Novel Investigative Study on the Anticancer Potential of Plant Extracts and Silver Nanoparticles as Inhibitory Agents for Lung Cancer

Hiba J.Hussein

Department of Chemistry, College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad, Iraq.

heba.jumaa1105a@ihcoedu.uobaghdad.edu.iq

Nijoud Faisal Yousif AL-Saarag

Department of Chemistry, College of Education for Pure Science, Ibn Al-Haitham, University of Baghdad, Iraq.

corresponding author: nijud.f.y@ihcoedu.uobaghdad.edu.iq

Abstract: Emergent therapeutic strategies that merge robust anti-cancer effects with antioxidant attributes are imperative for addressing lung cancer, a prevalent and often fatal ailment. This presentation explores the potential application of Ginkgo biloba silver nanoparticles (GB-AgNPs) and bilobtin as a viable avenue to impede the advancement of lung cancer while concurrently furnishing antioxidative benefits. In the current study, a total of 30 samples were examined from each of the three distinct groups: control, small cell lung cancer, and adenocarcinoma lung cancer. The results of the study indicate that the application of GB-AgNPs and bilobetein led to a significant reduction in elevated protein carbonyl and retinol binding protein (RBP) levels. This suggests that these interventions have the potential to effectively counteract crucial molecular markers linked to the onset and advancement of lung cancer. These findings open up a new and innovative pathway for the development of targeted therapeutic approaches for lung cancer, utilizing natural compounds as potential treatment agents.

Keywords: lung cancer, bilobetein, ginkgo biloba, Agnps, Human protein carbonyl, Retinol binding protein.

1.Introduction:

Reactive oxygen species (ROS), integral to a cell's oxygen metabolism, exhibit diverse physiological impacts through the initiation of signaling pathways crucial for cellular

division and growth. Nevertheless, an overabundance of ROS can result in the degradation of vital macromolecules such as proteins, lipids, and DNA[1,2].

The antioxidant activity attributed to flavonoids is believed to arise from their capacity to scavenge reactive oxygen species (ROS). In previous decades, it was widely hypothesized that the in vivo antioxidant effects of flavonoids primarily stemmed from their ability to counteract and reduce ROS through scavenging mechanisms, particularly during the 1980s and 2000s[3].

The flavonoid bilobetin is derived from Ginkgo biloba. Bilobetin is composed of two monoflavone molecules linked by C-C bonds, forming an atypical flavonoid structure. While it leans towards being biflavonoid in nature, its structural attributes and unconventional properties share resemblances with traditional flavonoids. Its chemical composition is represented by the formula $C_{16}H_{12}O_5$ [4].

The potential anti-tumor impacts of Ginkgo biloba extracts are thought to involve inhibiting tumor cell proliferation, triggering apoptosis, impeding tumor blood vessel formation, regulating tumor-associated genes, and exerting cytotoxic effects on tumor cells [5]. These outcomes might be attained through the plant's inherent antioxidant capabilities and its ability to scavenge free radicals [6].

Silver nanoparticles could potentially influence the transcriptional regulation of cancer protein biomarkers. [7,8]. By interacting with the cellular machinery involved in gene regulation, nanoparticles can influence the expression of the genes encoding the biomarkers, leading to reduced levels of protein [9].

In the serum, a biomarker denoted as protein carbonyl was detected. This biomarker is considered one of the irreversible manifestations of protein oxidation, speculated to represent an initial stage of protein alteration and indicative of protein irregularities induced by oxidative stress. The process of generating protein carbonyls involves oxidation reactions on specific amino acid residues like proline, arginine, threonine, and lysine, facilitated by metal catalysts [10].

The utilization of protein carbonyl (PC) can serve as a marker for detecting oxidative stress in a range of conditions, including cancer and various malignancies. [11,12].

