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Utilizing Beta-Glucan in Green Synthesis of Silver Nanoparticles for Safety and Cell Viability Testing

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Abstract

The study aimed to use barley-derived beta-glucan to prepare eco-friendly silver nanoparticles (AgNPs) via green synthesis. The barley variety "Abah 99" was used, yielding 78% and a concentration of 4.71%. Field effect scanning electron microscopy (FESEM) was used, revealing an irregular spherical shape with sizes ranging from 13.829 to 56.233 nm. Energy-dispersive X-ray (EDX) analysis showed the presence of Ag atoms at specific ratios in the sample. Zeta potential analysis confirmed the presence of negative charges on the green-synthesized particles (-18.7 mV). X-ray diffraction (XRD) analysis showed prominent peaks at 38.19, 44.37, 64.56, and 77.47, corresponding to the (111), (200), (220), and (311) planes, respectively. Furthermore, an MTT assay was conducted on HEK-293 cells (a type of human embryonic kidney cell) exposed to green-synthesized beta-glucan-integrated silver nanoparticles. Cell viability decreased in a dose-dependent manner following AgNPs treatment. However, treatment with Ag-beta-glucan green synthesis resulted in very high cell viability (approximately 100%) compared to the chemically synthesized silver nanoparticles at concentrations of 3.125, 6.25, and 12.5 µg/mL. The study demonstrated that cell viability decreased with higher concentrations, with the chemically synthesized AgNPs showing a notable reduction in cell viability, especially at concentrations of 25, 50, and 100 µg/mL. In contrast, the green synthesis of AgNPs-beta-glucan showed decreased cell viability only at 50 and 100 µg/mL, while the extract group maintained better cell survival at a concentration of 100 µg/mL.

Article Info.

Keywords:
AgNps, Characteristics, Beta-Glucan, Green Synthesis, Anticancer Activity.

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1. Introduction

Nanoparticles (NPs) and nanostructured materials (NSMs) have become a rapidly expanding and extensively studied topic within the techno-economic sector, with diverse applications across various fields. NPs and NSMs have gained great popularity due to their ability to modify their physicochemical properties. United States Food and Drug Administration (USFDA) and Environmental Protection Agency (EPA) have distinct definitions of nanometers (NMs). The USFDA specifies that NMs might have a minimum size within the range of 1-100 nm and have characteristics that vary with dimension [1]. Nevertheless, some legislation in the United States and the European Union (EU) expressly refers to non-national instruments (Nms), such as melting point, wettability, electrical and thermal conductivity, catalytic activity, light absorption, and scattering. A nanometer is a unit of measurement in the International System of Units (SI) with a length of 10^{-9} meters. Nanoparticles are often defined as existing within the size range of 1 to 100 nm. NMs are materials having nanoscale dimensions, whether external or internal, including nanofibers, nanoplates, nanowires, quantum dots, and other related words as defined by the International Organization for Standardization (ISO) [2].

Nanomaterials have seen explosive growth in their use in medicine, particularly in medication delivery, throughout the last several decades. Nevertheless, some nanomaterials may also have harmful consequences, with the primary one being their toxicity towards people and animals. Specifically, it has been shown that cadmium telluride (CdTe) quantum

dots substantially impact the characteristics of the mitochondrial membrane in a rat model [3]. Zinc oxide (ZnO) nanoparticles have been shown to induce hepatic impairment [4]. Dose-dependent silver nanoparticles (AgNPs) toxicity has been demonstrated in zebrafish embryos [5]. This has prompted researchers to devise methods of producing nanomaterials devoid of toxicity. Nevertheless, the waste generated while producing nanomaterials might pose an environmental risk. An approach to mitigate the environmental consequences of nanomaterial production is to adhere to the core concepts of green chemistry, as elaborated on elsewhere [6]. In this work, beta glucan (β-glucan), a widely available and chemically harmless polysaccharide, was used to synthesize AgNPs, which is an ecologically friendly approach. The biological response modifier function of β-glucan is well-established, it augments the host's immunological defence. Furthermore, it controls the levels of blood cholesterol [7-10]. Chemical reduction is the usual technique for synthesizing AgNPs as stable colloidal dispersions in water or organic solvents. Prior investigations have shown that triple-helix polysaccharides may serve as protective agents for stabilizing AgNPs. Therefore, β-glucan can function as both a reducing and a stabilizing agent [11–17].

