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Novel Study of the Effect of Bilobetein Compound and Silver Nano Particles of Ginkgo Biloba as Folate Antagonists by Inhibiting Enzyme Dihydrofolate Reductase in Iraqi Patients Serum of Small Cell and Adenocarcinoma Lung Cancer

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ABSTRACT

Background: The most common and deadly cancers are lung cancer. There are no symptoms or indicators in its early stages. In study silver nanoparticles of Ginkgo biloba herb were synthesized and prepared solutions of different concentration of this nanoparticles compound, solution of different concentrations of the bilobetein compound which is the main compound in the ginkgo biloba herb.

Objective: The purpose of this study was to prepare silver nanoparticles of ginkgo biloba herb and to study the effect of this compound at different concentration as an antifolate, through its reduction of the enzyme DHFR concentration

Patient and Methods: This study was divided into three groups as following: thirty samples of blood serum from men and women as a control group with age range 23-45 years, thirty samples of blood serum from men and women of small cell lung cancer patients with age range 45-80 years and finally, thirty samples of blood serum from men and women of adenocarcinoma lung cancer patients with age range 45-80 years.

Results: The 4 ppm concentration of the nanoparticles solution gave the best reduction in enzyme concentration in patients of small cell and adenocarcinoma lung cancer compared to control (465.94 ± 238.74 and 700.09 ± 324.59 vs 1680.38 ± 345.51 pg/ml). 8 ppm concentration showed reduction of enzyme concentration in small cell and adenocarcinoma lung cancer compared to control of 586.62 ± 225.57 and 1131.23 ± 415.33 vs 1860.06 ± 163.22 pg/ml.

Conclusion: This study concluded the bilobetein and silver nanoparticle of Ginkgo biloba had the ability in preventing growth of cancer cells by reducing the DHFR concentration in sera of small and adenocarcinoma lung cancer patients.

Keywords: Lung cancer, DHFR, Bilobetein, GinkgoBiloba**.**

INTRODUCTION

Cancer is a fatal illness in which normal body cell proliferation is constrained and aberrant alterations occur at an uncontrolled rate [(1)]. At this time, lung cancer in the globe, accounts for 23% of all cancer-related fatalities [(2, 3)]. Squamous-cell lung cancer (15 %–20 %) together with non-small cell lung cancer are the most common kinds of lung (NSCLC, 80%–85%). Primary lung cancer is mostly treated clinically by chemotherapy, surgery, and radiation [(4, 5).]

An ancient plant species known as ginkgo biloba is regarded to have a number of health advantages for living things [(6)]. It is chemically diverse and includes a lot of bioactive components. Ginkgo biloba has been shown to have a wide range of therapeutic and pharmacological properties, including anticancer, antidementia, antidiabetic, antiobesity, antilipidemic, antimicrobial, antioxidant, antilipid peroxidation, antiplatelet, anti-inflammatory, hepatoprotective, antidepressant, antiaging, immunomodulatory, antihypertensive, and neuroprotective effects [(7)].

Bilobetin, a naturally occurring chemical obtained from Ginkgo biloba, has a wide range of pharmacological actions, including those related to anti-oxidation, anti-cancer, antibacterial, antifungal, anti-inflammatory, antiviral, and promoting osteoblast formation [(8)].

Dihydrofolate reductase (DHFR) (EC 1.5.1.3) is a vital enzyme in biochemistry and pharmaceutical science (9). The importance of this family of drugs is shown by the fact that dihydrofolate reductase inhibitors are utilized as antibacterial, antimalarial, antifungal, and anticancer drugs.? The maintenance of tetrahydrofolate (THF) and its derivatives, which results in the production of purine and thymidylates, is regulated by DHFR, which is essential for healthy cellular development and proliferation [(10)]. The folate pathway, which uses the enzyme dihydrofolate reductase (DHFR) to catalyze the conversion of dihydrofolate to tetrahydrofolate utilizing NADPH- a crucial cofactor for the manufacture of purines, thymidylate, and a number of amino acids- has been a key target in the fight against cancer [(11)]. The success of antifolate drugs used to treat cancer by blocking DHFR, so depleting THF and decreasing DNA synthesis and cell proliferation, highlights the significance of this response [(12)].

