Synthesis, characterization, and cytotoxicity of Zinc phosphate nanoparticles on the HL-60 human leukemia cell line

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Abstract
Nanoparticles are successfully replacing anticancer medicines in medicine (NPs). In this study, the co-precipitation approach was used to create ZnP-NPs. ZnP-NPs particles in the XRD had an average size of 37.54 nm, whereas ZnP-NPs particles in the SEM had an average size of 78.56 nm. They were detected using various techniques, including FTIR, XRD, EDX, and SEM. According to EDX results, which showed that zinc (55.7%) and oxygen (24.7% of the sample) were present, the synthesized NPs have a high purity level. Furthermore, low Phosphorous concentrations (19.6%) suggest that the manufactured material is of sound purity. ZnP-NPs were evaluated on human cancer HL-60 cells at different concentrations (25, 50, 100, 200, and 400 µg/ml). After 24 hours, the killing rate was 6.5%, 15.2%, 23.8%, 38.2%, and 63.3%, in that sequence. Chemical precipitation is a better method for killing or inhibiting. Half-maximal inhibitory doses (IC50) for ZnP-NPs are 270.5 µg/ml. ZnP-NPs have potential therapeutic advantages as anticancer drugs. The drug was safe at all concentrations (not harmful).
Keywords: Zinc-phosphate nanoparticles, Apoptosis, Cytotoxic, and HL-60

Introduction

Zinc phosphate (ZP) is a non-toxic substance and one of the essential multifunctional metal phosphates because of its exciting properties and broad uses in various fields. Because of its low solubility in water and biological environments, ZP non-toxic building blocks has extensively used in the coatings business, medicine, and the electrical and electrical engineering disciplines, particularly in preventing corrosion [1,2]. Given the roughness of the metal basement's surface, the crystal size significantly impacts the anti-corrosion function. However, nano scaled goods were challenging to produce using the standard process for producing zinc phosphate. Consequently, various methods, including the hydrothermal approach [3-5]. Most applications made use of zinc phosphate. Zinc phosphate is manufactured on a large scale from zinc oxide and phosphoric acid or zinc salt and phosphates. This substance is non-toxic and can be utilized in a variety of binders. It would be most advantageous to manufacture a single particle with a size that does not exceed 100 nm, which is characteristic of nanoparticles [6]. The capabilities of zinc phosphate nanoparticles (ZP-NPS) depend, in large part, on the size distribution of the particles. Leukemia is one of the adult-specific hematologic malignancies that most usually affects bone marrow, the lymphatic system, and blood cells [7]. Unchecked development of poorly differentiated white blood cells and clonal hematopoiesis produced in the bone marrow are symptoms of leukaemia. Treatment options for leukaemia depend on several factors, including the patient's medical history, age, illness severity, and type of leukaemia. As part of their treatment regimens, patients frequently get targeted chemotherapy, radiation therapy, and bone marrow transplants [8]. Leukaemia manifests itself in a variety of unique shapes. Acute and chronic leukaemias are typically divided according to how quickly they develop. Based on the cell types present, leukaemia is generally divided into lymphoid and myeloid leukaemias [9]. Effective therapeutic pharmaceutical delivery, enhanced targeting, and controlled release is achieved. Recently, nanotechnology for drug delivery has been developed. Nanoparticles have the potential to overcome the limitations of conventional medicine and enable the development of personalized delivery platforms that include diagnostic, therapeutic, and other functions through intelligent design [10,11]. In nanomedicine, a recent but expanding field of study, materials at the nanoscale are employed for disease detection or finely targeted drug administration. The targeted administration of chemotherapeutic, immunotherapeutic, and biologic medications to treat diverse diseases is one of the best uses of nanomedicine [12]. The way that various human diseases are handled may alter due to therapeutics based on nanoparticles. The reduced bioavailability of medications hampers clinical translation due to nanoparticle instability and early release. Because all nanoparticles depend on control at the nano-size scale, even little modifications to the Nano formulation could have a significant impact [13]. According to the literature, nanomaterials may have an anticancer effect on many cancer cells. As a result, zinc phosphate (Zn3(PO4)2) has been used in a variety of industries and sectors, including electrical, medical, medication delivery, and industry [14, 15]. However, there hasn't been much research that discusses ZnPNPs as a cancer treatment. To the author's knowledge, zinc-phosphate nanoparticles were first introduced in the current work regarding their anticancer activity toward the HL-60 human leukaemia cell line.

doi.org/10.31838/hiv22.02.610 Received: 07.04.22, Revised:20.07.22, Accepted: 13.08.22

Experimental

The chemicals used in this study are Zn (NO3)2. 6H2O, (NH4)2HPO4, absolute ethanol, and Deionized Water. It was from excellent international companies of high purity.