Cancer is a multifaceted health condition characterized by the unregulated proliferation and dissemination of aberrant cells caused by a confluence of genetic, extrinsic, endogenous, and environmental elements [18]. Treatment of cancer includes therapies like chemotherapy, surgery, radiation, and immunotherapy. Despite research progress, challenges persist, such as side effects, limited specificity, and drug resistance. Nanomedicine is emerging as a promising field, using nanoparticles for better tumor detection, diagnosis, and personalized therapy. These nanoparticles, which work at the molecular level, offer significant advantages in precision medicine. They can be categorized into polymeric, metallic, magnetic, carbon nanotubes, liposomes, dendrimers, and quantum dots [19]. More recently, several nanoparticles, such as carbon nanotubes [20], nanoparticles with paramagnetic properties [21], liposomes [22], nanoparticles of gold [23], and several more [24], have been investigated for cancer diagnosis and treatment. In cancer therapy combinations, nanoparticles can potentially augment the pharmacokinetic and pharmacodynamic properties of existing anticancer medications [25,26]. In this context, certain metallic nanoparticles have shown substantial antitumoral activity against human cancer cells [27]. Due to their intriguing physical-chemical features, AgNPs are gaining increasing attention in cancer research, showing inherent antiproliferative action [28]. AgNPs were investigated for the development of an advanced cancer diagnosis and treatment [29]. Stable AgNPs may be synthesised easily and economically via a "green" synthesis approach. Silver, a noble metal, has favorable biological attributes, including antibacterial and antifungal. Recent studies have shown that different silver compounds have diverse effects on cancer cells [30]. Silver has low toxicity and limited absorption due to the body's detoxification mechanisms. However, AgNPs can efficiently bypass this issue as cells internalize them through endocytosis, releasing silver ions (Ag^+) at targeted sites [31]. AgNPs inhibit tumor cell growth and induce cell death while sparing healthy tissue. Unlike traditional treatments, which disrupt the cell cycle and suppress division, causing side effects, AgNPs offer a more targeted anticancer effect [32]. Still, certain cancerous growth forms resist this therapy [33]. Due to their distinctive physicochemical characteristics, AgNPs have emerged as a promising novel approach in cancer therapy. The enhanced bioavailability of drugs obtained by targeted drug delivery directly to cancer cells may enhance the efficacy of anticancer treatment and reduce adverse effects [34]. Silver nanoparticles and their conjugates with anticancer drugs induce reactive oxygen species (ROS), oxidative stress, DNA damage, cell cycle disruption, and tumor cell death through apoptosis and non-apoptotic mechanisms [35-37].

This study aims to evaluate the ability of beta-glucan extracted from natural sources to produce AgNPs using environmentally friendly methods (green synthesis), to examine the effect of green-synthesized nanoparticles on the viability of human cells (HEK-293) when exposed to different concentrations of AgNPs incorporated with beta-glucan, and to assess cell viability by measuring cytotoxicity when exposed to green-synthesized nanoparticles compared to chemically-synthesized nanoparticles at various concentrations.

2.Materials and Methodology

2.1. Materials

The materials used in this study were distilled water prepared in the laboratory; silver nitrate ($AgNO_3$) acquired from BDH England; trisodium citrate ($Na_3C_6H_5O_7$) purchased from CTS; 20% sodium carbonate (Na_2CO_3) obtained from CDH India; hydrochloric acid 2M (HCl) purchased from Merck, Germany; and ethyl alcohol (C_2H_6O) (99%) obtained from CHEM-LAB, Belgium.

2.2. Silver Nanoparticle Synthesis

AgNPs were synthesized using the chemical reduction technique proposed by Vigneshwaran et al. [29]. All the components of the reactive materials were dissolved in double-distilled water. For the standard experiment, 50 mL of a solution containing 1×10^{-3} M $AgNO_3$ was heated to boiling. 5 mL of a 1% trisodium citrate solution ($Na_3C_6H_5O_7$) was added dropwise to this initial solution. The solution was aggressively mixed and heated until an evident change in color (light brown) occurred. Ultimately, it was disengaged from the heating source and agitated until it reached ambient temperature (Fig. 1).