In the past, methotrexate (MTX) has been used to target the folate metabolic pathway and is a crucial part of the therapy of cancer, including lung, breast, head and neck, and osteosarcoma. Inhibiting de novo pyrimidine and purine production, which leads to cell death, is how anti-folate medicines combat folate-dependent enzymes. MTX is now utilized in chemotherapy [(13)]. The purpose of this study was to prepare silver nanoparticles of

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ginkgo biloba herb and to study the effect of this compound at different concentration as an antifolate, through its reduction of the enzyme DHFR concentration and to compare it with effectiveness of the compound bilobetetin at different concentrations as an antifolate, which is an active component of ginkgo biloba herb Keeping cancer cells from multiplying and possibly killing them is possible by preventing folate consumption.

MATERIALS AND METHODS

Subject

During the period from January 2022 to June 2022 blood samples were collected from patients complaining from lung cancer in Baghdad Teaching Hospital (Oncology Teaching Hospital) and they have been classified into three groups as the following:

Group one: 30 samples of men's and women's blood serum as a control group with age range (23-45 years).

Group two: 30 samples of men's and women's blood serum of small cell lung cancer patients with age range (45-80 years).

Group three: 30 samples of men's and women's blood serum of adenocarcinoma lung cancer patients with age range (45-80 years). And no previous disease which may interfere with the parameters analyzed in this study.

Collection and processing of specimens

8 ml of venous blood were drawn using disposable plastic syringes with a capacity of 10 ml. Blood samples from both the patients and the controls were placed into regular plastic tubes without anticoagulant, and the blood was allowed to coagulate for 20–30 minutes at 37°C. After being centrifuged for 10 minutes at 3000 rpm, the serum was collected, divided into tiny Eppendorf tubes, and kept at -20 °C until it was time for analysis.

Materials

- AgNO3 (Silver Nitrate,99.9%) from Merck, Germany; and 99.0%
- Bilobetein from China supplied the product, yirui bio-technology.
- Dihydrofolate reductase kit from china, finetest.
- Ginkgo biloba herb From Chain, Iraq Country Not a Product of Ginkobiloba.
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Ginkgo biloba extract used in the synthesis of silver nanoparticles: AgNPs were synthesized by adding (4 gm, 50 mL) of Silver nitrate (AgNO3) into extract from ginkgo biloba. The mixture was then swirled magnetically at 80 °C for 30 min. When the extract was being prepared, the hue abruptly changed from translucent green to black, signifying the creation of Ag NPs. AgNPs were powdered, conserved in an airtight bottle, and dried in an oven at 60 °C for 18 hours [(14)].

Preparation of solutions: Bilobetein and silver nanoparticles of ginkgo biloba herb solutions were synthesized by adding 0.005 mg of each to be dissolved in 100 ml of water to create a stock standard solution. Dilutions of stock solutions in water were used to create working solutions with various concentrations (1ppm, 4ppm, 8ppm, 10 ppm). Working standard solutions and all stock standard solutions were created the moment before usage.

Measurement the concentration of Dihydrofolate reductase in serum: The Dihydrofolate reductase concentration was measured according to the method of the measuring kit, which was manufactured by the company (finetest) and the principle of this method is the sandwich enzyme-linked immune-sorbent assay technology served as the foundation for this kit. On 96-well plates, capture antibody was already pre-coated. Additionally, the biotin-conjugated antibody was utilized as detecting antibodies and rinsed with wash buffer, HRP-Streptavidin was added, and unbound conjugates TMB substrates were utilized to visualize the HRP enzymatic reaction. TMB was catalyzed by HRP to yield a blue product that became yellow when acidic stop solution was added.

Ethical consent:

The Academic and Ethical Committee of the University of Baghdad approved the study. Informed written consent form was signed by each participant, who then consented to take part in the study. This study followed The World Medical Association's Declaration of Helsinki on the conduct of scientific research involving human beings.

Statistical investigation:

The means and standard deviations were provided, and Student's t-test was applied to contrast the importance of the discrepancy in typical values between any two groupings. $P \leq 0.05$ was regarded as statistically significant. The results' overall predictive values. The curriculum was followed by all groups studied of Microsoft Office XP 2010.