Synthesis of ZnP NPS Nanoparticles by co-precipitation

0.5M was prepared. Zn (NO3)2. 6H2O by dissolving 7.42 gm in 50 mL deionized water.0.5M of (NH4)2HPO4 was designed by dissolving3.3 gm in 50Ml deionized water. Drops of (NH4)2HPO4 were gradually added to the first solution, which was applied to the magnetic stirrer at a temperature of 40-50°C. With continuous stirring, 250-300, until a white misty precipitate appears at pH3. The precipitate was washed twice with non-ionic water and once with absolute ethanol. This precipitate was dried at 100°C for 12 hours.

ZnP NPs nanoparticles: characterization methods

The ZnP NPs nanoparticles were examined using a variety of methods, including X-ray diffraction (XRD), Fourier-transform infrared (FTIR) spectroscopy, and scanning electron microscopy (SEM). The nanoparticles' crystallite sizes were determined using XRD (Shimadzu, Kyoto, Japan). FTIR spectra of the samples were acquired with Shimadzu (Tokyo, Japan). A 200 kV Zeiss SEM was used for the SEM analysis (Germany).

Nanoparticles ZnP MTT assay

For this experiment, MTT dye (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) was used at a concentration of 10 mg/ml. In order to create concentration gradients of 25, 50, 100, 200, and 400 µg/ml, samples of ZnP NPs nanoparticles were dissolved in 0.2% DMSO. A sample of 200 µl suspended cells (1 104 cells/well) was distributed and prepared in RPMI media. The cells were grown for 24 hours in 5% CO2 at 37 °C. After receiving 20 µl of ZnP NPs treatment, the cell cultures were incubated for 24 hours under the same conditions. The MTT reagent was added to each sample and incubated for 5 hours at 37°C. The absorbance was measured at 570 nm [16].

Nanoparticle Hemolysis Assay for ZnP NPs

The hemolysis assay was used to screen for ZnP NPs nanoparticles at different dosages (50, 200, and 400 µg/ml) to identify harmful or non-toxic compounds. The blood sample was taken from the lab and put in an (EDTA) tube before being examined under a microscope at a magnification of (100). An (EDTA) tube was used to separate the blood cells from the plasma, and it was spun at high speed for 10 minutes. The cells were repeatedly washed with PBS after the plasma layer had been removed from them, adding 1ML of PBS each time, and the centrifuge cycle was repeated for 10 minutes. The cells were taken out of the PBS after two minutes had passed. The blood cell suspension was made by combining (1ML) with (9ML) PBS after the blood cells had been washed many times. Each tube receives a volume of (1200 µL) of the antagonist, which is added in increasing concentrations, and the final volume of (300 µL) of the cell suspension (1.5 ml). Each tube is spun for five minutes at a rate of 1000 cycles per minute after being incubated in the incubator for two hours. The difference in hemolysis was then measured using the Heh control settings (test tube containing blood and deionized water only, test tube containing blood, and PBS). After centrifugation, the (+) option shows the compound's toxicity when combined with blood constituents. The (-) option indicates that the drug was not harmful because the blood components did not combine after centrifugation [17].

Results and discussion

Characterization of ZnP nanoparticles by FTIR

Personalize (Zn3(PO4)2) nanoparticles by tracking their infrared (FT-IR) spectrum, as shown in Figure 1. The essential characteristic of the spectrum of zinc phosphate nanoparticles (Zn3(PO4)2) is the appearance of a sharp and distinct band at frequency 632 due to the stretching of the P-O bond (υ), which is a band that characterizes the phosphate structures. And the appearance of two bands at frequency 1010 and 948 due to the PO3-4 group (Zn-O). Moreover, the appearance of a distinctive wide band at frequency 3417 is related to the stretching of the υ (O-H) bond [18].

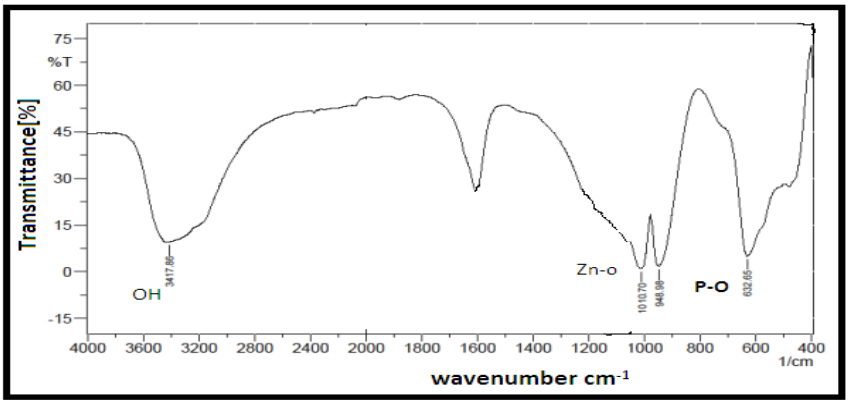

Fig. 1: Characterization of ZnP nanoparticles by FTIR

Utilizing X-ray diffraction to describe ZnP nanoparticles

The crystal structure of (Zn3(PO4)2) prepared by the co-precipitation method is characterized by X-ray diffraction, as shown in Figure 2. International Center for X-ray diffraction (ICDD) database card no. 00-001-0524.The average crystal size was calculated as 37.54 nm using the Debye-Scherer equation.

