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STUDY ON EXPLORING METAL NANOPARTICLES (MNPS) IN PHARMACOLOGY AND THEIR FUTURE ROLE IN THE DIAGNOSIS AND TREATMENT OF CANCER

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Abstract. In this study, DNA damage in MCF-7 cells by AgNPs was demonstrated AuNPs and AgNPs synthesized using Ginkgo biloba extract as a reducing agent are explored as possible candidates for the diagnosis and treatment of cancer. The cytotoxic effects of AgNPs on breast cancer cells (MCF-7), the antioxidant capacity of AgNPs and bilobetin in patients with lung cancer (small cell and adenocarcinoma), and the use of these treatments to control retinol binding protein I (RBP-1), a protein involved in cancer progression was studied in the research. Chemical reduction methods were utilized to synthesize AgNPs and AuNPs. AgNPs caused to damage DNA integrity as assessed by agarose gel electrophoresis. Commercials ELISA kits were used to quantify protein carbonyl (PC) and RBP-1 levels. I In addition, bilobetin or AgNPs significantly lowered RBP-1 in lung cancer patients and these agents may offer protective effects against cancer progression. This study concludes with the potent promise that metallic nanoparticles, in particular those from Ginkgo biloba, hold for cancer diagnostics and treatment through their cytotoxicity against cancer cells, free radical scavenging properties, and regulation of action of cancer related proteins.

Keywords: Nanoparticles, Cancer cells, Blood, AgNPs and AuNPs.
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1 Introduction

Metallic nanoparticles (MNPs) have emerged as revolutionary tools in nanotechnology with significant impact for clinical medical diagnostics and therapeutics (Chandrakala et al., 2022). A nanoparticle is a particle of matter that is between 1 and 100 nanometres (nm) in diameter. NPs are becoming popular tools in cancer diagnosis and treatment, making it one of the most promising areas (Chandrakala et al., 2022). MNPs are transforming the way cancer is being managed because of their capability to target specific cells, boost imaging (in the case of imaging MNPs) and accurate delivery of therapeutic agents (for targeted delivery MNPs). This introduction lays the ground work for more detailed examination of the effects of metallic nanoparticles in medical tests, specifically with respect to their influence on cancer cell activity (Alromi et al., 2021).

Recent studies have found that metallic nanoparticles substantially improve the sensitivity and selectivity of cancer diagnosis (Singla et al., 2016; Khursheed et al., 2022). For example, there are widespread applications of gold nanoparticles (AuNPs) and silver nanoparticles (Ag-

NPs) to molecular imaging owing to the tunability of their optical properties. Functionalised with targeting ligands that specifically bind to tumor cells, these nanoparticles allow for non-invasive imaging of malignant lesions. For gastrointestinal cancer, MNPs based technologies have demonstrated more selectivity and sensitivity than conventional diagnostic tools for early detection and target localization (Mahmood & Hammudi, 2024). In addition, MNPs are able to enhance contrast in some diagnostic imaging modalities such as computed tomography (CT) a magnetic resonance imaging (MRI), further increasing their use in diagnostic imaging. For example, consideration of gold nanoparticles with strong contrast effect on CT images allows early detection of cancer (Zhou et al., 2022a). The photothermal effect of gold nanoparticles can also be exploited for photothermal therapy that is based the absorption of light by nanoparticles and a resulting generation of heat to destroy cancer cells (Oliveira et al., 2023). MNPs possess such a multifunctional capability, which makes them good tools against cancer in the diagnostic arsenal.

Compared with strategies that rely on trial and error, such surface engineering platforms possess one of the significant advantages of enhancing the therapeutic efficacy and simultaneously reducing side effects by allowing these metallic nanoparticles to deliver therapeutic agents directly to tumor cells (Kalashgrani & Javanmardi, 2022; Tian et al., 2022). Because MNPs can be functionalized to carry a several drugs such as (chemotherapeutic drugs, antibodies, and nucleic acids), there are ample opportunities for tuning the properties of the MNP carrier depending upon the therapeutic agent of interest and on the therapeutic approach. It is then possible to engineer these nanoparticles production due to physiological conditions, such as pH or specific enzyme, guaranteeing the delivery of the drugs to tumor site (Zhou et al., 2022b). Reduced toxicity from systemic delivery of conventional chemotherapy is achieved with MNPs as the targeted delivery mechanism. MNPs help to reduce side effects, such as myocardial infarction or heart attacks, and blood clots, which are commonplace with traditional cancer treatments by distributing drugs to healthy rather than cancerous tissues to avoid (Selim et al., 2024). Additionally, the high surface area and adjustable properties of MNPs facilitate drug loading in several conditions which improves the therapeutic efficiency and makes the combination treatment of complicated diseases such as cancer more efficient.