RESULTS

Characterization of Ag NPs

UV visible spectra of synthesized Ginkgo Biloba as nanoparticle: The color of the reaction solution varied substantially throughout Ginkgo Biloba-AgNPs production, and subsequently, the color solution progressively became brown-black, indicating the surface formation. The produced AgNPs were excited via plasmon resonance. The UV-Vis spectra were

obtained as illustrated in (Figure 1). Ginkgo Biloba-extracts (GB) and artificial Ginkgo Biloba -AgNPs were measured at about 350-550 nm. Ginkgo Biloba absorption spectrum -AgNPs ranged between 320 and 600 nm, with a noticeable peak at 448 nm, however, the Ginkgo Biloba-extract failed to detect this peak from 200 to 400 nm [15].

FT-IR spectrum of synthesized Ginkgo Biloba as nanoparticle: FTIR analyses were performed to determine the potential Ginkgo biloba leaf extract contains biomolecules that cap, resulting in effective silver nanoparticle stability. The silver nanoparticle-infused plant extract's FTIR spectrum (Figure 2). The plant extract's FTIR spectrum before and after have showed significant changes showed a shifting in the peaks at 3506.59 $cm^{-1}$and 3402cm^{-1} (due to N–H bending and amides). The spectrum in the band 1450.47 cm^{-1} and 1381.03cm^{-1} results from the C–N, C-O stretching modes were responsible for the aromatic amine group's stretching mode. Signed peak in 1654.92 cm^{-1}, 1246.02 cm^{-1} and 2924 cm^{-1} can be attributed to the vibrational stretching of the C–OH bond in proteins, polyphenols, and alkene groups, respectively, in the plant extract. The FT-IR study revealed that polyphenols, amides, and amino groups were present in the produced AgNPs [15].

X-Ray Diffraction analysis of synthesized Ginkgo Biloba as nanoparticle: XRD measurement revealed the crystalline form of Ag nanoparticles. As observed, there are four prominent Bragg refections at around 38.34, 44.47, 64.65 and 77.69° corresponding to the four diffraction peaks, which are identified as fcc silver planes (111), (200), (220), and (311). Bragg reflections are faint and widened in comparison to the powerful (111) reflection (Figure 3). This property showed that the nanocrystals are extremely anisotropic. Debye-equation Scherrer's was used to compute the mean size of nanoparticles by measuring the width of the (111) peak. Other peaks were also identified, indicating that the nanoparticles' surfaces were coated with AgO [15].

FESEM analysis: The FESEM was used to investigate the surface morphology of the Ag "nanoparticles [16]. Figure (4) showed FE-SEM photographs of the prepared Ag samples. The FESEM images showed the presence of nanoparticles of spherical and cubic shapes that have different sizes and are irregularly distributed. The presence of aggregation of nanoparticles was observed due to the different nanoparticle sizes and solvent evaporation during sample preparation. The obtained results confirmed the success of the silver nanoparticles synthesis process from the extract, which is consistent with the results of previous studies [16]. It was found that the average nanoparticles of the FESEM images are 50-62 nm.