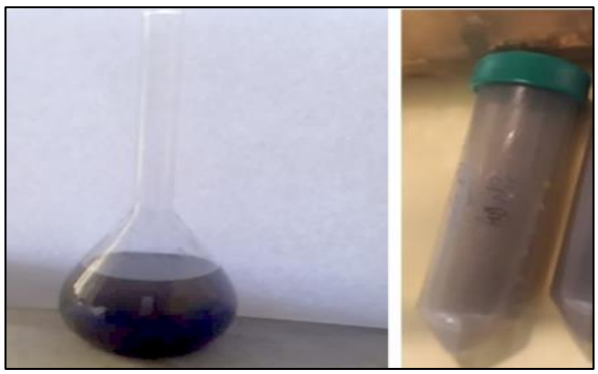

Figure 1: The Chemical synthesis of silver nanoparticles.

2.3. Preparation of β-glucan

Beta-glucan derived from grains was obtained according to the method described by Din et al. [30]. 50 g of whole barley flour was combined with 500 mL of distilled water, then buffered to pH 7 by adding a 20% solution of Na_2CO_3. The resultant mixture was subjected to a 30-minute heat treatment at 55°C. Following a 15-minute centrifugation at 4°C at 15,000 x g, the sediment was discarded, and the eluent was recovered. The pH was fine-tuned to 4.5 by adding a 2M hydrochloric acid solution to induce protein precipitation. Next, the liquid was separated by centrifugation at 12,000 x g for 30 minutes at 4°C, while the precipitate was discarded. In a 1:1 ratio (H:H), ethyl alcohol (C_2H_6O) (99^+) was introduced to the mixture, which was then allowed to stand at 4°C for 12 hours. The precipitate was separated by centrifugation at 3,000 x g for 15 minutes at 4°C. It was then dried in a lyophilizer at -50°C for 4 hours, and the beta-glucan ratio was quantified. Beta-glucan concentration was determined according to the method described by Hughes et al. [31] using a High-Performance Liquid Chromatography (HPLC) system (Shimadzu Corporation, Japan). A standard solution was prepared with a concentration of 30 mg/mL, and

then 20 µL of the standard solution was injected into the HPLC system using a C18 Lichrospher column (50mm, 4.6mm) with a particle size of 3 µm. The mobile phase consisted of distilled water with a flow rate of 1.2 mL/min at 30°C, and detection was carried out using a Shimadzu Refractive Index Detector (RF). Separation was performed using a Shimadzu 10AV-LC liquid chromatography system equipped with an LC-10A dual pump (Shimadzu). Peaks were identified using a UV-Vis A-SPD spectrophotometer. To estimate the beta-glucan concentration in the sample, one gram of the extract was dissolved in 25 mL of distilled water, and the same procedure as for the standard solution was followed. The concentration of beta-glucan in the sample was calculated using the following equation

Sample concentration (mg/g) = (Dilution inverse×Standard concentration×Sample peak area\ Standard peak area).

Finally, the resulting powder was stored at a refrigeration temperature of around ±5°C (Fig. 2).

Figure 2: Preparation of Beta-Glucan extract.

2. 4. Green Synthesis of the AgNPs using β-Glucan

The green-synthesized silver nanoparticle colloidal solution was prepared utilizing a previously known polysaccharide technique [41]. β-Glucan was heated in an oil bath at a temperature range of 130–140ºC for about 30 minutes with continuous stirring by magnetic rotation. The β-glucan was promptly combined with $AgNO_3$ [33]. The mixture was further heated at 90ºC. Following a 5-minute mixing period at this temperature, the pH of the chosen solutions was modified to the target level by adding 0.1M NaOH while stirring continuously. The whole process was carried out in the absence of illumination. The solution mixture was autoclaved at 121ºC and 15 pressure for 20 minutes, followed by cooling to ambient temperature (Fig. 3).

Figure 3: The green production of AgNPs using β-Glucan extract.