Cellular retinol-binding protein-1 (CRBP-1), a cytosolic binding protein with a molecular weight of 15 kDa, plays a crucial role in the uptake and subsequent esterification of retinol by modulating its availability within the cell [13].

Retinol, a constituent of the retinoid signaling pathway, associates with RBP1, facilitating its transportation to target cells. After entering the cell, retinol engages with retinoic acid receptors (RARs) and retinoid X receptors (RXRs). Subsequently, retinol undergoes conversion into active vitamin A forms. The activation of these receptors, integral to gene expression regulation, can influence various cellular functions including cell differentiation, regulation of the cell cycle, and induction of apoptosis [13,14].

2.METHOD AND MATERIALS:

From January 2022 to June 2022, blood specimens were gathered from individuals diagnosed with lung cancer at the Oncology Teaching Hospital of the Baghdad Teaching Hospital. These samples were subsequently categorized into the following three distinct groups:

-(G1) comprised of 30 blood serum samples collected from healthy individuals aged between 23 to 45, encompassing both genders.

- (G2) included 30 blood serum samples from patients of both sexes diagnosed with small cell lung cancer, with an average age range of 45 to 80.

-(G3) consisted of 30 blood serum samples from patients with lung adenocarcinoma, aged between 45 to 80.

It's worth noting that none of the participants had any medical history that could potentially influence the traits under investigation in this study.

2.1. Collection and preparation of specimens:

A total of 8 milliliters (mL) of venous blood was collected using disposable plastic needles of 10 milliliter capacity. Subsequently, the blood samples were placed in basic plastic containers containing patient and control samples, and clotting was allowed to occur at a temperature of 37 degrees Celsius for a duration of 20 to 30 minutes. The separation of blood was carried out using small Eppendorf tubes, followed by centrifugation at 3000 revolutions per minute (rpm) for 10 minutes. The resultant samples were then stored at a temperature of -20°C until the time of analysis.

2.2. Chemicals

- The product (Yirui Biotechnology) was delivered by Bilobetein from China.

- ginkgo biloba herb.

- Protein carbonyl kit from China.

-Retinol binding protein-1 from China.

2.3. Silver nanoparticles were made using ginkgo biloba extract:

A combination of Ginkgo biloba extract and 4 grams (50 mL) of silver nitrate (AgNO3) was utilized to generate AgNPs. The subsequent procedure entailed magnetic stirring of the mixture at 80°C for a duration of 30 minutes. Notably, the color shift from a translucent green to black occurred during the extract preparation, signifying the creation of AgNPs. The resulting AgNPs were transformed into powder form, preserved in an airtight container, and subsequently subjected to a drying process at a temperature of 60°C for a span of 18 hours.

2.4. Preparation of solutions:

Within this investigation, solutions derived from the ginkgo biloba herb encompassing bilobate and silver nanoparticles were concocted. This process involved adding (0.005mg) of each component and dissolving them in 100 milliliters of water, leading to the creation of a foundational standard solution. Subsequently, this standard solution was diluted using water to generate operational solutions encompassing a spectrum of concentrations, namely (1ppm, 4ppm, 8ppm, and 10ppm). It's noteworthy that the preparation of both practical and standard stock solutions was carried out immediately prior to their utilization.

2.5. Measurement of the concentration of Human Protein Carbonyl (PC) and retinol-binding protein-1 (RBP-1) in serum:

The assessment of concentrations for both human protein carbonyl (PC) and retinol-binding protein-1 was conducted utilizing a measuring kit provided by the company (finest). The kit's methodology was grounded in the competitive-ELISA detection technique. Notably, the target of interest had already been coated onto the microtiter plate included within the kit. Following the elimination of any surplus conjugate and unbound samples or standards through

washing, each well of the microplate was subsequently treated with HRP-Streptavidin (SABC). Subsequently, a TMB substrate solution was introduced to each well. The interaction between the enzyme and substrate was then halted using a solution of sulfuric acid, and the subsequent alteration in color was measured spectrophotometrically at a wavelength of 450 nm [15,16].