Besides their role as a diagnostic and therapeutic tool for cancer, metallic nanoparticles are antimicrobial and anti-viral as well, which are essential in preventing infections that confound cancer treatment (Souri et al., 2022). For example, conventional nanoparticles of silver and copper have demonstrated broad-spectrum antimicrobial activity against a myriad of pathogens including bacteria and viruses. Thus, these nanoparticles might be used to coat medical devices or include in wound dressings to halt the development of infections harmful to the overall health of the cancer patient (Deng et al., 2022). Interactions of MNPs with microbial membrane cause loss of cellular function and the death of the microorganisms, explaining the antimicrobial action of MNPs (Ribeiro, 2022). This is especially helpful when treating cancer because, in these cases, patients typically have immune compromised systems and are prone to infections. integration of the antimicrobial MNPs into cancer therapies reduces the risk of secondary infections and improves patient's outcomes as healthcare providers.

Despite the many advantages afforded by their use in cancer diagnosis and treatment, metallic nanoparticles are fraught with difficulties (Oehler et al., 2024). The biggest concern is that the nanoparticles could be toxic. It has been shown in studies that metal NPs based on NPs have immunogenic, nephrotoxic, and neurotoxic consequences. MNPs cytotoxicity can affect both cancer and healthy cells, so their profiles of toxicity should be thoroughly understood before they could be safely applied in humans. Therefore, the risk of these MNP is mitigated by the strategies of the researchers for the design of safer MNP (Al-Eryani et al., 2021; Hammudi & Mahmood, 2025). This includes green synthesis methods, surface modifications and the development of new methods for nanoparticle toxicity appraisal (Samuel et al., 2022).

The aim of the work

To uncover NP toxicity at low exposures and to discover new biomarkers and targets of nanotoxicity, high throughput omics studies such as genomics and proteomics are being used in order to screen for such toxicity. The aim of these efforts is to develop these MNP applications in order that the beneficial effects of MNPs in cancer treatment are achieved, and the possible risks potential hazards to human health are mitigated.

2 Materials and Methods

2.1 Design of the experimental

From January to June 2023, blood samples were taken from patients with lung cancer at the Baghdad Teaching Hospital. Three primary groups were created from the samples: (Ctrl) included 25 blood serum samples from both male and female healthy people between the ages of 23 and 45. Thirty blood serum samples from patients aged 45 to 80 with small cell lung cancer were included in the study (SCLC). Thirty blood samples from Adenocarcinomacondition in the lung, at ages ranged (45-80) and the average period of time between diagnosed cancer and receiving treatment from 1-6 months on the first and second stage. were included in the ADCA trial. Notably, none of the individuals had any medical histories that would have affected the characteristics being examined in this study. The cytotoxicity was evaluated using an MTT test. In short, cells were treated with different doses of the chemical after being seeded in 96-well plates. MTT reagent was applied after a day, and the formazan crystal formation was detected at 570 nm. Cell viability as a proportion of the control group was reported. Utilizing the DPPH radical scavenging assay, antioxidant capability was assessed. Using spectrophotometry, the compound's capacity to scavenge DPPH radicals was assessed at 517 nm. The compound's IC50 values, which indicate the concentration needed to scavenge 50% of DPPH radicals or impede cell growth, were computed.

2.2 Gathering and getting ready the samples

Venous blood was collected into a total of 8 mL in sterile plastic tubes with a capacity of 10 mL. Plastic containers with patient and control samples were placed into a 37°C incubator for 20–30 minutes in order for blood samples to coagulate. Small Eppendorf tubes were used for separating the blood and then to centrifuge the blood at 3000 rpm for 10 minutes. All samples were held in -20 degrees until analysis. The extraction process, including the solvent used, extraction time 25 minute, and temperature 28^{O}C. Additionally, critical parameters such as the incubation temperature and duration for various assays were mentioned in Živanović et al. (2024).