3. Results and Discussion

X-ray diffraction (XRD) is used to characterize the nanoparticles produced by the green method [34] to verify the elemental composition as silver and to determine the structural details. Fig. 4 displays the XRD pattern of AgNPs. The pattern shows prominent peaks at 2θ of 38.19 °, 44.37 °, 64.56°, and 77.47°, corresponding to the (111), (200), (220), and (311) vibrational planes, respectively. The analysis of JCPDS (card number 89-3722) reveals that the characteristic pattern of green-synthesized AgNPs has a face-centered cubic (FCC) structure. In a study by Khan et al. [35], bioactive silver-coated nanostructures were prepared using embedded beta-glucan (β-glucan) and nano-hydroxyapatite. The XRD pattern analysis revealed the organic and inorganic phases in bioactive nanostructures. A low density of organic materials (β-glucan and acrylic acid) was observed at around 26°, accompanied by a decrease in crystallinity due to hydrogen-bond formation and chemical interactions between β-glucan and acrylic acid. The presence of nano-hydroxyapatite (n-HAp) in the polymer matrix resulted in an amorphous behavior. Sharp peaks for HAp were observed at 2θ angles such as 25.3°, 31.4°, and others, while the peak at 39.69° indicated the presence of Ag coating.

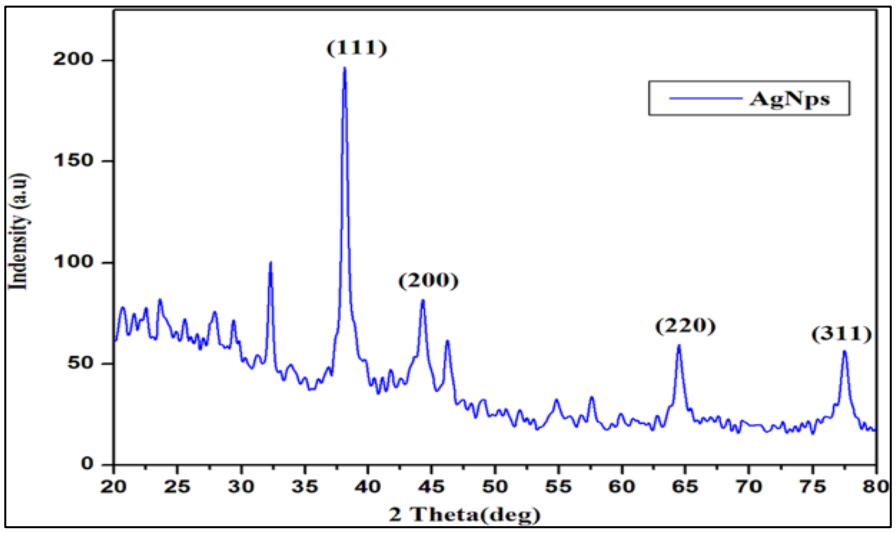

Figure 4: XRD pattern of the synthesized AgNPs.

Field effect scanning electron microscopy (FESEM) images showed the size and shape of the AgNPs. The particles appeared spherical, hexagonal, or irregularly shaped. Green synthesis using beta-glucan resulted in more uniform shapes and sizes due to the controlled reduction process. The images in Fig. 5 depicted how uniformly the nanoparticles were distributed. A successful green synthesis process would ideally yield well-dispersed nanoparticles without agglomeration. The FESEM images might reveal surface modifications or nanoparticle coatings due to beta-glucan. This could show as a thin layer or changes in the surface texture compared to chemically synthesized AgNPs. FESEM also gave insights into the crystalline structure of the AgNPs. The presence of distinct crystal facets or orientations indicates the crystallinity level achieved during the synthesis process. The FESEM characterization of AgNPs synthesized using beta-glucan was aimed to evaluate the quality, size, shape, distribution, and surface characteristics of the nanoparticles and compare them to those synthesized using chemical methods. The emphasis would be on understanding the advantages and potential applications of green synthesis using natural compounds like beta-glucan in producing nanoparticles with desirable properties for various purposes, including biomedical, environmental, or industrial applications. The mean

dimensions of AgNPs varied between 50 and 121 nm in diameter. The nanoparticles exhibited excellent uniformity and had an average diameter of 81.02 nm. The majority of the particles were spherical, uniform, and did not exhibit significant agglomeration. In a study by Pino et al. [36], on antibacterial β-glucan/zinc oxide nanocomposite films for wound healing, the morphology of the nanoparticles was examined using FESEM to determine the particle size distribution. The results showed that the ZnONPs had a spherical shape with a size of approximately 32 nm. The specific surface area was also determined, which was around 50 m^2/g.

