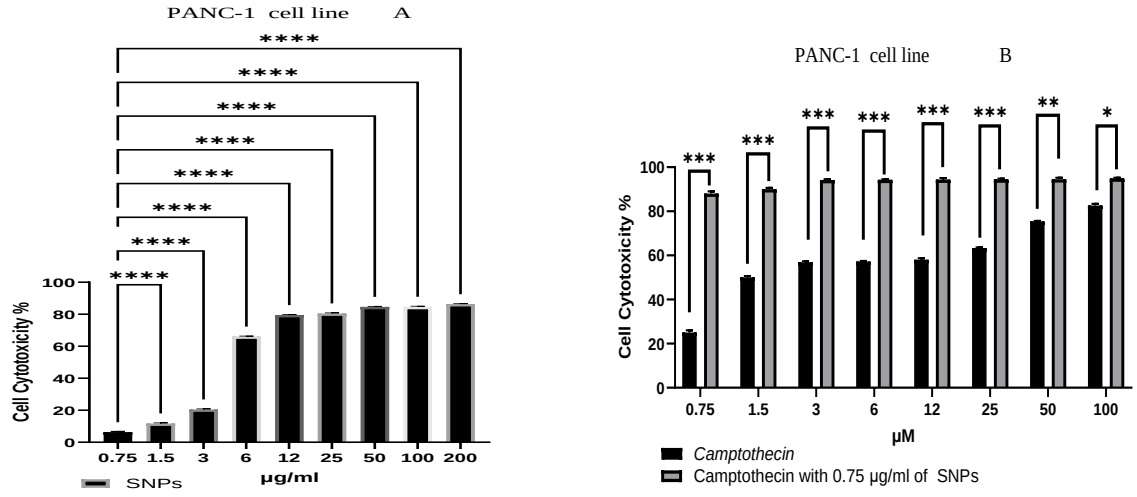


**Figure 7.** cytotoxicity effect of A- SNPs, B- Camptothecin and Camptothecin with 0.75 µg/ml of SNPs on PANC-1 cell line. The averages of the three measurements ± standard deviation are used to describe the results (n = 3–4 independent repetitions).

### *3.3. Angiogenesis, Inflammation and antioxidant results*

VEGF contributes significantly to controlling angiogenesis, which is the generation of blood vessels for tumor development and spreading, something crucial to the tumor's survival and growth. MMP-9, on the other hand, is an enzyme that contributes to the breakdown of the extracellular matrix to assert tumor invasiveness and metastasis. Thus, MMP-9 facilitates the degradation of

structural barriers and invasion of cancer cells into tissues and surrounding stroma and their distal dissemination to distant organs. Both, VEGF and MMP-9, stimulate tumor growth, invasion, and metastasis(Alves *et al.*, 2023, Uslukaya *et al.*, 2023, Rashid and Bardaweel, 2023), and therefore both targets were investigated in this research. In the current study, the influence of the treatments on VEGF levels is seen in Figure 8A. The simultaneous administration of camptothecin along with SNPs, as well as camptothecin alone, significantly reduced VEGF levels in comparison to the control group. Notably, there was an even more significant reduction in VEGF levels when camptothecin and SNPs were combined. Moreover, the effect of the treatments on MMP9 levels is seen in Figure 8 B. MMP9 levels were much greater in the control group than in the treatment groups. In contrast to the control, MMP9 levels were significantly decreased by both SNPs and camptothecin alone. However, no significant difference between camptothecin and SNPs alone or in combination was observed. This suggests that the combination did not lower MMP9 levels any further than the individual treatments did.

Moreover, cytokines and antioxidants are involved in the progress of cancer, and both have antagonistic functions. TNF α and IL-1β the main pro-inflammatory cytokines support tumor growth through chronic inflammation which enhances cell growth. Some of these cytokines are known to phosphorylate both the NF-κB and the STAT3 pathways that increase tumor cell invasion and metastasis besides inhibiting anti-tumor immunity. On the other hand, antioxidant enzymes such as CAT and GPX have the responsibility of eliminating what is known as reactive oxygen species (ROS) generated during inflammation. It recognizes that the regulation of stress and the reactivity of the tumor microenvironment in terms of cytokine oppression, as well as the antioxidant stability of the cancer cell, determine the chemo-sensitivity and progress of cancer (Acevedo-León *et al.*, 2023, Bardelčíková *et al.*, 2023). Thus, TNF α, IL-1β, CAT and GPX were selected in this study.