2.3 Constructing and describing AuNPs

In order to stop the particles from aggregating and growing further, sodium citrate was used as a capping material during the chemical reduction of $AuCl_{4.3}H_2O$ to create spherical gold nanoparticles (AuNPs)(Baborák, 2023; Hammudi et al., 2023). A boiling of $HAuCl_4$ ($1x10^{-3}$ M, 100 mL) mixed with tri-sodium citrate (0.0388 M, 10 mL) used hot plate stirrer. The color of the solution changed from yellow to without color to wine red, that was think is a sign which AuNPs were product. In order to manage the particle size and produce particle size distribution, the solution is refluxed for fifteen minutes. After that, the heater is switched off, and the mixture is agitated until it reaches $25C^0$. The acquired sample's absorption spectra were examined with a UV-VIS spectrophotometer, and a TEM was used to analyze the size and form of the particles.

2.4 Ginkgo biloba extract was used to create silver nanoparticles

The solution of 4 grams (50 mL) of $AgNO_3$ and Ginkgo biloba extract was used to synthesize AgNPs. The general steps to prepared material are (collection and identification of raw material of plant, dry, grinding, pulverization, suitable solvent extraction, maceration, filtration, separation, remove solvent and final steps storage).Magnetically stirring the slurry for 30 minutes at 80°C was the next step. One of the important findings is the extraction processing during which hue transitioned from a translucent green to black implying the formation of AgNPs. The resulting AgNPs were converted into powder, kept in an airtight container, and dried at 60°C for 18 hours (Aslam et al., 2021).

2.5 DNA fragmentation

The fragmentation of cellular DNA was analyzed in relation to the established IC50 after the introduction of AgNPs to MCF-7 cells at both low and high concentrations (10 μM and 100 μM, IC50, respectively). DNA fragmentation was assessed in relation to the anticipated IC50 after treating MCF-7 cells with AuNPs at low and high concentrations (10 μM and 100 μM, respectively). A constant quantity (100 ng) of cellular DNA (Genomic DNA Purification Kit, Amersham Biosciences) obtained from treated and untreated cells was analyzed using 1.5% agarose gel electrophoresis in Tris-acetate buffer at pH 8.2, and then stained with 0.5 μg/ml ethidium bromide. The bands underwent UV transillumination and were then imaged. The occurrence of low-molecular-weight DNA fragments, or smearing, indicates cellular demise.

2.6 Human Protein Carbonyl (PC) and retinol binding protein-1 (RBP-1) concentrations in serum are measured

The company's premier testing kit was used to quantify the quantities of human protein carbonyl and retinol-binding protein-1. The competitive-ELISA detection technique was the basis for the kit's methodology. Notably, the target that was intriguing was located on the microtiter plate of the kit. After each microplate well, any surplus conjugate and unbound samples or standards were removed by washing. A TMB solution for the substrate was thereafter introduced to each well. The enzyme and substrate were halted with a sulfuric acid solution, and the resultant color change was quantified by spectrophotometry at 450 nm.

2.7 Statistical investigation

To evaluate the significance of the variations in group mean values, the standard deviations of mean values were also supplied. Using Prism 9 in GraphPad Analysis of the student's test was also done. Thus, a P value < 0.05 was considered statistically significant. Analyzing the data using a one-way ANOVA.

3 Results

3.1 Silver NPs induce DNA distruction in cells of the breast tumor

The results of agarose gel electrophoresis for the DNA of MCF-7 cells of the breast tumor that expoture to several levels of AgNPs are shown in Figure 1. Lanes 2 and 3 show smearing that indicates the cytotoxic effect of the AgNPs on all three groups of cells (control, lung tumor cells and adenocarcinoma) and appear with the appearance of smearing in all three groups, namely (control, small cell lung cancer and adenocarcinoma). It was observed that the smearing intensity increases with AgNP concentration, indicating a dose-dependent effect related to volume and according to DNA extraction from MCF-7 cell lines (untreated and treated) with many levels of AgNPs was electrophoresed on EB-stained gel in the three groups ctrl(A), SCLC (B), and

