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FACIAL APPROACH FOR THE SYNTHESIS OF ZINC OXIDE/ BOSWELLIA GUM RESIN NANOPARTICLES

Mervat Magdy Shahen[a]*, Nadia Mohammed Ahmed [b], Emad Tolba [c], Omali Youssef El-Khawaga[a]

[a] *Chemistry Department, Faculty of Science, Mansoura University, Mansoura, 35516, Egypt*
[b] *Medical Biochemistry Dept, Medical researches and clinic studies institute, Dokki, Cairo, 12311, Egypt*
[c] *Polymers and Pigments Department, National Research Centre, 33 El Bohouth St, Dokki, Cairo, 12311, Egypt*

Abstract

Research on the synthesis of multifunctional metal-based nanoparticles using natural extracts is expanding quickly in the biomedical field. Zinc oxide nanoparticles (ZnO-NPs) are well-known bioactive inorganic nanoparticles that have been extensively investigated in regenerative medicine. Herein, ZnO-NPs were prepared using water soluble zinc chloride salt with sodium hydroxide in the presence of Triethanolamine (TEA), as a surfactant agent. ZnO-NPs were functionalized with the Oleo gum resin (BSE) using an in-situ loading approach. The morphology and structure of the as-prepared materials were characterized using TEM, XRD, and Ƶ-potential & DLS. In addition, the cell viability of normal human skin fibroblasts (HSF) after its exposure to varying concentrations of the as-prepared ZnO-NPs and BSE-loaded ZnO-NPs. Although, the XRD data indicates the formation of Zincite phase in the presence/absence of BSE. The TEM results show that ZnO-NPs changed from a rod-like structure with an average particle size 172±29nm in width and 40±12nm in length to an irregular spherical NPs with an average particle size 49±8nm for 10BSE-ZnO-NPs and 15±3nm for 20BSE-ZnO-NPs. The MTT test proves that 20BSE-ZnO-NPs enhance cell growth and show reduced toxicity against HSF cells compared to 10BSE-ZnO-NPs. the obtained results suggest that the ZNO-NPs containing BSE provide a better stimulatory response against Human fibroblast cells, which confirm that the BSE loaded-ZnO-NPs may be utilized in skin tissue healing application.

Keywords*: Zinc oxide nanoparticles (ZnO-NPs); green synthesis; glycerol; Triethanolamine (TEA); Oleo gum resin (BSE); characterization; cytotoxicity.*

1. Introduction

Nanotechnology is a revolutionary way to manipulate atoms, molecules or molecular clusters into novel materials at the nanoscale (one billion times smaller than a metre) **[1]**. Nanoparticles with size ranging from 1 to 100 nm are known as nanoparticles (NPs). Based on their features, forms or size; they can be divided into several classes such as: Metal NPs, ceramic NPs, polymeric NPs and hybrid/composite NPs. Because of their large surface area and nanostructures; NPs have special physical and chemical characteristics **[2, 3]**. Ceramic or metal oxide NPs offer a wide range of applications in medicine and other related applications due to their unique physical, chemical and biological properties **[4, 5]**. Among them, zinc oxide particles (ZnO) are generally recognized by the US Food and Drug Administration (FDA) as a highly safe synthetic food additive material with less risk and considered more useful than traditional food additives **[6, 7]**.

Because of their unique physical and chemical properties, ZnO particles are one of the inexpensive and readily available metal oxide materials with high catalytic and photochemical activities. Nanosized ZnO particles are known to be applied in several biomedical sectors due to their biocompatibility, low toxicity, bioactivity, anti-bacterial potential and anti-inflammatory activity. In addition, Numerous recent studies have demonstrated that ZnO nanostructures may effectively encourage the growth, proliferation and differentiation of many cell types in order to support cell functions throughout the regeneration

*Corresponding author e-mail: Mervat25@yahoo.com .; (Mervat M. Shahen)
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process and facilitate the formation of new tissue **[8-12]**. In the recent past years, ZnO-NPs have garnered significant attention in skin tissue healing due to their novel low-cost, intrinsic biocompatibility, antibacterial, antioxidant, antidiabetic, and anti-inflammatory properties **[11, 13, 14]**. ZnO NPs can also promote numerous biological activities including cell migration, re-epithelialization, and angiogenesis.