2.6. Statistical investigation:

To assess the significance of variations in mean values across different groups, mean values along with their corresponding standard deviations were provided. Additionally, the Student's t-test was employed for analysis. Statistical significance was defined as a (P value < 0.05). These outcomes hold predictive implications for the broader finding [17].

3.Result and Discussion:

3.1Ginkgo biloba nanoparticles with a UV–visible spectrum:

During the synthesis process of Ginkgo Biloba-AgNPs, a remarkable color transformation of the reaction solution was observed, transitioning from its initial hue to a brown-black shade as the surface formation progressed. The AgNPs generated gained energy through plasmon resonance. The UV-Vis spectra presented in Figure 1 exhibited distinct characteristics of both synthetic Ginkgo Biloba-AgNPs and Ginkgo Biloba extracts (GB) within the range of 350 to 550 nm. Notably, the peak in the range of 200 to 400 nm was absent in the Ginkgo Biloba extract. In contrast, the absorption spectrum of Ginkgo Biloba-AgNPs extended from 320 to 600 nm, prominently featuring a significant peak at 448 nm. This synthesis was carried out in a solvent of water [18].

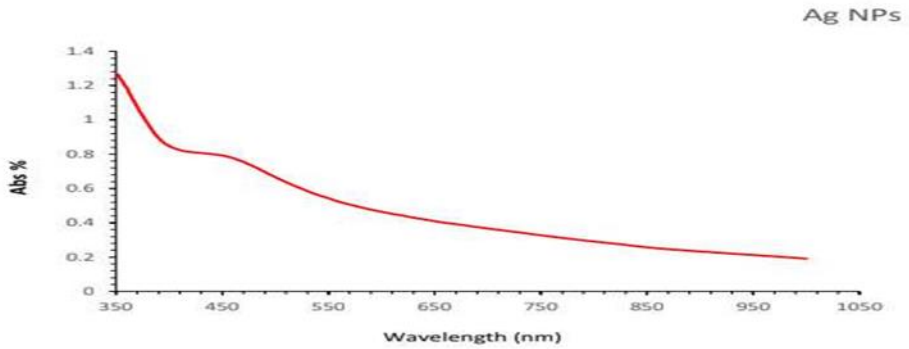

Figure 1: UV visible spectra of Ginkgo Biloba produced as silver nanoparticles

3.2. FT-IR spectrum of synthesized Ginkgo Biloba as nanoparticle:

The analysis employing FTIR indicated that the Ginkgo biloba leaf extract likely contains biomolecules that could influence the stability of silver nanoparticles. The FTIR spectrum displaying the plant extract with silver nanoparticles is illustrated in Figure 2. A noticeable shift in peak positions was observed between the before and after FTIR spectra of the plant extract, specifically at 3506.59 cm^{-1} and 3402 cm^{-1}, indicative of modifications in N-H bend and amide products. Notably, distinct changes were observed in the 1246.02 cm^{-1} and 1381.03 cm^{-1} bands, which revealed elongation modes associated with the aromatic amino group C-N and C-O. The distinct peak at 1654.92 cm^{-1}, 1072.42 cm^{-1} and 1091.71cm^{-1}, and 2924 cm^{-1} and 3402.43$cm^{-1}$was attributed to the vibrational stretching of the C-OH bond in plant proteins, polyphenols, and alkene groups [18].