The effect of both camptothecin and SNPs together and separately on TNFα expression levels is shown in Figure 8C. Comparing the silver particle-treated group to the control group, there was a significant rise in TNFα levels. Nevertheless, in comparison to the control, camptothecin individually did not significantly affect TNFα levels. Interestingly, when compared to the other treatment groups, the combination of camptothecin with SNPs led to a substantial decrease in TNFα levels. Furthermore, the effects of SNPs and camptothecin on IL-1β expression levels are shown in Figure 8D, both separately and in combination. IL-1β levels were notably greater in the silver particle-treated group relative to the control group. It is noteworthy that despite no significant difference between camptothecin alone compared to the control, the incorporation of silver particles into camptothecin considerably increased IL-1β levels relative to the control group and compared to camptothecin alone. The effect of different treatments on GPX levels is displayed in Figure 8E. When evaluated against the control group, the group treated with SNPs showed a substantial decrease in GPX levels. In the same direction, GPX levels significantly decreased in comparison to the control when camptothecin was used alone. Furthermore, in comparison to the control, there was also a decrease in GPX levels when camptothecin and SNPs were combined. Figure 8F shows the impact of various treatments on CAT levels. The group treated with SNPs exhibited no significant increase in CAT levels in comparison to the control group. Moreover, CAT levels did not alter significantly from the control in the groups treated with camptothecin alone or combined with SNPs. Furthermore, there was no notable difference in CAT levels between the combination therapy group and the camptothecin group, suggesting that the addition of SNPs to camptothecin did not further change CAT levels.