Therefore, ZnO-NPs have been designed, synthesized and investigated for the design of multifunctional wound dressing materials, either by the antimicrobial properties or their antioxidant activities. These qualities are all very relevant to skin tissue regeneration and healing **[15-17]**. Indeed, much research has been done on wound healing in an effort to speed up healing process and minimize scarring. Unfortunately, the healing potential of the current clinical options are not enough to aid in the healing of chronic wound or burns. With the current development in nanomedicine, functional ZnO-NPs offer a novel clinical application in the treatment of impaired, infected, diabetic wound.

In general, the physical and chemical features of metal oxide nanoparticle can be easily controlled for a given application by changing the synthesis route. Metal oxides can be made in a number of various synthesis rouetes, including chemical, physical, and biological methods **[13, 18, 19].** The biological "green" synthesis of NPs is a more promising and distinct method from chemical and physical synthesis.

Metal nanoparticles could be made safely and effectively, without the use of dangerous materials, by using safe reagents like: water and natural extracts. The biosynthesis encompasses two steps: collection, cleaning, drying, pulverization and dissolution of plant components to obtain its extract is the first step, where, the growth of ZnO-NPs precipitate from the plant extract is the second step **[20-22]**. As a result, stable and well dispersed ZnO-NPs can be produced by active components found in plant extracts **[23]**. Many studies successfully synthesis ZnO-NPs from natural source using green synthesis are performed, for example: Osuntokun et al., used the aqueous extract of broccoli **[24]**, Obeizi et al., used *Eucalyptus globulus* essential oil **[25]** , Dhayalan et al., used *Aegle marmelos* L. fruit extract **[26]**, Xu et al., used the Moringa oleifera extract **[27]**, Salem et al., used *Solanum rantonnetii* leaves aqueous extract **[28]**, Alahdal et al., used leaf extract of *P. austroarabica* **[22]** and recently, Manimegalai et al., used *Hardwickia binata* leaves extract for the production of ZnO-NPs **[29]**.

Boswellia Serrata, also called Indian olibaum, is a branching tree, used as Indian frankincense, found in India, Middle East and Northern Africa **[30]**. It is traditionally used in Ayurvedic medicine owing to its diuretic, stimulant, expectorant, anti-inflammatory, astringent, antipyretic and antiseptic properties which making it important to cure ulcer, goitre, piles and diarrhoea **[31, 32].** Boswellia Serrata resin is composed of (5-9%) essential oil, (21-22%) mucopolysaccharides and (65-85%) pure resin containing monoterpenes, diterpenes, triterpenes, tetracyclic. Also, the most notable components are; a natural mixture of pentacyclic triterpene acids called boswellic acids (BAs) **[33, 34]**. Among them; the major acids identified are beta-boswellic acid, 3-acetyl-β-boswellic acid, 11-keto β-boswellic acid (KBA) and 3-acetyl-11-keto β-boswellic acid (AKBA). These components have demonstrated a therapeutic potential against microbial, hormonal, gastrointestinal, and inflammatory illnesses. They are the most significant bioactive compounds utilized in pharmaceutical formulations and the widely used ingredients in complementary and alternative medicine systems. **[32, 35-37]**.

This paper demonstrates the green synthesis of ZnO-NPs using sodium hydroxide (NaOH) and Triethanolamine (TEA); regarded as a viscous organic compound that is a tertiary amine in the presence of the basic solution. Besides, the paper discusses the effect of loading BSE with different concentrations on the biosynthesised ZnO-NPs characteristic and morphology. Moreover, the in-vitro cytotoxic effect of different prepared formulas on normal human skin fibroblast cells (HSF) has been estimated by the conventional MTT reduction assay.