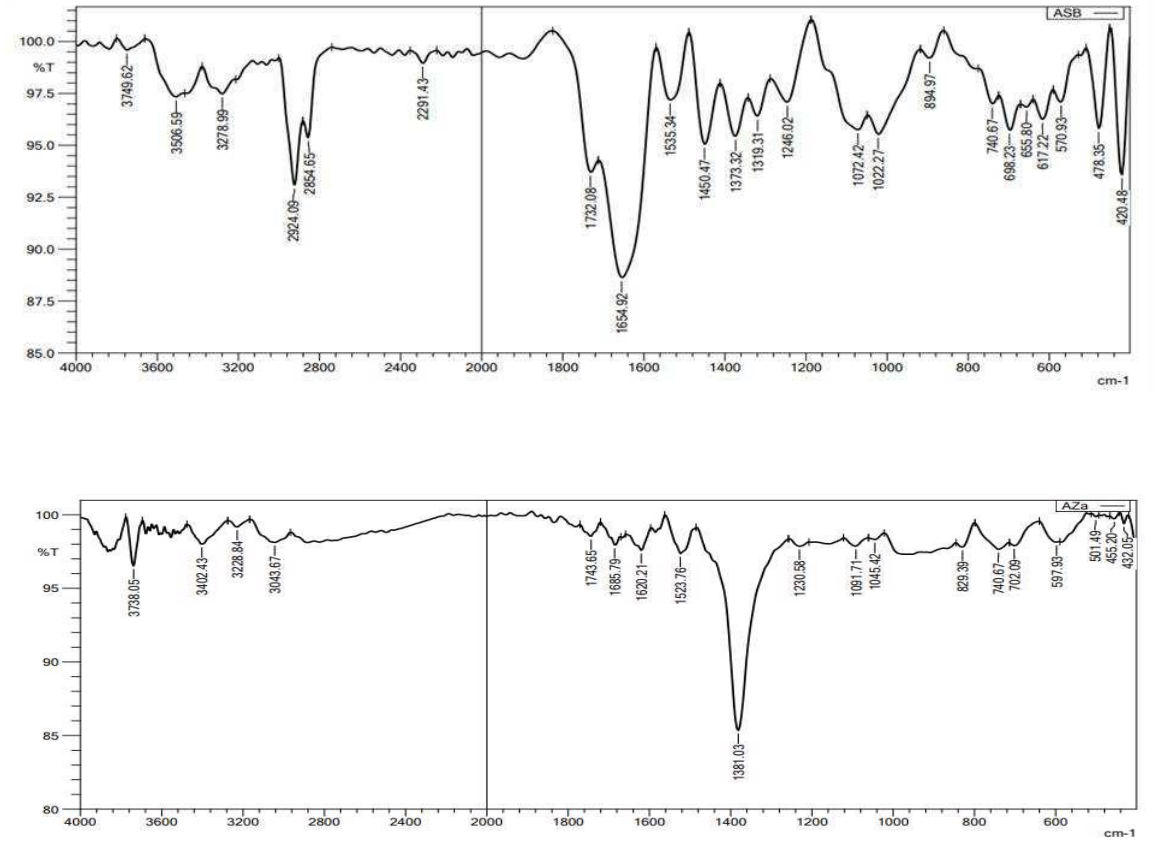

Figure 2 shows the FT-IR spectra of ginkgo biloba as silver nanoparticles in (a) and (b), respectively.

3.3. Ag nanoparticles were found to be crystallized by XRD analysis.

The XRD analysis unveiled the crystallization of Ag nanoparticles. The presence of four distinct diffraction peaks, associated with fcc silver planes (111), (200), (220), and (311), corresponded with the pronounced Bragg reflections observed at approximately 38, 34, 44, 47, 64, 65, and 77.69. Notably, the Bragg reflections exhibited variations in both contraction and expansion as compared to the robust reflection (111), signifying the pronounced anisotropy inherent in nanocrystals. To gauge the average nanoparticle size, the Debye-Scherrer equation was employed, with measurements taken from the peak width of (111). Furthermore, supplementary peaks were identified, indicating the presence of AgO-coated nanoparticles on the surfaces[18].