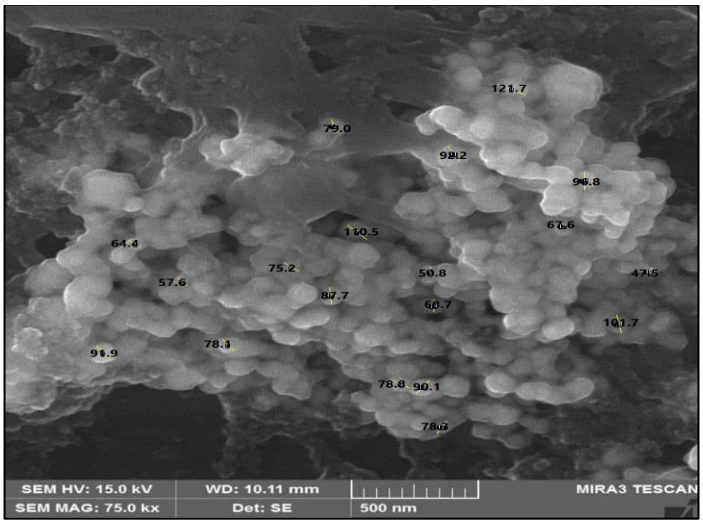

Figure 5: FESEM image of the synthesized AgNPs.

An energy-dispersive X-ray (EDX) spectrometer scrutinized the constitution and clarity of AgNPs created through eco-friendly means. Within the EDX spectrum, distinct signals of silver particles emerged around 3 keV, unveiling an even spread of these particles (Fig. 6A). Consistent with previous findings, the EDX pattern showed a peak absorption at approximately 3 keV. The analysis of the elemental composition of the commercially produced AgNPs (as seen in Fig. 6B) identified the sample with the greatest concentration of silver components. These results are consistent with those of Al-Khafaji [37], which showed that the nano solution of rosemary extract recorded a sharp peak at 3 keV in the EDX spectrum. EDX analysis also revealed the presence of several elements other than silver Ag, such as oxygen (O) at about 0.5 keV, and silicon (Si) at about 1.7 keV.

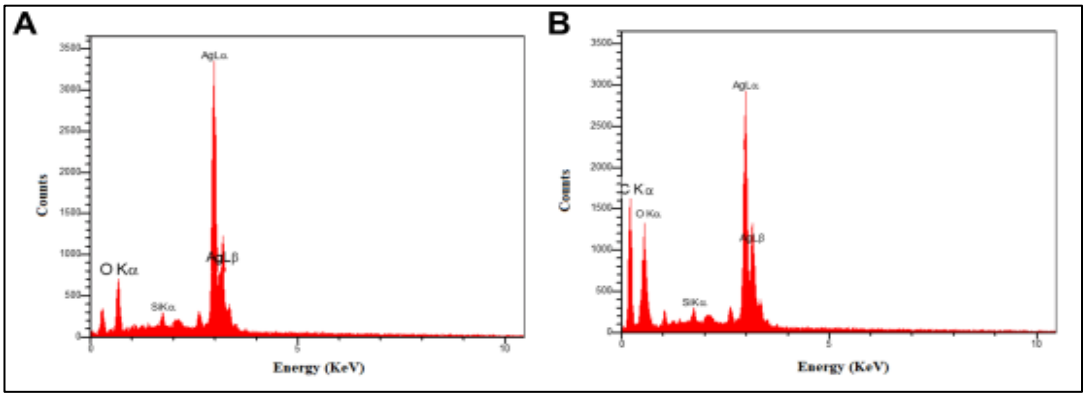

Figure 6: EDX assay of (A) Chemically-synthesized AgNPs. (B) Green-synthesized AgNPs.