2. Experimental

a) Extraction process of Oleo gum resin (BSE):

Prior to extraction; Boswellia serrata were grinded and sieved using a 250 µm sieve. Briefly, finely powdered resin (10gm) was added to 200 ml of 96% ethanol solution. The mixture was then kept under slow string at room temperature for 24 h in the dark condition before filtration using Whatman Grade 50 Filter Paper. To assess the extraction yield (%). The obtained BSE was collected and stored at 4 °C for further studies.

b) Encapsulation of Oleo-gum-resin loaded zinc oxide nanoparticles (BSE-ZnO-NPs):

ZnO-NPs were synthesized according to previously reported method **[38]** with a slight modifications. In a typical synthesis, 0.6 M $Zncl_2$ solution was prepared by dissolving 1.7 gm of $Zncl_2$ salt in 20 ml of distilled water. The solution was stirred for 10 min, and then glycerol (gly) added to the solution at gly/Zn^{2+} molar ratio of 1:5, then TEA was added to the solution at TEA/gly molar ratio of 1:2. After that, about 2ml of NaOH solution (6 M) was added to the mixture till reaching pH~12 with

continuous stirring. The white precipitate was collected via centrifugation of the mixture at about 5000 rpm for 10 min at room temperature and washed three times with distilled water to remove unreacted regents. Subsequently, the wet precipitates were air dried at 50°C.

For BSE-ZnO-NPs: The loading of BSE was performed via an in situ -loading approach. In brief, 10ml or 20ml of BSE was added to 0.6 M $Zncl_2$ solution previously stirred with 6ml glycerol then 4ml of TEA added. After that, about 2ml of (6 M) NaOH solution was added to the mixture till reaching pH~12 with continuous stirring. The BSE-ZnO-NPs mixtures were centrifuged at 5,000 rpm for 5 min. At the end, the collected BSE-ZnO-NPs were dispersed in water and collected again via centrifugation at 5000 rpm for 5 min. This process was repeated three times to ensure the removal of free BSE, and subsequently dried at 50 °C.

c) Microstructure Characterization:

To confirm the formation of the ZnO-NPs; X-ray diffraction (XRD) was performed to determine the crystal structure of the as-prepared materials. Also, Transmission electron microscopy (TEM) performed to study the particle size of the material in nano scale & crystal structure and examine the morphological characteristics of nanoparticles formulas by a high-performance digital imaging transmission electron microscopy (JEOLH-7650, Hitachi High-Technologies Corp., Tokyo, Japan) with an acceleration voltage operating at 200 kV. Additionally, Zeta potential (*Z*-potential) & dynamic light scattering (DLS) by (Nano-ZS, Malvern Instruments Ltd., UK) that used for measuring the average diameter & the size distribution. And *Z*-potential; the assessment of surface charges of the formed nanoparticles which measured by using a particle size analyser (Nano-ZS, Malvern Instruments Ltd., UK). For measuring Size distribution and *Z*-potential, the sample was sonicated for 10-20 min. just before assessment.

d) In Vitro Cytotoxicity (MTT) Assay:

The cytotoxic effect of the as-prepared ZnO-NPs formulas (ZnO, 10BSE-ZnO, 20BSE-ZnO) was investigated by the conventional MTT reduction assay; as reported previously **[39]**. Cytotoxicity test was determined in HSF Cells that was grown in DMEM cells medium containing 10% FBS, 100 units/ml of penicillin, and 100 mg/ml of streptomycin. Cultures were preserved in a humidified atmosphere with 5% CO_2 at 37 °C. Each sample was diluted in DMEM complete medium at 37 °C to make a stock solution. Different concentrations of two-fold serial dilutions were performed. Briefly, Confluent monolayers of cells were seeded in 96 well-microtiter plates for 24 hrs, the Cells were incubated with the different samples of varying concentrations in triplicate at 37 °C in a CO_2 environment for 48 hrs. Then, 20 µl of 5 mg/ml MTT was added gently to each well and incubated at 37°C for 4 hrs. After that, carefully media was removed and 150 µl MTT reagent were added. The cells were covered with tinfoil and agitate on orbital shaker for 15 min. Finally, the OD was measured at 570 nm using microplate reader. half-maximal inhibitory concentration (IC_{50}) value of ZnO-NPs that inhibits 50% of the cells were calculated using the following formula:

$$\text{Growth Inhibition} = \frac{\text{OD of control - OD of treated sample}}{\text{OD of control}}$$