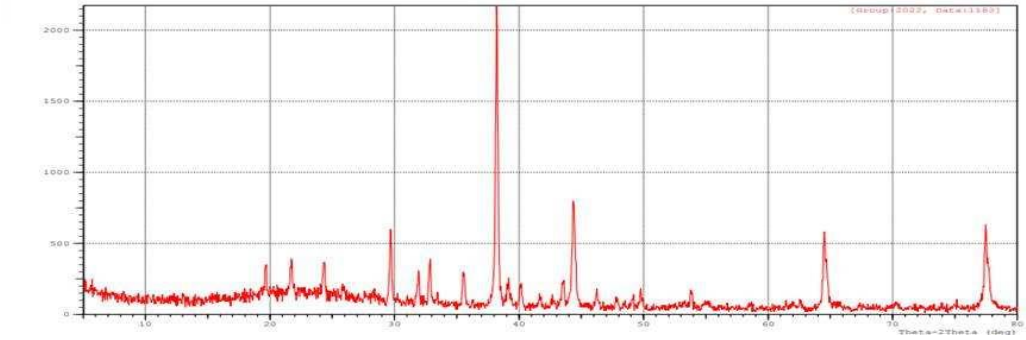

Figure (3): XRD examination of Ginkgo Biloba silver nanoparticle

3.4. FESEM analysis:

The morphology of the Ag "nanoparticles" surface (16) was studied using the FESEM. Figure (4) displayed FE-SEM images of the ready-made Ag samples. The FESEM pictures revealed the existence of nanoparticles with uneven distributions of spherical and cubic forms in various sizes. Due to the various nanoparticle sizes and solvent evaporation during sample preparation, the existence of nanoparticle aggregation was seen. The acquired results supported the validity of the extraction-based synthesis of silver nanoparticles (19), which is consistent with other research findings. It was discovered that the FESEM pictures' average nanoparticle size is between 50 and 62 nm [19].

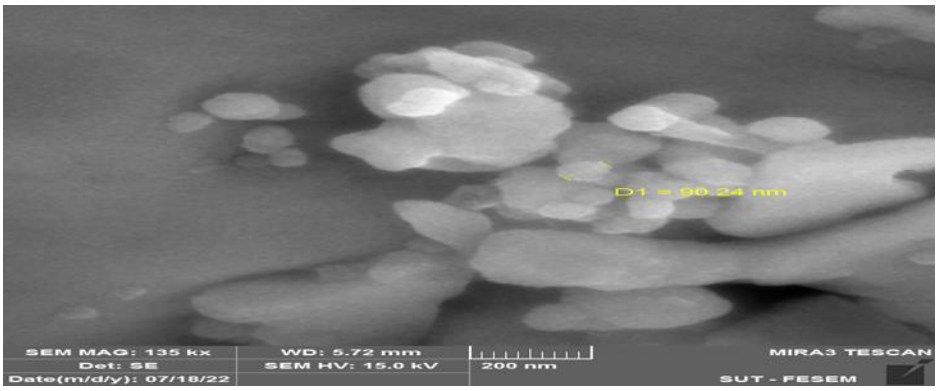

Figure (4): Analysis of Ginkgo Biloba silver nanoparticle using FESEM

Groups	No.	PC (nmol/l) Without add Mean± SD	P	PC (nmol/l) With bilobetein Mean± SD	P	PC (nmol/l) With Ginkgbiloba nanoparticles Mean± SD	P
Control (G1)	**30**	1.11 ±0.221		0.88 ± 0.28		0.715 ±0.19	
Small cell Lung Cancer (G2)	**30**	2.53 ± 0.84	1.46×10^{-12}	1.11 ± 0.122	0.000145	0.995 ±0.421	0.001555422
Adenocarcinma (G3)	**30**	6.39 ± 2.03	1.59×10^{-20}	2.155 ± 0.134	2.736×10^{-30}	1.51 ±0.0354	5.71589×10^{-30}
			1.149×10^{-13}		3.1696×10^{-38}		1.06471×10^{-08}