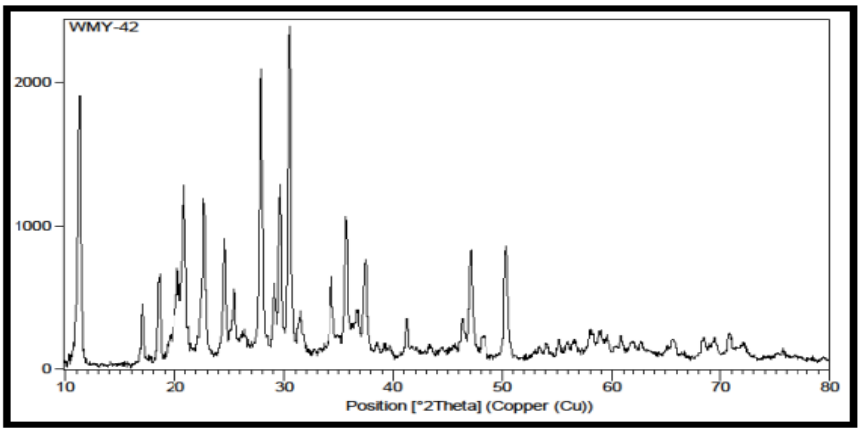

Fig. 2: X-ray diffraction spectrum of ZnP nanoparticles

ZnP nanoparticle characterization with energy-dispersive X-rays

The proportion of elements in the prepared Zn3(po4)2 nanoparticles was determined by co-precipitation by energy-dispersive X-ray EDX. As shown in Figure 3, the results showed the presence of zinc (55.7%) and oxygen (24.7%). Moreover, small percentages of Phosphorous (19.6%) indicate the prepared substance of sound purity.

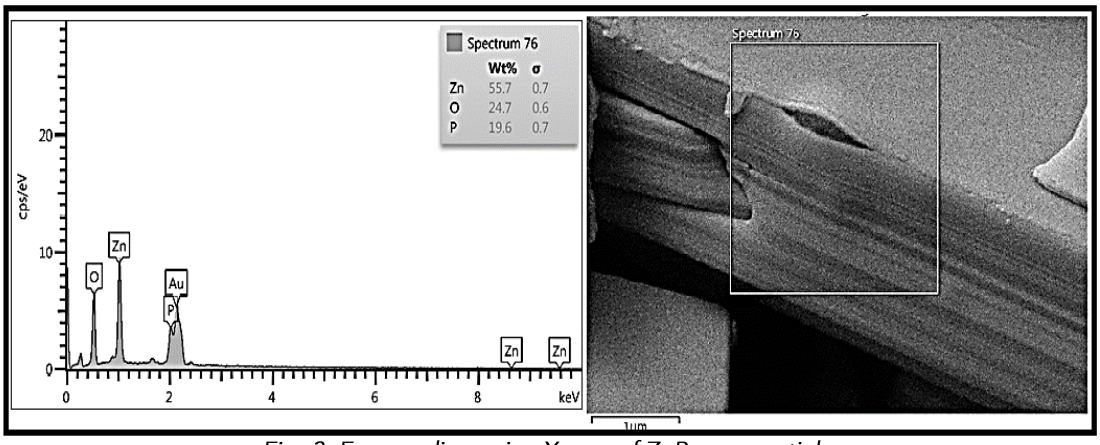

Fig. 3: Energy-dispersive X-rays of ZnP nanoparticle

ZnP nanoparticle characterization using SEM

The Zn3(po4)2 nanoparticles' morphology and structural makeup are depicted in Figure 4. The nanoparticles were made in the nanometer range, and SEM images revealed that while most were present in agglomerated form due to electrostatic effects, some were well separated from one another. These particles have a 78.56-nanometer diameter.