3. Results & Discussion

i. XRD analysis

The crystalline nature of the as-prepared nanoparticles was studied by XRD measurement. The patterns of XRD in the range of 5° to 80° of the as-prepared samples (ZnO, 10BSE-ZnO & 20BSE-ZnO) nanoparticles are shown in Figure 1. The XRD pattern shows a number of main diffraction peaks for all the samples with 2Θ values of 30.3, 33.98, 34.3, 48.01,

56.7, 59.2, 61.9, 67.3, 67.4 and 68.4°. The XRD patterns clearly illustrate the formation of a wurtzite structure of zinc oxide that is hexagonal with a space group P63mc (JCPDS card no. 79-0208). In addition, there were some other diffraction peaks in the range of 10° to 20° observed for 10BSE-ZnO and 20BSE-ZnO nanoparticles, which can be attributed to the presence of BSE. The average crystalline size and the degree of crystallinity among the samples were determined using Debye–Scherrer and Hermans–Weidinger methods **[12, 40-42]**. As a result, the crystallite sizes were 79.9, 36.23 and 10.02 nm for ZnO-NPs, 10BSE-ZnO-NPs and 20BSE-ZnO-NPs, respectively.

Furthermore, it was observed that the incorporation of BSE results in an obvious decrease in the degree of crystallinity of ZnO-NPs from 65.7% for ZnO-NPs to 49.2% for 10BSA-ZnO-NPs and 45.3% for 20 BSA-ZnO-NPs. In agreement with other previous studies; these results indicate that the addition of BSE led to an increase in the amorphous nature of the prepared ZnO-NPs and a decrease in crystallinity **[43, 44]**.