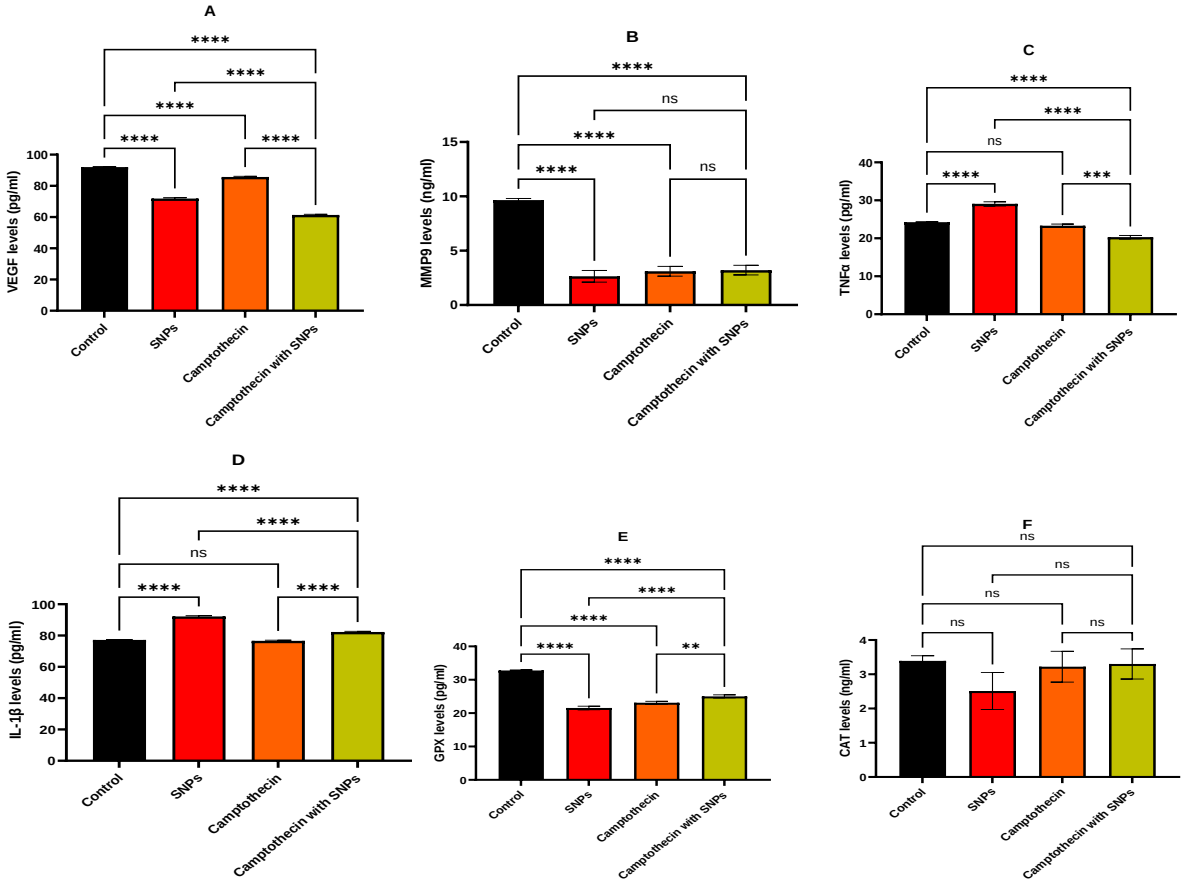


**Figure 8.** The expression level of A- VEGF, B- MMP 9, C- TNFα, D- IL1β, E- GPX and F- CAT, under the effect of 0.75 µg/ml of SNPs, 0.75 µM of Camptothecin and 0.75 µM of Camptothecin with 0.75 µg/ml of SNPs on PANC-1 cell line. The averages of the three measurements ± standard deviation are used to describe the results (n = 3–4 independent repetitions).

## 4. Discussion

Nanomedicine has become an important area of research with the advancement of nanotechnology, especially in the design of nanoscale materials for imaging and drug administration (Mir *et al*., 2017). Nanoparticles are extensively utilized as a therapeutic approach against different types of cancer due to their targeted delivery and enhanced therapeutic index (Crucho and Barros, 2017, Al-Qaraleh *et al*., 2024, Alahmad *et al.*, 2022). Because of their remarkable impair in vital cellular functions as well as their role in severe cellular damage or death, in particular, silver particles hold great promise for future developments in nanotechnology (Kalantari *et al.*, 2020). Aside from their exceptional thermal behavior and unusual visual qualities, they also display remarkable use in a wide range of healthcare, such as Antimicrobial dressings, wound treatment, and anticancer drugs (Dutt *et al.*, 2023, Jdayea and Neamah, 2024). In addition, SNPs are used in other biological applications, including diabetes, antiviral, and anti-inflammatory medicines (Konduri *et al*., 2024). Nevertheless, During the chemical production of nanoparticles, hazardous byproducts that might damage the environment are frequently produced (Khan *et al.*, 2021). Therefore, Biological synthesis is the best option to allay these worries since it uses inexpensive, easily handled, non-toxic, plentiful, and ecologically friendly microbes and plant materials (Gunti *et al.*, 2022, Lakshmeesha *et al.*, 2019).

In our previous study, we successfully characterized the biogenesis of SNPs using *Aspergillus flavus* through UV–vis absorption spectroscopy, ATR-FTIR, and XRD analyses (Al-Soub *et al*., 2022, Alqaraleh *et al*., 2023). Furthermore, in the present study, the SNPs were characterised using Zetasizer and SEM; the findings show that lyophilization has a substantial effect on the size, charge, and PDI. The SNPs had a PDI of 0.27, a surface charge of -25 mV, and an average size of 150.455 nm before lyophilization, indicating a reasonably homogeneous and stable nanoparticle dispersion appropriate for biological applications. The cause of the increase in particle size that results from lyophilization is the formation of aggregates through the nanoparticles, and this is known to occur especially when nanoparticles go through solvent elimination processes such as freezing and drying (Trenkenschuh and Friess, 2021, Eliyahu *et al.*, 2020). Furthermore, the reduction of the surface charge from -25 mV to -14 mV also supports this because the lower surface charges are associated with reduced electrostatic repulsive forces acting between particles and thus encourage the formation of aggregates (Shrestha *et al.*, 2020, Hedberg *et al*., 2012). Despite these changes in the size, charge and PDI, the SNPs remain within acceptable ranges for nanoparticulate formulations, indicating that none of their fundamental characteristics has been lost for possible therapeutic use (Abu Hajleh *et al.*, 2023, Al-Soub *et al*., 2022, Alqaraleh *et al*., 2023).