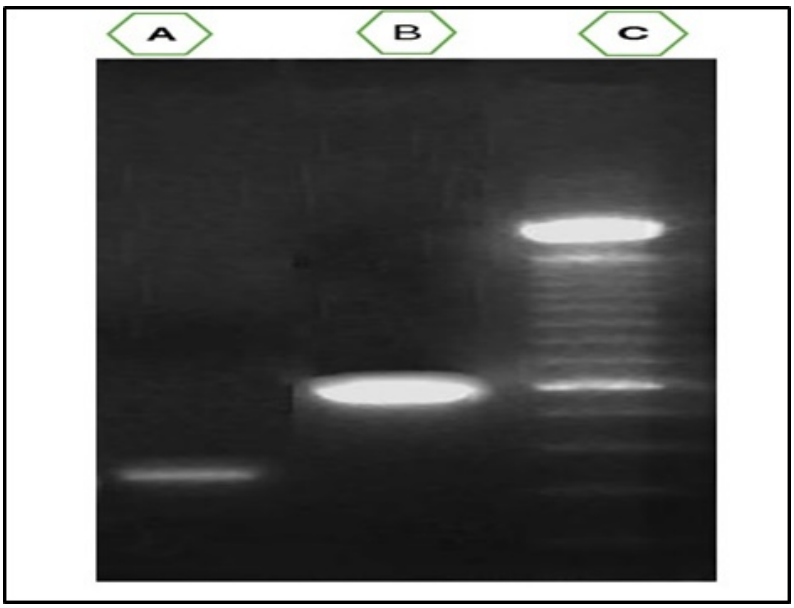

Figure 1: DNA extraction from untreated and treated MCF-7 cell lines with varying concentrations of AgNPs was electrophoresed on EB-stained gel in the three groups ctrl(A), SCLC (B), and ADCA (C). Lanes 2 and 3 treated MCF-7 with 10 μM/ml and 100 μM/ml, respectively, whereas lane 4 was left untreated (cell control) and included a 100 bp.

ADCA (C). Lanes 2 and 3 treated MCF-7 with 10 $\mu M/ml$ and 100 μM/ml, respectively, whereas lane 4 was left untreated (cell control) and included a 100 bp.

3.2 Effect of bilobatin and silver nanoparticles on oxidative stress in Lung Cancer Patients

The serum levels of RBP-1 in the three patient groups with and without addition of bilobetin and Ginkgo biloba silver nanoparticles. SCLC and ADCA (cancer) showed significantly higher RBP1 levels than in the control ($p < 0.0001$). Treatment with both bilobetin and the silver nanoparticles negatively influenced RBP-1 levels in the cancer groups, particularly as shown in Table 1 and Figure 2, thereby suggesting that the latter may provide a protective contribution to restrict cancer progression. anoparticles can interact with cancer cells through various mechanisms.

A prevalent method is surface functionalization using ligands that selectively bind to receptors overproduced on cancer cells. This precise administration improves cellular absorption and minimizes off-target impacts. Once internalized, nanoparticles can release their therapeutic payload, such as chemotherapeutic drugs, directly into the cancer cells. Additionally, nanoparticles can induce cellular stress responses, leading to oxidative stress, DNA damage, and apoptosis. Some nanoparticles can also trigger immune responses, activating immune cells to attack cancer cells. By manipulating the surface chemistry, shape, size, and payload of nanoparticles, researchers can design targeted therapies that maximize efficacy and minimize toxicity.

Table 1: Showed, both with and without the bilobetin (8 ppm) and Ginkgo biloba silver NPS (4 ppm), the protein carbonyl in with small cell and adenocarcinoma lung tumor as compared with the control (Mean± SEM)

Groups	**Groups**			**P-values**
	Control (Ctrl)	**Small cell lung cancer** (SCLC)	**Adenocarcinma** (ADCA)	
Number of paction	**25**	**25**	**25**	**-**
PC (nmol/l) Without add	1.410 ±0.3512^a	2.877 ±0.2021^a	6.343 ± 0.5144^b	0.0002
PC (nmol/l) With bilobetein	0.9400±0.03464^a	1.367±0.1309^a	2.485±0.1768^b	0.0004
PC (nmol/l) With Ginkgbiloba nanoparticles	0.7380± 0.05632	1.138±0.3283	1.473± 0.07446	0.0988