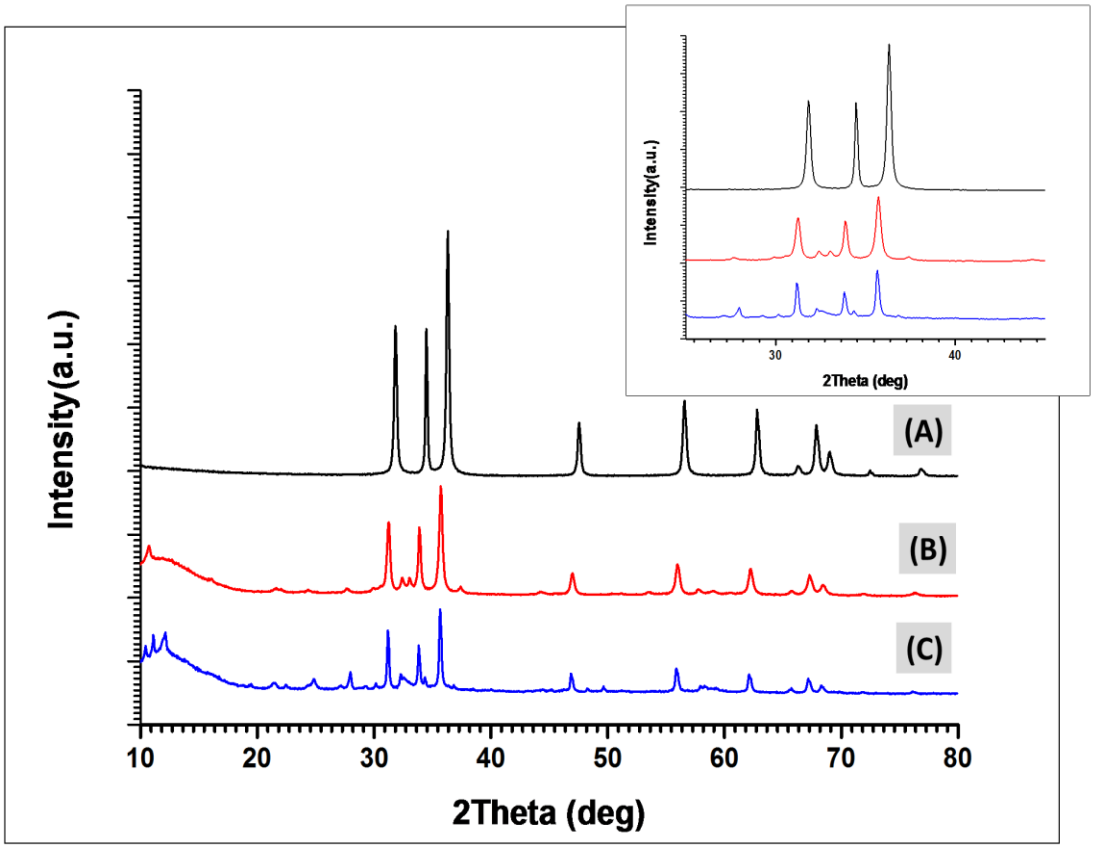

Figure 1. XRD pattern of ZnO nanoparticle synthesized in the presence of Boswellia serrata extract (BSE) (A) ZnO-NPs, (B) 10 BSE-ZNO-NPS & (C) 20 BSE-ZNO-NPs.

ii. Morphological analysis

The shape and size of the as-prepared ZnO-NPs & BSE-loaded ZnO-NPs samples were examined using the TEM analysis **[45]**. Figure 2 ; presents the images of TEM and selected area electron diffraction (SAED). The particles were mainly rod-like structure in shape with average particles sizes ranges of 172±29 nm in width and 40±12 nm in lengths. On the other hand, TEM (Fig. 2 D, E) micrographs indicate that, the sample of BSE-loaded ZnO-NPs samples exhibited bound globular clusters of irregular spherical nano-size particles with average diameter 49±8 nm for 10BSE-ZnO-NPs sample and 15±3 nm for 20BSE-ZnO-NPs sample. ZnO-NPs size and shape change could be related to the adsorption of BSE on the surface of ZnO-NPs. SAED patterns indicate the crystalline nature of the as-prepared ZnO-NPs. The lack of the diffraction rings for BSE-ZnO-NPs samples can be due to the poorly ordered state of the nano-size crystalline structure of the obtained ZnO-NPs, as indicated from XRD analysis.