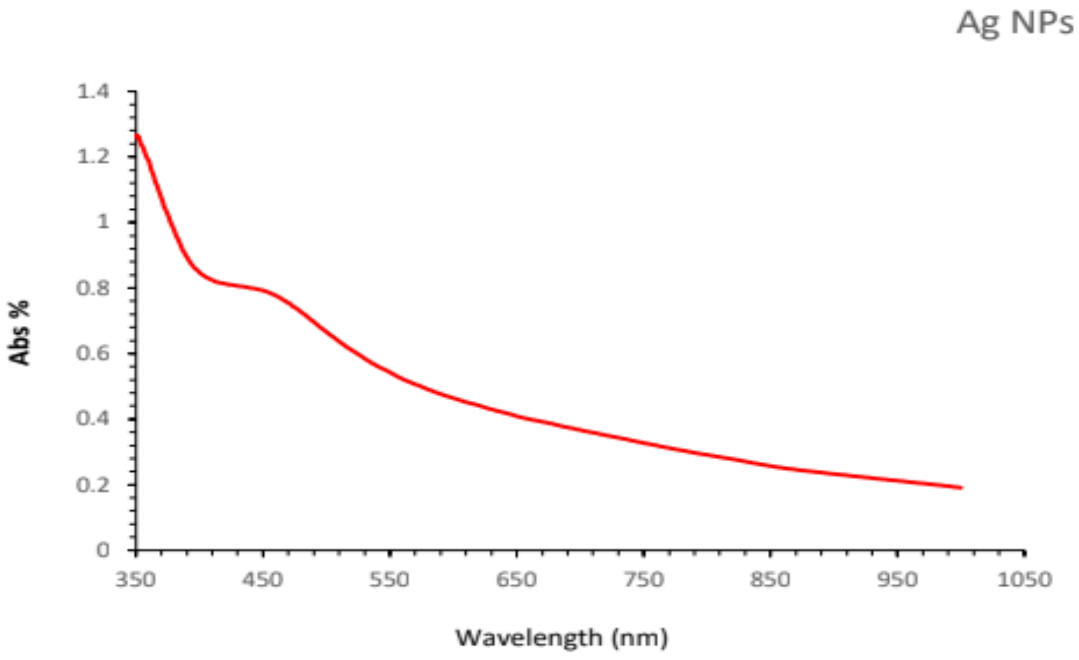

Figure (1): UV visible spectra of synthesized Ginkgo Biloba as silver nanoparticle.

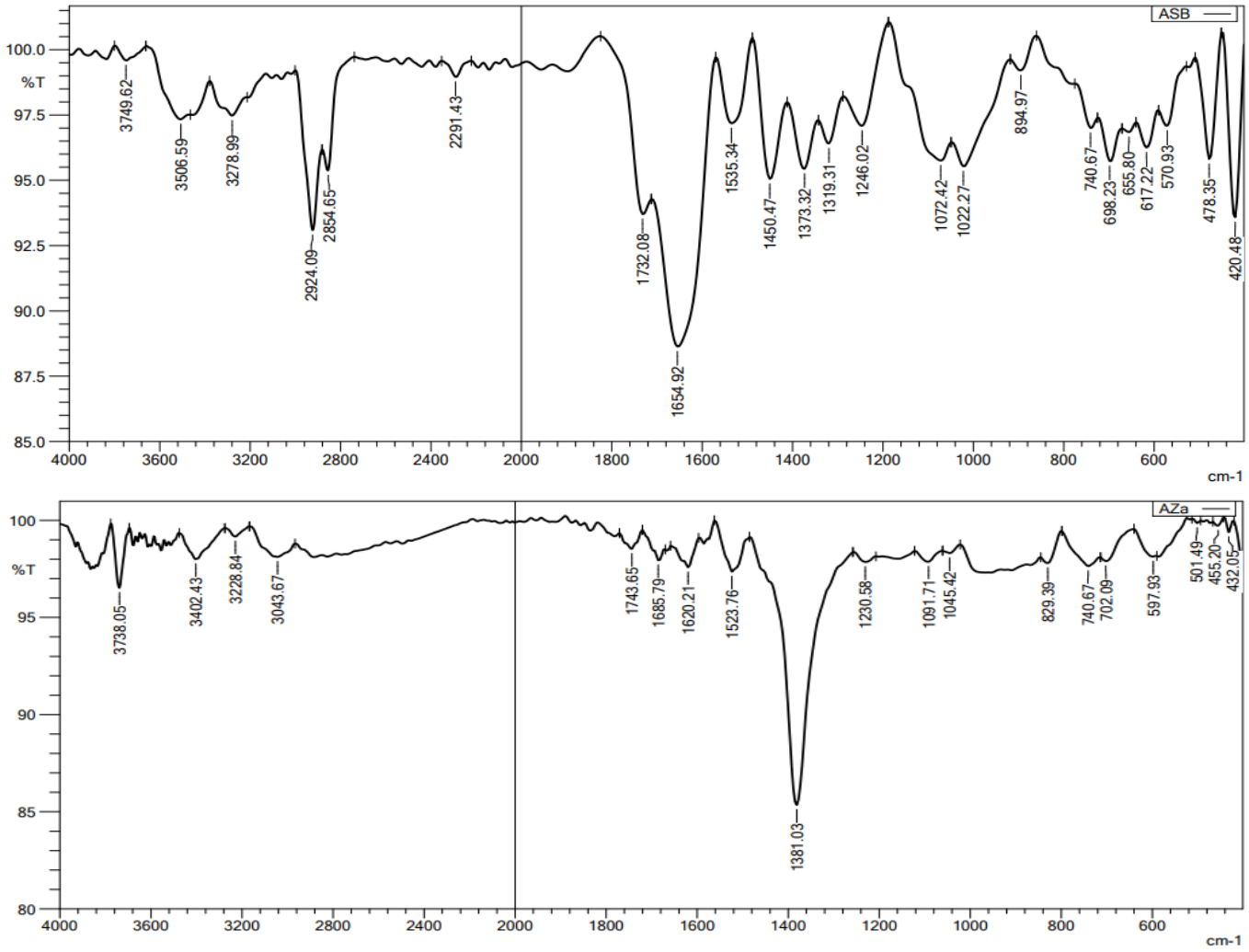

Figure (2): FT-IR spectrum of (a) Ginkgo biloba (b) Ginkgo biloba as a silver nanoparticles.