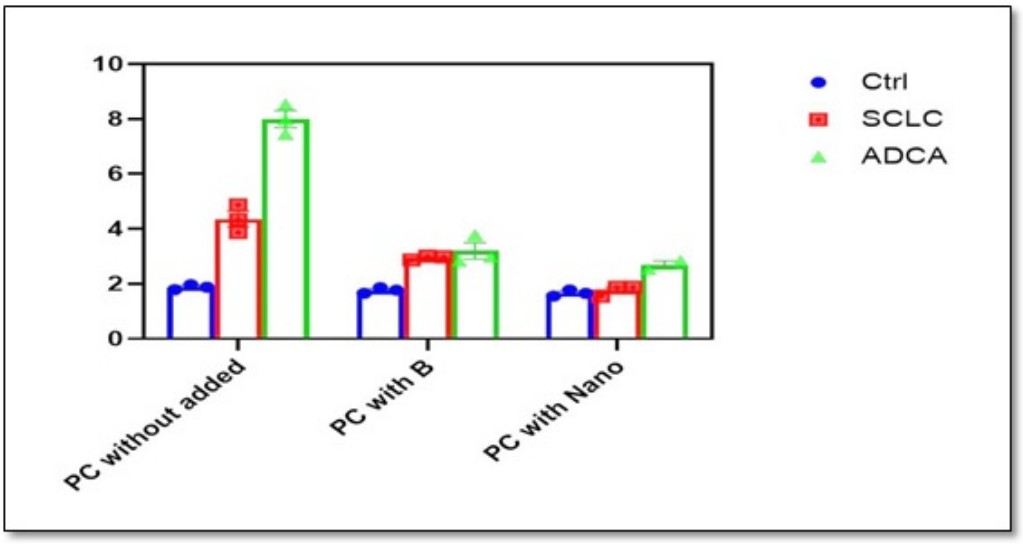

Figure 2: The protein carbonyl in the serum with small cell and adenocarcinoma lung tumor was reveals with and without the bilobetin solution (8 ppm) and Ginkgo biloba silver NPS solution (4 ppm), in comparison to the control groups

3.3 Visualization of Silver Nanoparticles Synthesized Using *Ginkgo biloba* Extract

With and without the addition of bilobetin and Ginkgo biloba silver nanoparticles, the levels of RBP-1 were measured in the serum of the three patient groups. RBP-1 levels were significantly elevated in both cancer groups (SCLC and ADCA) ($p < 0.0001$) when compared with the control group. Compared with the untreated breast cancer groups, treatment with the nano-silver particles and bilobetin significantly reduced the level of RBP-1, as shown in Table 2, thus providing a possible protection against cancer progress, although the addition of the silver nanoparticles did not lead to a protective effect against breast cancer progression compared with the treatment with either the silver nanoparticles or with bilobetin.

Table 2: RBP-1 levels in each of the three research groups' sera. (Mean± SEM)

Groups	Groups			P-values
	Control (Ctrl)	Small cell lung cancer (SCLC)	Adenocarcinma (ADCA)	
Number of paction	25	25	25	-
RBP-1 (ng/ml) Without add	3.037 ± 0.3018^{a}	2.226 ± 0.1650^{a}	5.890 ± 0.2893^{b}	0.0001
RBP-1 (ng/ml) With bilobetein	2.221 ± 0.05198^{a}	2.276 ± 0.02598^{a}	2.848 ± 0.03732^{b}	0.0001
RBP-1 (ng/ml) With Ginkgbiloba nanoparticles	2.081 ± 0.02906^{a}	2.115 ± 0.01027^{a}	2.539 ± 0.04826^{b}	0.0001

3.4 Visualization of Silver Nanoparticles Synthesized Using Ginkgo biloba Extract

Through FESEM (Field Emission Scanning Electron Microscopy), it provides visual confirmation for the presence of silver nanoparticles produced through Ginkgo biloba extract. Figure 3 confirms that it is possible to obtain this structure, by following the arrows in the image at the beginning.