We also observed the cytotoxic activity of SNPs with and without camptothecin on several types of cancer cell lines such as A549, HT-29, MCF7 and PANC-1. For selective cytotoxicity of SNPs, a range of doses, from 0.75 to 200 µg/ml, were tested in this study. Fibroblast cells showed significant cell death at higher doses and insignificant toxicity at 0.75 µg/ml. To balance safety and efficacy, a concentration of 0.75 µg/ml of SNPs was chosen for further testing. This dose enhances the camptothecin cytotoxic effects from 0.75 to 200 µm, in PANC-1 cell lines, while achieving minimal toxicity to normal fibroblast cell lines. This indicates that the combination of silver particles and camptothecin is considered a viable combination for improving cancer treatment, particularly for pancreatic cancer, which is resistant to traditional chemotherapy (Mizrahi *et al*., 2020). The synergistic effect may be caused by the unique properties of SNPs, such as their capacity to boost camptothecin intracellular transport and effectiveness by raising its bioavailability or ease of absorption by cancer cells. Furthermore, it has been demonstrated that SNPs produce silver ions and ROS, which can impair essential biological processes including protein synthesis and DNA replication. Because of these modifications, cancer cells may become more cytotoxic, especially since camptothecin inhibits topoisomerase I and degrades DNA (Mikhailova, 2020, Alfei *et al.*, 2024, Patel *et al.*, 2024, Hamad *et al.*, 2020, Flores-López *et al.*, 2019, Choudhary *et al.*, 2022).

Additionally, the results of this study indicate that the combination of SNPs has a significant effect on several indicators associated with oxidative stress, inflammation, and angiogenesis in cancer cells. In particular, the combined administration of SNPs and camptothecin demonstrated a significant reduction in VEGF levels, that outweighed the effects of either treatment alone. This observation implies a synergistic impact in suppressing angiogenesis, a crucial process for tumor development and metastasis. Nevertheless, while either camptothecin or SNPs alone decreased MMP9 levels, which is an indicator of cancer invasion and metastasis, their cooperation did not intensify this decrease. This aligns with findings from several studies where SNPs alone and in combination with chemotherapy were shown to downregulate angiogenic factors (Zhan *et al*., 2024, Abdelfattah *et al*., 2022, Gurunathan *et al*., 2018). Moreover, Different inflammatory indicators were affected by the treatments. TNFα and IL1β levels were significantly elevated by SNPs alone, but not by camptothecin alone (Yuan and Gurunathan, 2017). Nevertheless, their combined effect was a considerable reduction in TNFα and IL1β, suggesting the possibility of an anti-inflammatory effect that might lower inflammation in cancer cells (Yuan and Gurunathan, 2017). Furthermore, when both treatments were administered at the same time, there was no further decrease in GPX and CAT levels as there was when they were administered separately, which dramatically reduced the levels of the enzymes. These results highlight the intricate ways in which SNPs and camptothecin interact to address inflammation, angiogenesis, and oxidative stress, and they point to possible therapeutic advantages in the management of cancer (Zhan *et al.*, 2024, Abdelfattah *et al*., 2022, Gurunathan *et al.*, 2018, Yuan and Gurunathan, 2017).

These results indicate that augmentation of proinflammatory cytokines may promote the growth of tumor, and that proinflammatory cytokines and ROS could be up-regulated by SNPs. SNPs, however, can induce cellular injury and may be linked to increased production of ROS that may surpass the production of proinflammatory cytokines. The present study has also revealed that even though combination treatments decrease the synthesis of pro-inflammatory cytokines, they do not enhance the levels of antioxidant enzymes that protect against ROS, amplifying the cytotoxic effect on cancer cells and which could account for the synergistic effect of the combination treatments for pancreatic cancer. Hence, this combination is a potentially effective approach in offering more selective and powerful methods of cancer therapy given that standard chemotherapy treatment is not as effective as required.

## 5. Conclusion

This study showed how camptothecin may be administered with SNPs to increase the effectiveness of cancer therapy. It was found that 0.75 µg/ml was the ideal dose of SNPs to reduce toxicity to normal cells and greatly increase camptothecin's anticancer effects, particularly in cell lines associated with pancreatic, lung, and breast cancer. The results of the research on selective cytotoxicity served as the foundation for these conclusions. It is quite probable that the SNPs' capacity to impede cellular functions and trigger oxidative stress is what gives them their synergistic effect on cancer cells. These results demonstrate how SNPs may be used as an adjuvant method in cancer treatment, reducing adverse effects and enhancing the therapeutic benefits of traditional chemotherapy. Furthermore, this work has some limitations, including the following: the study used only a small number of cancer cell lines, which may be inadequate to give the best picture of the cell's behavior. Furthermore, the action mechanism of camptothecin and the silver particles on cancer cells needs to be fully explored, especially regarding the use of different ratios of camptothecin to silver particles as well as testing the expression levels of a different biomarker, which involved invasion and metastasis process, under the influence of such a combination. Future research should focus on testing the combination across a larger variety of cancer cell lines and enhancing the selectivity and efficiency of the novel formulation in vivo since it may lead to significant improvements in clinical experiments.

## Acknowledgment

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