ZETA potential analyses in the chemical synthesis of AgNPs, especially those produced using beta-glucan extract in green synthesis, were expected to reveal distinctive characteristics. For ZETA potential, the synthesized AgNPs utilizing beta-glucan extract were anticipated to display altered surface charges compared to chemically synthesized AgNPs (Fig. 7A and B). The presence of biological molecules from the beta-glucan extract might have influenced the surface charge of the nanoparticles, potentially leading to a different ZETA potential value [38]. This could have indicated enhanced stability or other interactions with surrounding environments. In a study by Ashraf et al. [39], the nanoreduction technique was used as a means to utilize beta-glucan extracted from cereals and fungal sources, where the zeta potential in this study ranged between -27 and -6.3 millivolts.

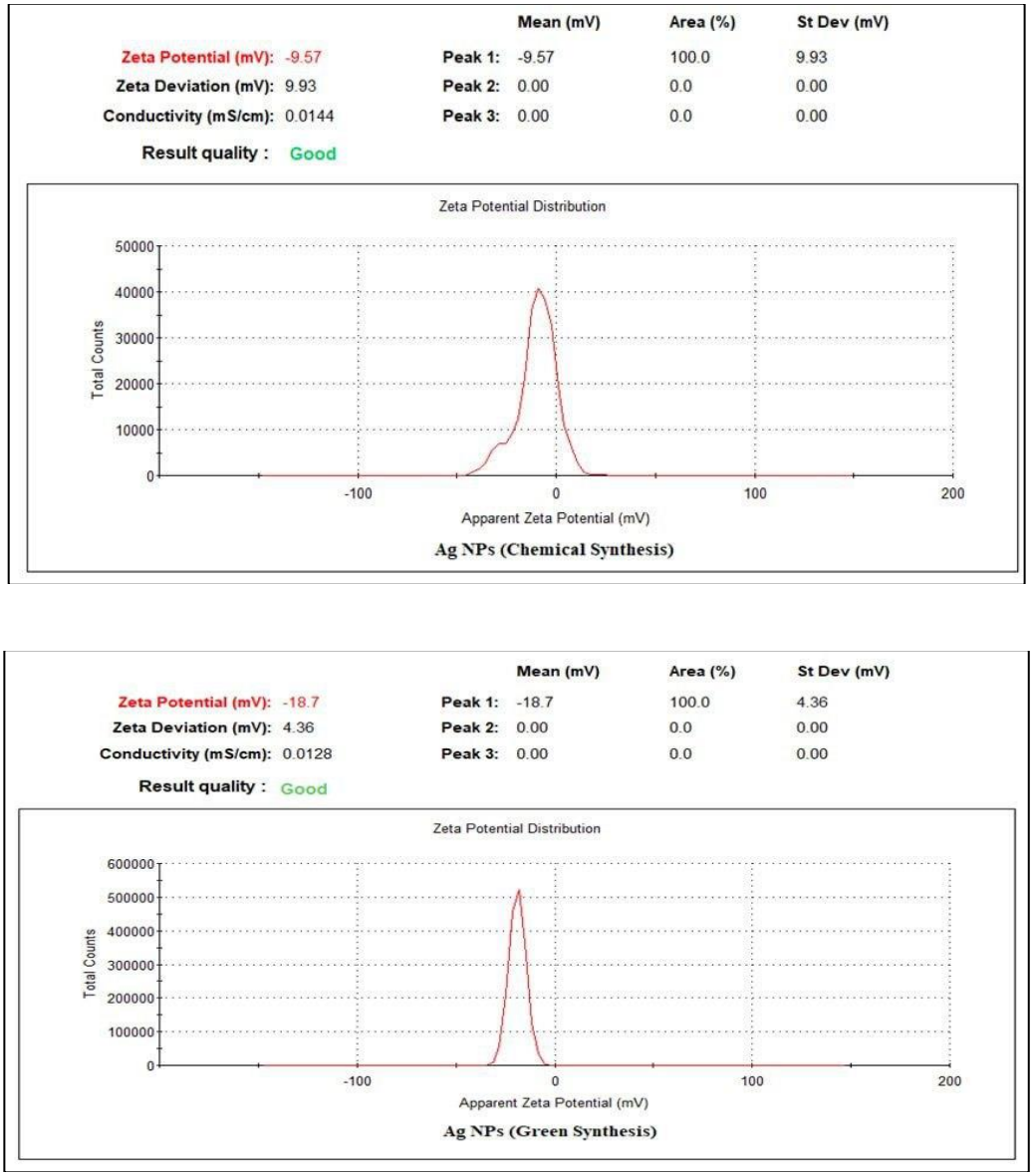

Figure 7: Zeta potential analysis of (A) Chemically-synthesized AgNPs ; (B) Green-synthesized AgNPs