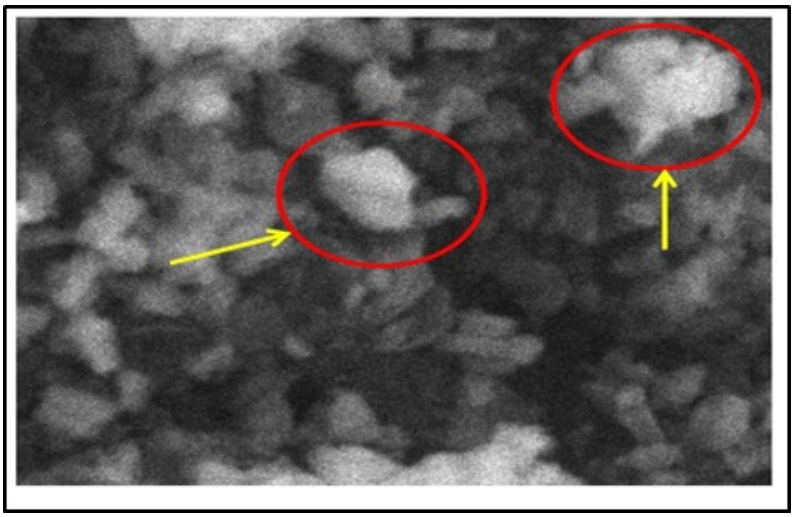

Figure 3: Using a FESEM, analyze the silver nanoparticles in Ginkgo biloba.

4 Cellular Uptake and Localization of Silver Nanoparticles in MCF-7 Cells

Distribution of silver nanoparticles (AgNPs) inside MCF-7 cells was visualized using Transmission electron microscopy mapping. The localization of the nanoparticles within the Cyto, CM, and individual NPs indicates cellular uptake and cellular component interaction. This result combats the possibility of targeted drug delivery by AgNPs as presented in Figure 4.

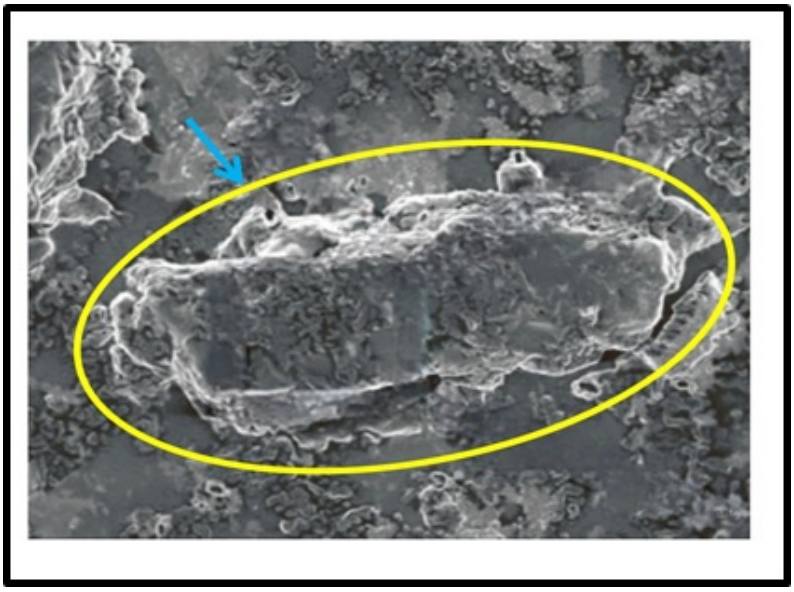

Figure 4: Transmission electron microscopy mapping of AgNPs in MCF-7 cells revealed the following locations: NPs, CM, and Cyto.

5 Discussion

This work used agarose gel electrophoresis to dtermine the silver NPS effects on DNA integrity in MCF-7 breast cancer cells. Here, a clear dose dependent increase in DNA fragmentation, evident by the smearing in lanes treated with AgNPs relative to the untreated control lane (Figure 1). This result further supports previous work showing that AgNPs are cytotoxic against a variety of cancer cell lines through mechanisms including DNA damage (Williams & Serpell, 2020) induced by AgNPs. The generation of ROS and direct interaction of AgNPs with DNA are probably responsible for this cytotoxic effect (Ilić et al., 2021). The higher amounts of smearing due to higher amounts of AgNPs also testify to stronger correlation with cytotoxic effect as stronger correlation between AgNP concentrations and cytotoxic effect. Finally, DNA damage and cytotoxic effects inflicted by AgNPs on MCF-7 breast cancer cells as confirmed by this work provides additional support for treatment of cancer with AgNPs. Yet, more work in optimizing both dose and delivery methods is needed to mitigate what may be toxic to healthy cells.