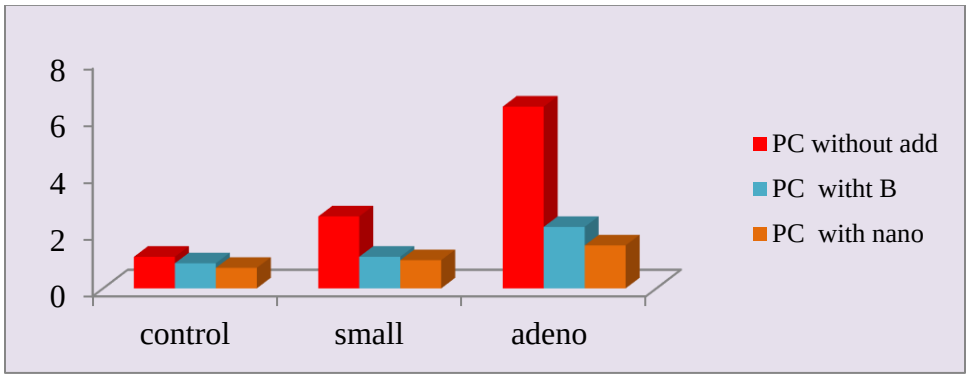

Fig.5: The Levels of PC in the plasma of the three study groups.

Table 1 displayed the (mean± SD) of the concentration of protein carbonyl (PC) in the serum of patients with small cell and adenocarcinoma lung cancer in comparison to the control groups, with and without the addition of a solution of bilobetin in concentrated form (8 ppm) and a solution of silver nanoparticles of Ginkgo biloba in concentrated form (4 ppm).

The PC concentration values in the patient groups G2 and G3 were substantially greater ($P \leq 0.05$) than those in the control group G1 with no addition, as shown in Table (1) and Figure (5).

Within patient groups G2 and G3, there was a noteworthy reduction ($P \leq 0.05$) observed in the levels of PC concentrations when compared to the control group G1. This decrease was observed particularly upon the administration of additional solutions of bilobetein (8 ppm) and silver nanoparticles (4 ppm).

These results align with previous research that has shown an elevated blood serum protein carbonyl level in Basal cell carcinoma compared to that of healthy control subjects [20].

These outcomes are in line with recent research that demonstrates a notable rise in serum protein carbonyl levels following exposure to low doses of nanosilver, particularly in the presence of ginkgo biloba silver nanoparticles, when contrasted with control groups [21].

Protein oxidation is predominantly initiated through processes facilitated by metal-catalyzed reactions. In the context of the Fenton reaction, metal ions, H_2O_2, and hydroxyl radicals collaborate to generate hydroxyl radicals, which then target adjacent amino acid residues. This oxidative environment prompts the breaking of peptide bonds, leading to a range of outcomes such as peptide cleavage or cross-linkages between proteins. The amino

acids methionine and cysteine, possessing heightened reactivity, are particularly susceptible to oxidative alterations due to their sulfur content. These amino acids' susceptibility is due to their sulfur content. Additionally, amino acids can swiftly undergo changes through interactions with ROS, stemming from their side chain interactions. Generally, plant extracts are recognized for their antioxidant effects owing to phenolic compounds, which not only serve as ROS scavengers but also as chelators for metal cations. These attributes position them as potential remedies for conditions characterized by ROS-related ailments [22].