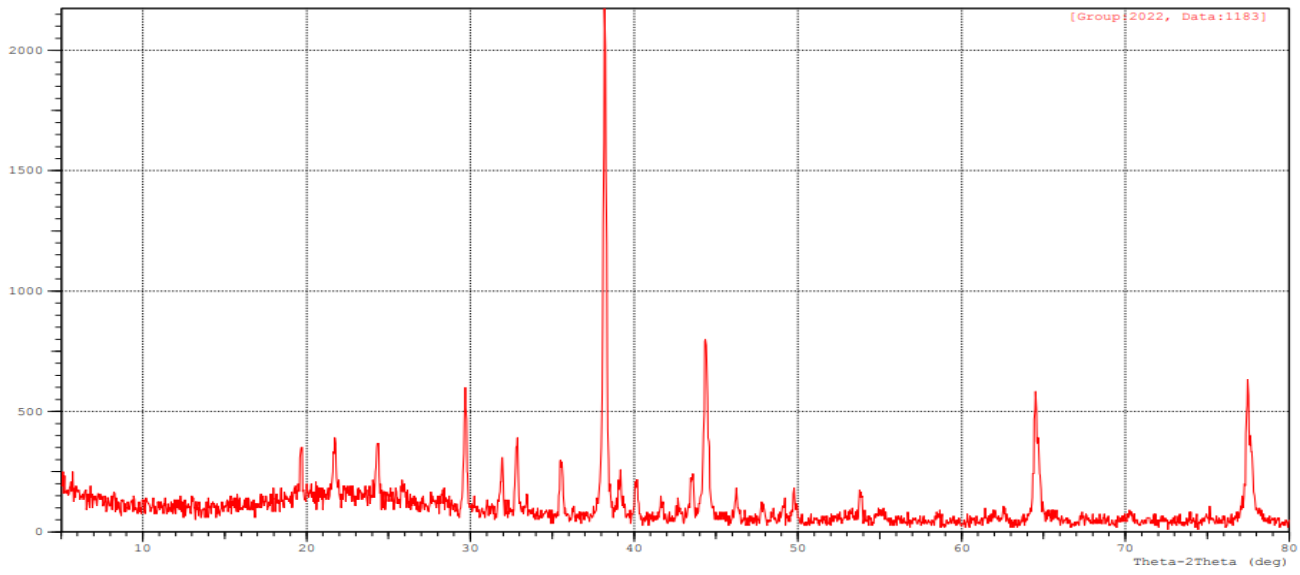

Figure (3): XRD analysis of Ginkgo Biloba as silver nanoparticle

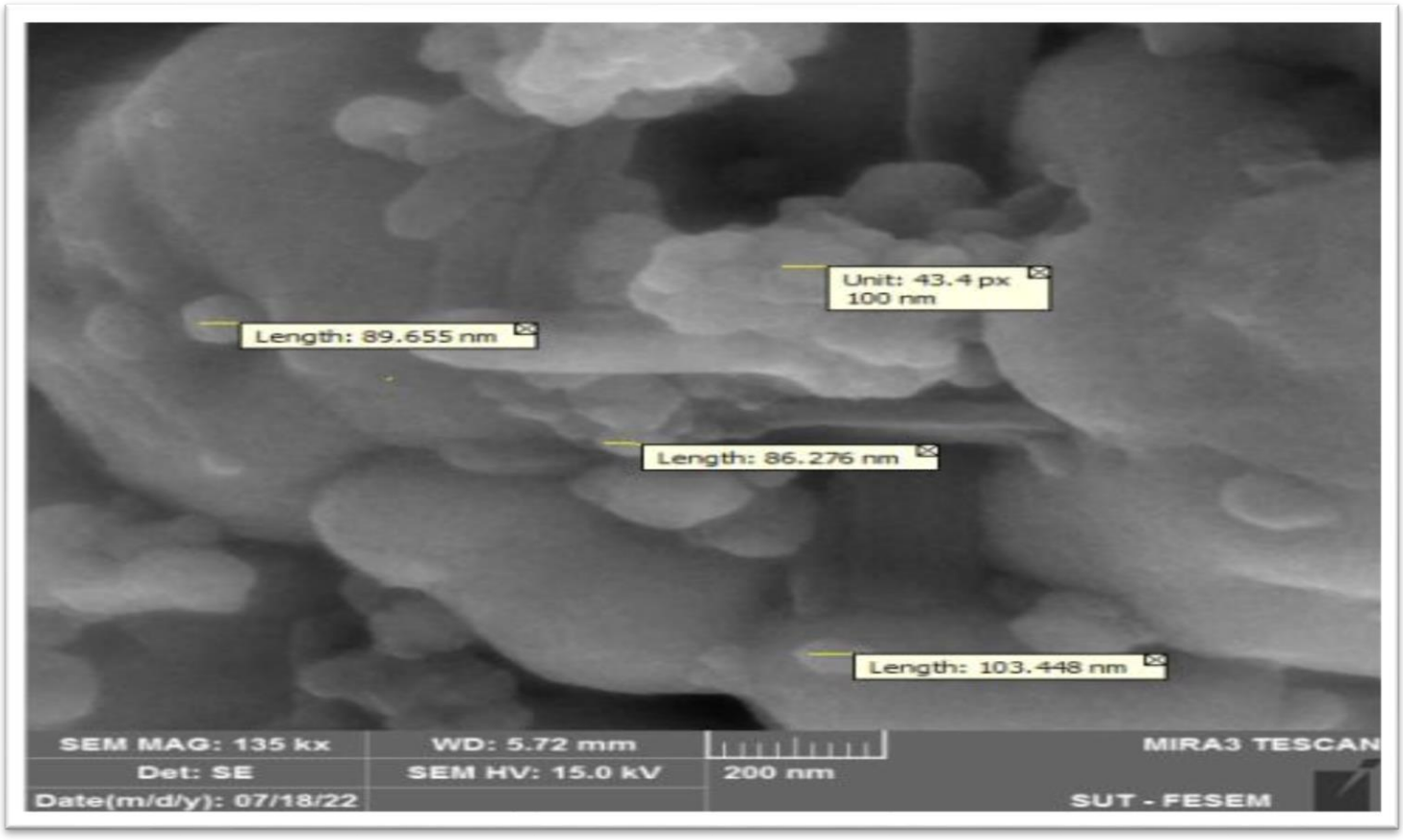

Figure (4): FESEM analysis of Ginkgo Biloba as silver nanoparticle

The effect of Bilobetin and silver nanoparticles of Ginkgo Biloba as antifolate: Table (1) illustrated that the mean of the concentration of dihydrofolate reductase in serum of patients small cell and adenocarcinoma lung cancer compared to control group without any added and with added solution of Bilobetein in concentration of 8 ppm and the solution of silver nanoparticle of Ginkgo biloba in concentrated of 4ppm.

Table (1): DHFR concentrations in sera of three studies groups.

Groups	No.	DHFR (pg/ml) Without add Mean± SD	P	DHFR (pg/ml) With bilobetein Mean± SD	P	DHFR (pg/ml) With Ginkgbiloba nanoparticles Mean± SD	P
Control (G1)	30	661.19 ±223.57		586.62±255.57		465.94 ± 238.74	
Small cell Lung Cancer (G2)	30	2233.56±234.10	3.43×10^{-34}	1131.23±415.33	0.00230	700.09 ± 324.59	0.00234
Adenocarcinma (G3)	30	2780.14 ±759.51	3.66×10^{-21}	1860.06±163.22	7.74×10^{-31}	1680.38 ± 345.51	9.95×10^{-23}
			0.00038		1.63×10^{-12}		1.63×10^{-12}