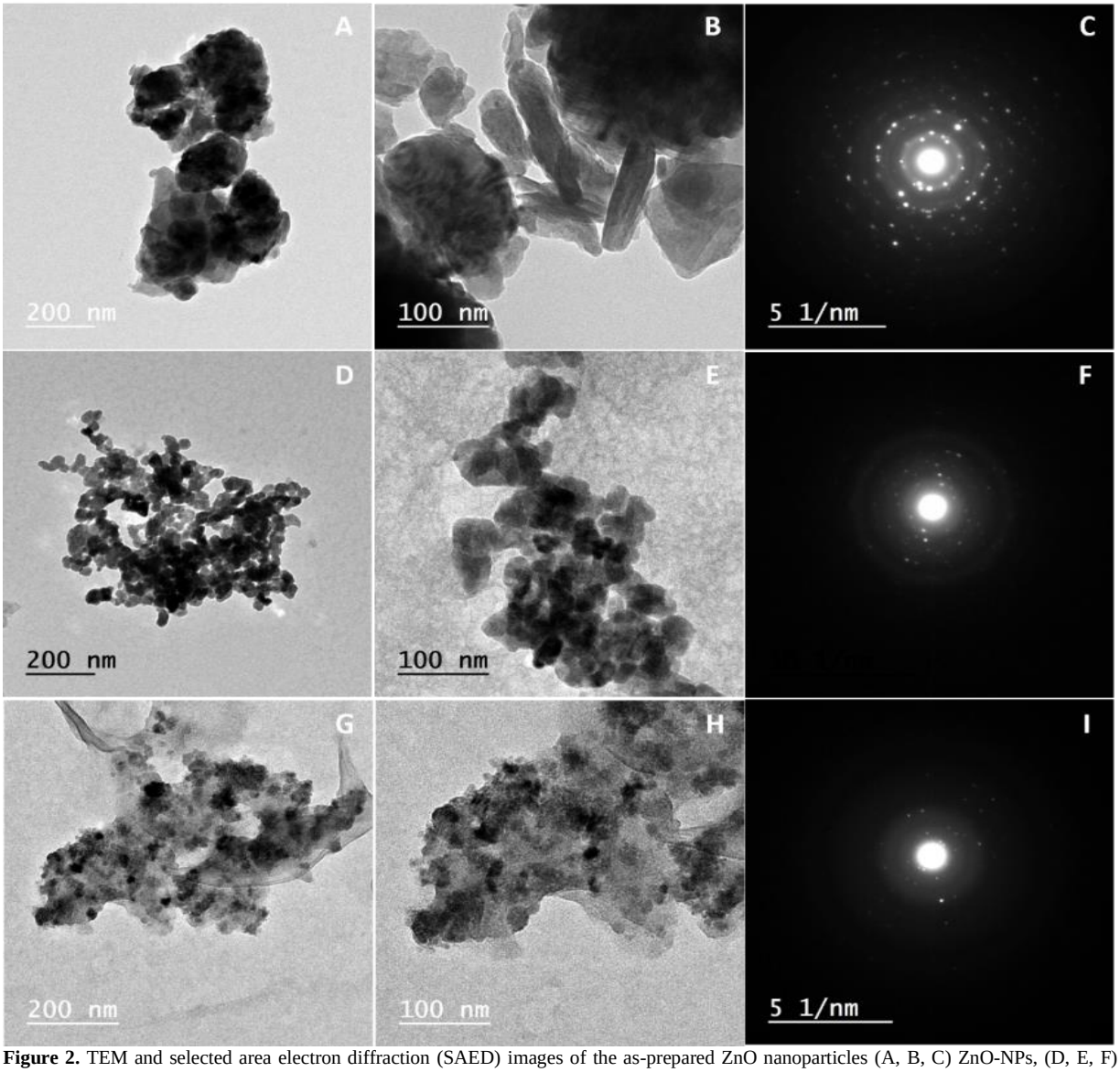

Figure 2. TEM and selected area electron diffraction (SAED) images of the as-prepared ZnO nanoparticles (A, B, C) ZnO-NPs, (D, E, F) 10BSE-ZnO-NPS & (G, H, I) 20BSE-ZnO-NPs.

iii. Particle size distribution and Ζ-potential analyses

The measurement of nanoparticle size distribution of the as-prepared ZnO-NPs and related surface charge expressed as Ζ-potential were performed using the DLS method. The summary of DLS measurements is presented in Figure 3. It can be observed that; the values obtained for the Ζ-potential of the ZnO-NPs and 10BSE-ZnO-NPs suspensions showed surface potentials of -11.8 ± 3.52 mV and -9.39 ± 5.43 mV, which indicates not only good colloidal stability, but also chemical reactivity in aqueous environment such as biological system. In addition, figure 3 shows; size distributions obtained by DLS for the as-prepared ZnO-NPs and 10BSE-ZnO-NPs. The sample exhibited average sizes of 1044±136.8 nm for ZnO-NPs suspension and 893.9±123.5 nm in10BSE-ZnO-NPs suspension. According to the TEM observation, the large particles sizes recorded with DLS measurements could be due to aggregation of the ZnO-NPs **[46]**. In addition, the change in the surface charge can be attributed to the binding affinity of BSE extract components with ZnO-NPs, conferring the colloidal stability of ZnO-NPs and alleviating their aggregation.