The presence of an aromatic ring paired with a carbonyl molecule serves as a shield against protein oxidation. In the case of bilobetin, its polyphenolic nature is characterized by the inclusion of a hydroxyl group and aromatic rings, both of which play a crucial role in preventing carbonyl compound formation – a principal category of protein oxidation products. Polyphenols exert their impact by averting reactive oxygen species (ROS) production, reducing protein nitration and carbonylation, and augmenting thiol groups within proteins, thereby mitigating protein oxidation. The action of antioxidants revolves around scavenging free radicals, chelating metal ions, curtailing the generation of oxidative precursors such as ROS, and enhancing both the synthesis and effectiveness of antioxidant enzymes. The efficacy of phenolic compounds in interacting with sites of oxygenation is pivotal to their antioxidant potency, alongside their inherent structural attributes. Notably, phenolic compounds featuring a minimum of two adjacent hydroxyl and carbonyl groups have been substantiated to possess potent antioxidant properties [23]. AgNPs have the capability to induce oxidative stress, carbonylation, and DNA damage within cancer cells, leading to their demise. Furthermore, AgNPs exhibit the potential to regulate cancer cells by intervening in signaling pathways that curtail their uncontrolled proliferation. The observed reduction in tumor vasculature proliferation, migration, and invasive tendencies can be attributed to the enhanced permeability and retention (EPR) effect induced by AgNPs. This phenomenon contributes to the control of cancer cells by impeding their unregulated growth [24].

An excess of ROS within cells triggers the liberation of cytochrome c from the mitochondrial intermembrane space and triggers the activation of caspase-9. This enzyme is responsible for cleaving cellular proteins, culminating in cell death [25].

Activation of signaling complexes, initiated by ROS activation of TRAIL-R1/2 and TRAIL-R1, leads to the activation of effector caspases, eventually instigating apoptosis. Procaspases 8 and 10 are recruited to the cytoplasmic surface as a result. Moreover,

Groups	No.	RBP-1 (ng/ml) Without add Mean± SD	P	RBP-1 (ng/ml) With bilobetein Mean± SD	P	RBP-1 (ng/ml) With Ginkgbiloba nanoparticles Mean± SD	P
Control (G1)	30	2.72 ± 0.633		2.218 ±0.1984		2.074 ±0.1341	
Small cell Lung Cancer (G2)	30	2.547 ± 0.193	0.167645	2.276 ±0.2533	0.24583688	2.099 ± 0.0499	0.342571
Adenocarcinma (G3)	30	5.80 ± 0.573	1.768×10^{-27}	2.91±0.4587	3.068×10^{-10}	2.62 ± 0.14989	1.5213×10^{-21}
			1.293×10^{-36}		1.237×10^{-08}		1.3453×10^{-25}

Caspase-8 and Caspase-10 participate in the cleavage of Bid, generating truncated Bid (tBid), which subsequently relocates to mitochondria. There, tBid inhibits the anti-apoptotic functions of Bcl-2 and Bcl-XL while activating Bax and Bak. This sequential cascade sets in motion the apoptotic mitochondrial pathway. Thus, ROS serves as a catalyst for both the intrinsic and extrinsic apoptotic pathways. Notably, the nanocomplex aids in the restoration of damaged joints where intrinsic and extrinsic apoptosis has been provoked by altered separator macrophages [25,26].