Fig. 8 illustrates the MTT Assay, which measures the MTT activities of human embryonic kidney (HEK-293) cells exposed to green nanocomposite particles supplemented with beta-glucan. According to the ISO 10993-5 standards, the interpretation of cell viability

data is as follows: A value above 80% shows the absence of cytotoxicity, 80-60% represents mild cytotoxicity, 60-40% represents moderate cytotoxicity, and less than 40% represents significant cytotoxicity [40]. When exposed to chemically synthesized Ag nanoparticles, cell viability declined in a way that was dependent on the dosage and the active ingredient. Consequently, treatment with green-synthesized Ag NPs using beta-glucan and beta-glucan extract exhibited almost very high cell viability (approximately 100%) compared to the chemically-synthesized AgNPs at concentrations (3.125, 6.25, 12.5 µg/mL). Cell viability dropped below 80% and even below 70% when treated with the green-synthesized Ag-beta glucan at a concentration of 25µg/mL. While treating with the chemically-synthesized AgNPs at the same concentration, cell vitality was maintained at a high rate. However, the beta-glucan extract group maintained cell viability at rates close to 95% at the same concentration of 25µ/mL. At higher concentrations of 50 and 100µg/mL, cell viability significantly dropped below 40% when treated with chemically-synthesized Ag nanoparticles, approximately 60% for green-synthesized AgNPs, and between 70 and 80% for the botanical extract groups. The study revealed that cell viability decreases with higher concentrations, which is consistent with what was reported by Gabriel et al. [41]. Despite the effectiveness shown by the (AgNPs, green-synthesized AgNPs, and beta-glucan extract) compounds in maintaining cell viability, the study revealed that cell viability decreased with higher concentrations. Specifically, the chemically-synthesized AgNPs group exhibited reduced cell viability, particularly at dosages of 25, 50, and 100 µg/mL. Subsequently, the group produced via green synthesis (AgNPs-beta-glucan) exhibited reduced cell viability at concentrations of 50 and 100 µg/mL. In contrast, the group treated with the extract had superior cell survival even at a high dose of 100 µg/mL.

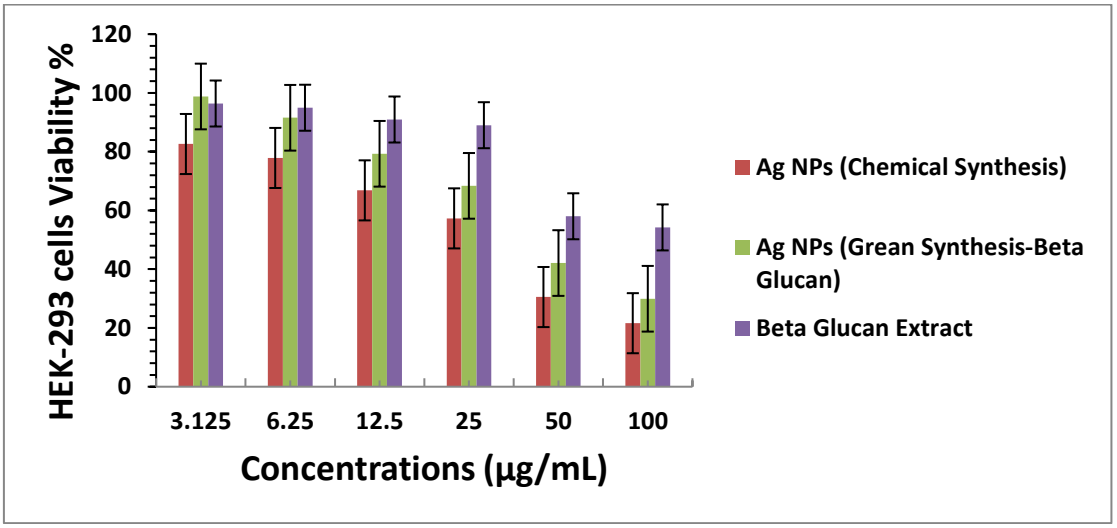

Figure 8: MTT assay of chemically and green synthesized AgNPs using β-glucan and phenolic compounds extract.