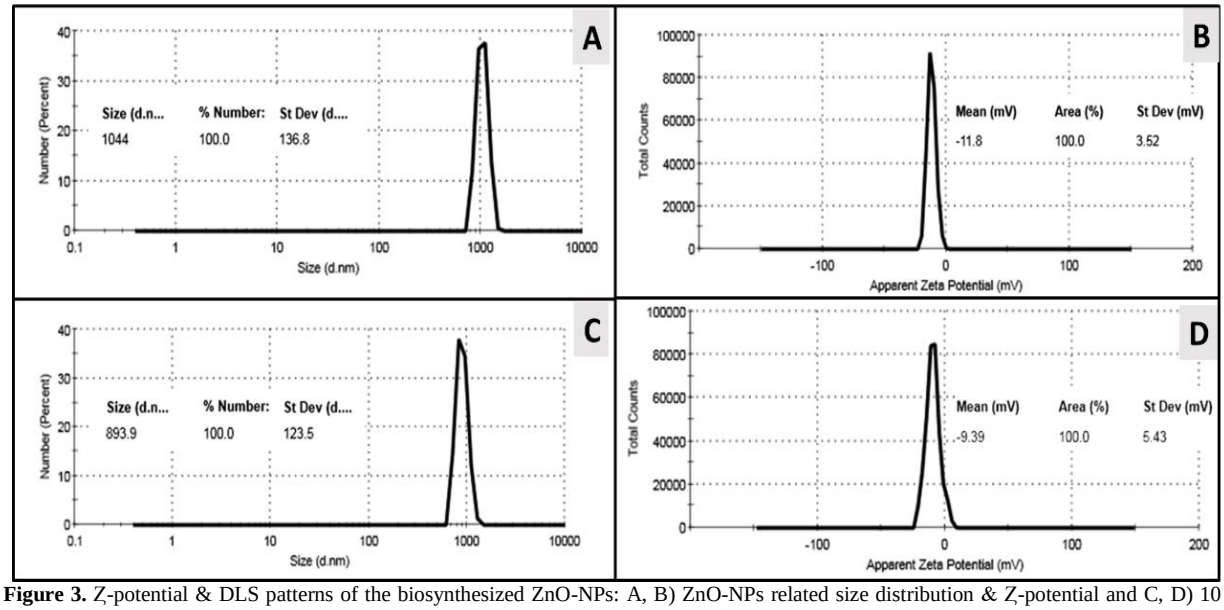

Figure 3. Ζ-potential & DLS patterns of the biosynthesized ZnO-NPs: A, B) ZnO-NPs related size distribution & Ζ-potential and C, D) 10 BSE-ZnO-NPs related particle size distribution & Ζ-potential.

iv. MTT assay

Cell viability, a widely used technique, allows one to monitor how cells respond to materials in culture by counting the number of growing (or surviving) cells in the presence or absence of a certain item over time. So, the effects of the as-prepared ZnO-NPs and BSE-loaded ZnO-NPs samples on cell growth, human skin cells (HSF) have been treated with various concentrations of the prepared NPs (1, 10, 20, 30, 50, 100, 200 and 1000 µg/ml) for 72h. Figure 4; shows the cytotoxicity of the prepared samples ZnO, 10BSE-ZnO & 20BSE-ZnO nanoparticles on HSF cells, where their IC_{50} values measured by MTT as 176.3 µM ,258 µM and 214.5 µM, respectively. It could be observed that BSE loaded nanoparticles showed better cell viability compared to Zno-NPs. The results as presented here show that ZnO and BSA have a dose dependent cytotoxic behavior on the cultured HSF cell line. The cytotoxicity data and IC_{50} values varied significantly among the samples. Since, the particle size plays a vital role in controlling the particular cytotoxic behavior of Nanomaterials. In agreement with other findings **[47, 48]**, our results demonstrate that the as-prepared NPs trigger cell specific responses resulting in variable degree of cytotoxicity, which could be attributed to the change in particle sizes, as well as the BSE content.