Table 2: RBP-1 concentrations in the sera of the three study groups**.**

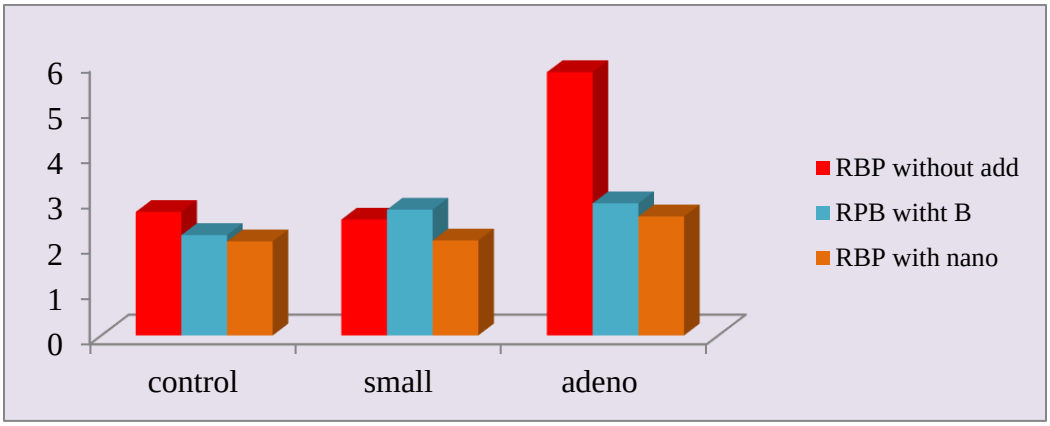

Fig.6: Retinol binding protein-1 (RBP-1) concentrations in the sera of the three study groups.

The concentration of retinol-binding protein-1 (RBP-1) in the blood of individuals afflicted with small cell and adenocarcinoma lung cancer was illustrated in Table 2. This comparison was drawn against control groups, both with and without the supplementation of a concentrated bilobetein solution (8ppm) and concentrated Ginkgo biloba silver nanoparticle solutions (4ppm).

Based on the data presented in Table 2 and Figure 6, it is evident that patient groups G2 and G3 exhibited notably elevated levels of retinol-binding protein-1 (RBP-1) concentration in comparison to the untreated control group G1 ($P \leq 0.05$).

These results align with recent discoveries indicating a substantial escalation in the susceptibility to hepatocellular carcinoma due to heightened cellular retinol-binding protein-1 levels [27,28].

Apigenin, a natural flavonoid found in various plants, has been documented to possess diverse biological attributes, notably including its anticancer properties. Apigenin can interact with and modify the expression and function of retinol-binding protein-1 (RBP1), leading to apoptosis induction and suppression of proliferation through RBP1 upregulation. This modulation profoundly influences the development and progression of lung cancer. The amplification of the p53 tumor suppressor pathway acts as an intermediary for this phenomenon [29,30], exerting a pivotal role in regulating cellular survival and

proliferation. Additionally, interactions with various signaling networks and transcription factors, including the NF-B signaling pathway, have been observed [31,32].

Van der Waals forces are the attractive interactions that occur between nonpolar molecules. Apigenin possesses carbonyl groups capable of engaging in van der Waals-style interactions with hydrophobic regions of retinol-binding protein (RBP). These interactions, arising from temporary shifts in electron distribution, lead to weak attractive forces between nonpolar groups. Through interactions with hydrophobic regions or structural modifications, apigenin can modulate the activity of RBP, thereby affecting its functionality [33]. The non-covalent interactions between the functional groups present on AgNPs and RBP-1 facilitate the binding of AgNPs to RBP-1. Electrostatic interactions occur between oppositely charged groups, such as the COOH group on AgNPs and positively charged amino acid residues on RBP-1. Additionally, hydrogen bonds form between specific functional groups on AgNPs, like COOH or NH2 groups, and complementary groups on RBP-1. Hydrophobic interactions take place between the hydrophobic regions of apigenin and the hydrophobic surface of RBP-1, contributing to the inhibition of cancer through these contacts. [34,35]. Much like Apigenin, the polyphenolic compound bilobetein could hinder the noncovalent interaction and reduce the expression of RBP-1 protein, potentially arresting the progression and dissemination of lung cancer. The interaction between RBP-1 and AgNPs might amplify the anticancer effectiveness of AgNPs by suppressing the PI3K/Akt/mTOR pathway and fostering apoptosis in cancerous cells [36-38].

4.Conclusion:

Research investigating the impact of Ginkgo biloba silver nanoparticles (GB-AgNPs) and bilobetein on the modulation of retinol binding protein (RBP) and protein carbonyl levels within the context of cancer has yielded promising outcomes. The study revealed that the application of GB-AgNPs and bilobetein resulted in a substantial reduction in the heightened expression of protein carbonyl and RBP. This indicates their potential as effective interventions capable of attenuating significant molecular markers associated with the initiation and advancement of lung cancer.

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