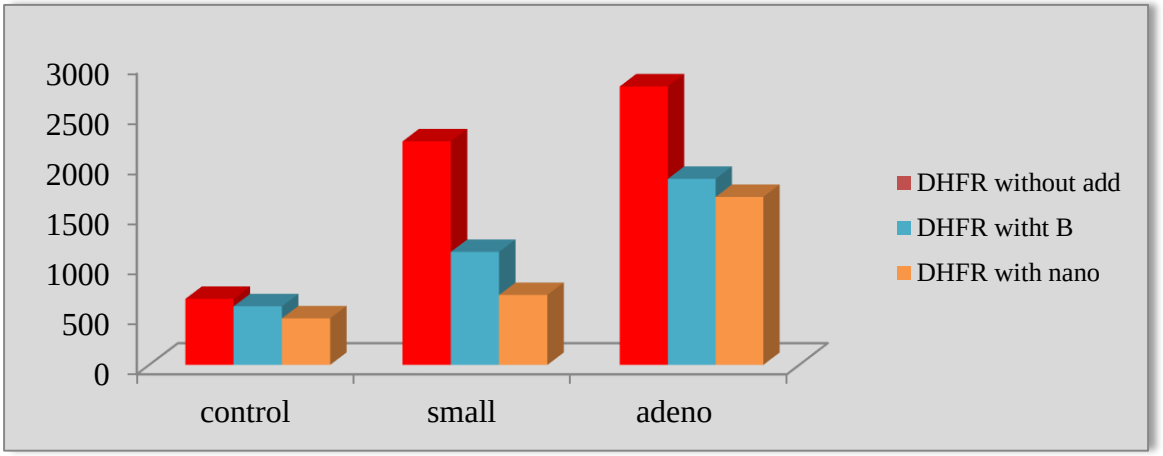

Figure (5): DHFR concentrations in sera of three studies groups.

From table (1) and figure (5) a significantly higher ($P \leq 0.05$) in the values of DHFR concentration was noted in the group of patients. G2 and G3 compared to control group G1.

Without any addition and after adding solutions bilobetein with concentration of 8 ppm and the silver nanoparticles of Ginkgo biloba with concentration of 4 ppm. It is also observed that the values of DHFR concentration after adding the solution of silver nanoparticles with concentration of 4 ppm reduced more when compared to the values of DHFR concentration after adding bilobetein solution with concentration of 8 ppm and without any addition in all study groups.

DISCUSSION

In this study it is possible to suggest the bilobetein compound and silver nanoparticles of Ginkgo biloba herb as antifolate materials that prevent the growth of cancer cells and thus kill them through its ability to reduce the concentration of DHFR enzyme thus, it is similar in its action to the methotrexate medication which inhibits the DHFR enzyme [(17)].

Methotrexate was discovered by **Hutchings *et al*.** [(18)]. After one year, Sidney Farber, a medical expert, hypothesized that folate is necessary for cancer cells to maintain. Hence methotrexate would limit cancer progression. They established that methotrexate was beneficial in lowering symptoms in children with acute lymphoblastic leukemia. Several years later, the medication was approved for the management of psoriasis and rheumatoid arthritis. Humans now routinely use methotrexate to treat cancer and autoimmune diseases. Methotrexate interferes with DNA synthesis, repair and cellular replication because inhibition of DHFR by folate antagonists (methotrexate) causes shortage in the cellular pools of midylate and purines and a corresponding decrease in nucleic acid synthesis [(10)].

One of the key actions of MTX in oncology has been thought to be its mechanism as a folate antagonist. Folates are the structural constituents that support cellular growth. The folate receptor protein family is responsible for the cellular absorption of MTX. The major enzyme that MTX inhibits is 5-aminoimidazole-4-carboxamide ribonuclease (AICAR) transformylase (ATIC) and stops DHFR, an enzyme that is accountable for dihydrofolate (DHF) catalyzed to tetrahydrofolate (THF). The end product of this process is prevention of thymidylate production. synthetase (TYMS), which is essential, residues of thymidine, it lowers the human T cells' purine and pyrimidine pool levels by boosting UTP levels and lowering levels in tandem of both GTP and ATP. It inhibits T cell expansion and boosts apoptosis ([19]).

CONCLUSION

Though the results of this study, it is possible to suggest the bilobetein and silver nanoparticle of Ginkgo biloba act as antifolate materials leading to prevention of the growth of cancer cells and thus kill them through its ability to reduce the Dihydrofolate reductase concentration in sera of small and adenocarcinoma Iraqi lung cancer patients.

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Sources of funding: Nil**.**
Author contribution: Authors contributed equally in the study.

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