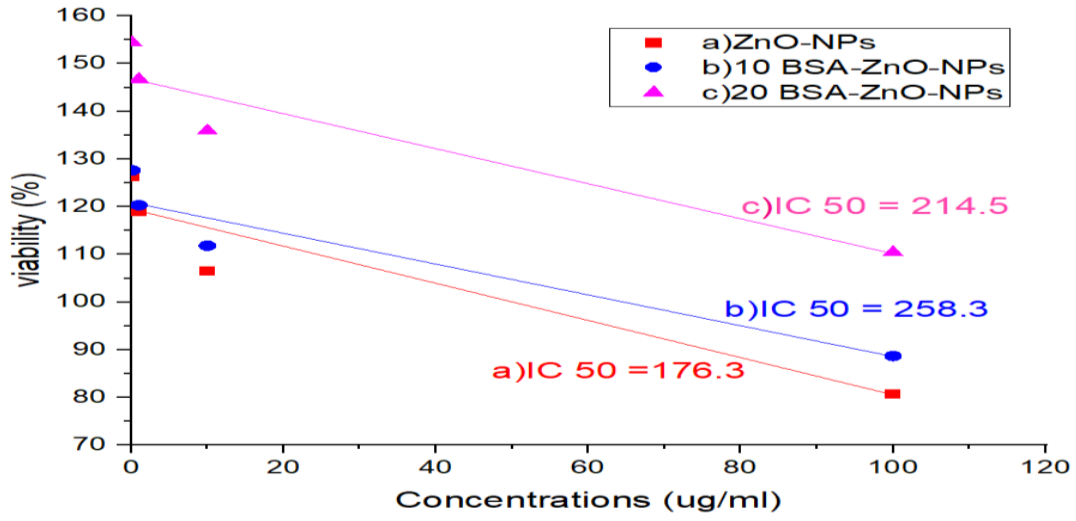

Figure 4. IC_{50} of different biosynthesized nanoparticles against HSF cells. a) ZnO-NPs cytotoxicity on HSF, b) 10BSE-ZnO-NPs cytotoxicity on HSF & c) 20BSE-ZnO-NPs nanoparticles cytotoxicity on HSF

4. Conclusion

Homogenous spherical ZnO-NPs were successfully biosynthesized using glycerol, as a reducing agent, and in the presence of Boswellia Serrata Extract (BSE). The prepared NPs characterized by XRD, TEM & DLS which clearly illustrate: the formation of ZnO-NPs with mainly rod-like structure & an increase in the amorphous nature of the prepared ZnO-NPs and a decrease in the crystallinity with a notable change in the shape and size of ZnO-NPs when BSE loaded. This may be due to the adsorption of BSE on the surface of ZnO-NPs. In addition, the incorporation of BSE results in a slight change in the surface negativity of ZnO-NPs (-11.8 ± 3.52 mV) to (-9.39 ± 5.43 mV) for BSE-ZnO-NPs. The IC_{50} values of the prepared samples measured by MTT were 176.3 µM for ZnO-NPs, 258 µM for 10BSE-ZnO-NPs and 214.5 µM for BSE-ZnO-NPs. Finally, the in vitro outcomes showed that; the BSE-ZnO-NPs possess remarkable effects on HSF cell growth.

However, antibacterial activity, more in-vitro studies against other cell lines, and in-vivo research are necessary for a conclusive analysis concerning the wound healing potential of BSE-ZnO-NPs.

5. Recommendations

- It is recommended to demonstrate the enhanced cell growth potential by performing of cell proliferation assays.

- Immunofluorescence staining of cell components (nucleus, cytoskeleton etc.) after nanoparticle treatment is recommended to clarify the cell-nanoparticle interactions."

- In vivo biocompatibility tests in animal models are recommended to confirm the manuscript results.

6. Conflict of interest

There is no conflict of interest

7. Acknowledgment

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