The protein carbonyl ('PC') levels of small cell lung tumor and adenocarcinoma were assessed in this study looking at the effect of bilobetin and Ginkgo biloba silver nanoparticles (AgNPs). Results, shown in Table 1, show that both bilobetin and AgNPs reduced PC levels in both cancer groups to that of the untreated controls ($p < 0.0004$), suggesting a potent antioxidant activity. These findings are consistent with other research demonstrating the antioxidant properties of bilobetin (Sreelekha et al., 2021). Possible mechanisms responsible for the observed reduction in PC levels include the capacity of these substances to counteract reactive oxygen species (ROS), trigger lipid peroxidation and boost the oxidative activity of endogenous antioxidant enzymes (Fornari Laurindo et al., 2023). that bilobetin and Ginkgo biloba derived silver nanoparticles have antioxidant properties that allow them to reduce oxidative stress in lung cancer patients and may serve as potential therapeutic agents to mitigate oxidative damage during cancer progression. The specific mechanisms by which these antioxidants exert protective effects and the effectiveness of this therapy in larger patient populations has not been explored further.

In this study, impact of bilobetin and Ginkgo biloba silver nanoparticles (AgNPs) on serum retinol binding protein 1 (RBP1) levels in patients with SCLC and adenocarcinoma (ADCA) were investigated. Table 2 reveals that RBP1 was significantly higher ($p < 0.0001$) in SCLC and ADCA groups than healthy control group. However, in both cancer groups, treatment with

either bilobetin or AgNPs significantly suppressed RBP-1 levels. Such findings are in keeping with previous observations that in many cancers in which RBP-1 is frequently Overexpressed, including lung cancer, RBP-1 is Overexpressed and that its Overexpression has a role in tumor growth and metastasis (Nag et al., 2022). Bilobetin, and AgNPs, each displayed the potential to downregulate cellular signaling pathways regulating RBP-1 such as JAK/STAT pathway or PI3K/AKT pathway, and could thus be responsible for their observed reduction in RBP-1 levels after treatment (Morais et al., 2024). Moreover, the antioxidant properties of bilobetin and AgNPs, as demonstrated in previous studies Živanović et al. (2024) may also participate in reducing RBP-1 levels by reduction of oxidative stress, a well-known cause of RBP-1 overexpression. Finally, these results show that bilobetin and Ginkgo biloba derived AgNPs can protect against lung cancer progression by lowering RBP-1 levels with both cellular signaling modulations and oxidative stress reduction. These findings need to be replicated with larger patient cohorts and investigate further the potential of these findings as a basis for new therapy.

Synthesis and cellular uptake of Ginkgo biloba extract coupled silver nanoparticles (AgNPs) were studied in this work. The successful synthesis of AgNPs has been confirmed by FESEM analysis shown in Figure 3, which shows distinct nanoparticle structures indicated by arrows. Moreover, cellular uptake and interaction with cellular components were further evidenced by transmission electron microscopy mapping in Figure 4, which clearly showed localization of AgNPs in the cytoplasm, cell membrane and as single particles within the MCF-7 breast cancer cells. While many studies have demonstrated cellular internalization of AgNPs, this finding is consistent with several studies that suggest application of AgNPs as targeted drug delivery vehicles (Malysheva et al., 2021). The cellular uptake of AgNPs is perhaps mediated through endocytosis, the process by which cells engulf extracellular materials (Talarska et al., 2021). However, because AgNPs can penetrate cellular barriers and interact with intracellular components, they may carriers for treament chemical substances that targeted delivery with the potential to increase quality of the treatment while minimizing off-target effects. In this study, we confirm the successful synthesis of the AgNPs with Ginkgo biloba extract and their uptake by MCF-7 cells, making them a potential candidate for targeted drug delivery in cancer therapy. Futher research, however, is required to optimize nanoparticle functionalization and targeting strategies for specific therapeutic applications. In accordance with the ethics of scientific research, consent was obtained from cancer patients from whom samples were taken and their condition was determined by the Iraqi-German Center for Functiona l Diagnostics, which diagnosed the presence of breast cancer by means of a PET scan.

6 Conclusion

The study successfully demonstrated the potential of metallic nanoparticles, particularly silver NPS that formed by Ginkgo biloba extract, as a promising approach for cancer diagnosis and treatment. The cytotoxic effects of AgNPs on MCF-7 breast cancer cells and the antioxidant activities of bilobetin and AgNPs in lung cancer patients highlight the multifaceted applications of these nanoparticles in combating cancer. Furthermore, the observed reduction in RBP-1 levels suggests a potential role in inhibiting cancer progression. However, further research is necessary to determine the optimal dosage, delivery method, and long-term safety profile to effectively translate these promising findings into clinical applications. Addressing these limitations will be crucial for realizing the full potential of nanoparticle-based therapies in cancer treatment.

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