4. Conclusions

The comprehensive evaluation of AgNPs synthesized through chemical and green synthesis using beta-glucan unveils critical insights into their structural, biological, and therapeutic implications. FESEM characterization offers a detailed glimpse into the morphology and distribution of nanoparticles. The green synthesis method provides better control over nanoparticle shape and size, resulting in more uniform, well-dispersed nanoparticles. The presence of beta-glucan might contribute to surface modifications, as evidenced by subtle textural changes. The EDX assay further verifies the constitution of the

eco-friendly synthesized AgNPs, showcasing the dominance of silver elements. Elemental mapping pinpoints the highest silver concentration in the samples, validating the synthesis process's purity. Zeta potential analyses show that the beta-glucan extract alters particle surface charge and size. Variations in ZETA potential and broader size distributions underscore the impact of natural extracts on the synthesis process, potentially enhancing stability or altering interactions in diverse environments. MTT assays highlight cell viability trends. While both the chemically and green synthesized AgNPs showed promising cell viability, the green synthesis with beta-glucan extract exhibits higher viability at lower concentrations, emphasizing its potential for reduced cytotoxicity.

Conflict of Interest

Authors declare that they have no conflict of interest.

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استخدام البيتاكلوكان في تخليق جسيمات الفضة النانوية الخضراء لاختبار بقاء وسلامة الخلية

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الخلاصة

هدفت هذه الدراسة إلى استخدام بيتا جلوكان المستخلص من الشعير لتحضير جسيمات نانوية فضية صديقة للبيئة (AgNPs) عبر عملية تصنيع خضراء. استُخدم صنف الشعير "أباه 99"، بنسبة إنتاج 78% وتركيز 4.71%. أظهر الفحص المجهري الإلكتروني الماسح ذو التأثير الحقلي (FESEM) شكلاً كروياً غير منتظم بأحجام تتراوح بين 13.829 و56.233 نانومتر. وأظهر تحليل حيود الأشعة السينية المشتتة للطاقة (EDX) وجود ذرات الفضة بنسب محددة في العينة. وأكد تحليل جهد زيتا وجود شحنات سالبة على الجسيمات المصنعة بطريقة خضراء (-18.7 ملي فولت). وأظهر تحليل حيود الأشعة السينية (XRD) قممًا بارزة عند 38.19 و44.37 و64.56 و77.47، والتي تُقابل المستويات (111) و(200) و(220) و(311) على التوالي. علاوة على ذلك، أُجري اختبار MTT على خلايا HEK-293 (نوع من خلايا الكلى الجنينية البشرية) المعرضة لجزيئات نانوية من الفضة مُدمجة مع بيتا جلوكان مُصنّعة بطريقة صديقة للبيئة. انخفضت حيوية الخلايا بشكل متناسب مع الجرعة بعد المعالجة بجزيئات الفضة النانوية. ومع ذلك، أسفرت المعالجة بجزيئات الفضة المُدمجة مع بيتا جلوكان المُصنّعة بطريقة صديقة للبيئة عن حيوية عالية جدًا للخلايا (حوالي 100%) مقارنةً بجزيئات الفضة النانوية المُصنّعة كيميائيًا عند تركيزات 3.125 و6.25 و12.5 ميكروغرام/مل. أظهرت الدراسة أن حيوية الخلايا تنخفض مع زيادة التركيز، حيث أظهرت جزيئات الفضة النانوية المُصنّعة كيميائيًا انخفاضًا ملحوظًا في حيوية الخلايا، خاصةً عند تركيزات 25 و50 و100 ميكروغرام/مل. في المقابل، أظهر التخليق الأخضر لـ AgNPs-beta-glucan انخفاضًا في حيوية الخلايا فقط عند 50 و 100 ميكروغرام/مل، بينما حافظت مجموعة المستخلص على بقاء أفضل للخلايا عند تركيز 100 ميكروغرام/مل.

الكلمات المفتاحية: بيتا جلوكان، جسيمات الفضة النانوية، التوليف الاخضر، مضاد للسرطان.