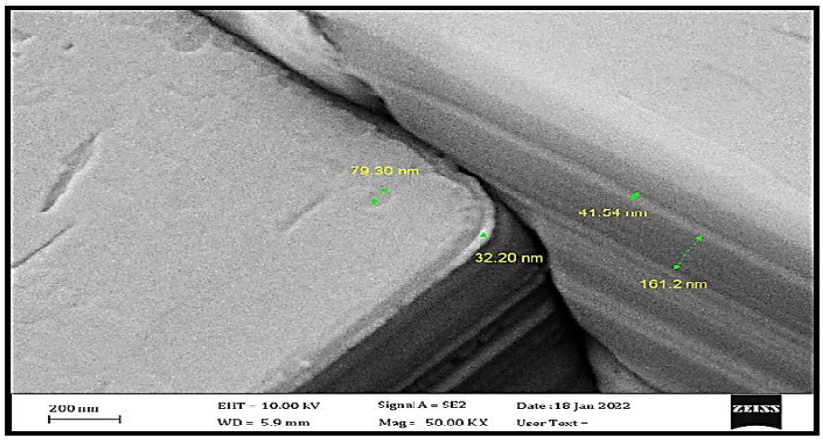

Fig. 4: SEM of ZnP nanoparticle

Inhibition of ZnP nanoparticle for HL-60 human cancer cells

According to Figure 5, HL-60 human cancer cells survived longer when mixed ZnP nanoparticles were added using Chemistry Precipitation Method A at varying concentrations (25-400 µg/ml) than when the cells were left in the blank condition. The killing rate was 6.5% at a concentration of 25 and 15.2% at a concentration of 50. At a concentration of 200, the killing rate increased from 23.8% at a concentration of 100 to 38.2% at 200, demonstrating a correlation between concentration and the percentage of inhibition or killing. And 400 µg/ml presents a killing rate of 63.3%.

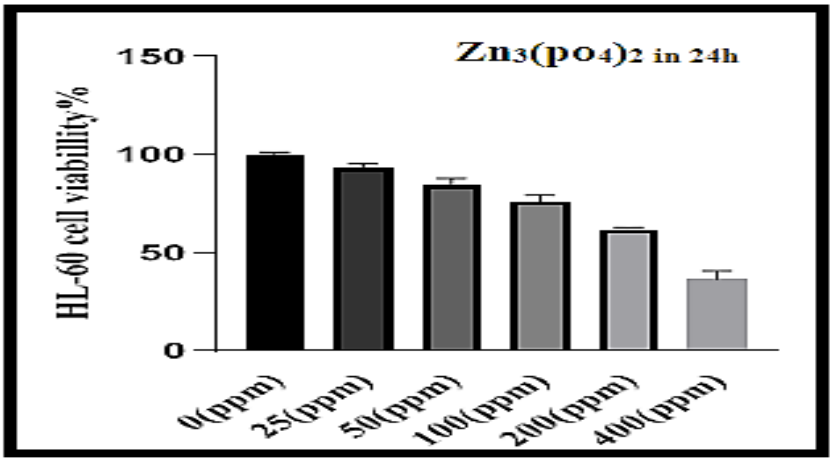

Fig. 5: Inhibition of ZnP nanoparticle for HL-60

The half-maximal inhibitory concentration (IC50) of ZnP nanoparticles by chemical precipitation was evaluated after 24 hours of incubation with HL-60 cells using a normalized response. Fig. 6 shows a value of 270.5 µg/ml for the IC50.

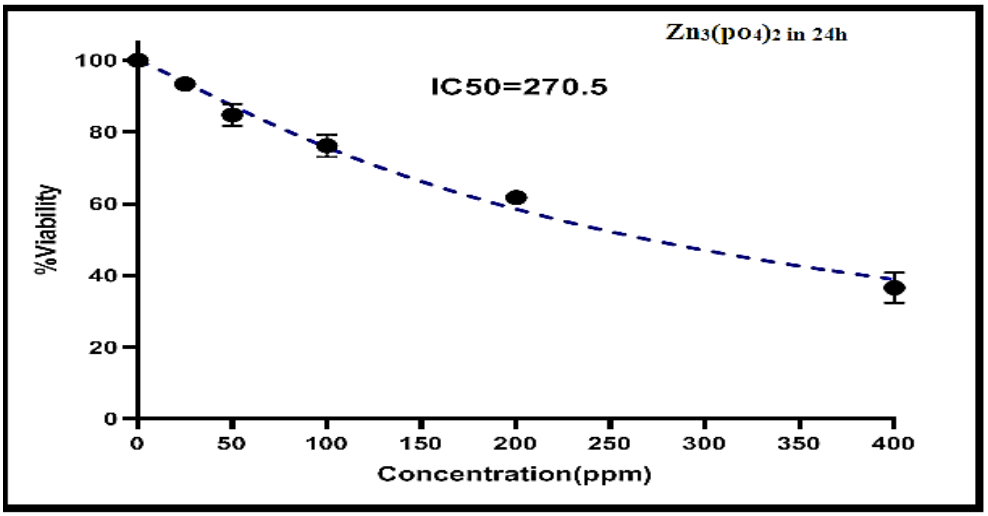

Fig. 6: IC50 of ZnP nanoparticle for HL-60

Fig. 7. Investigation of the cytotoxicity of the ZnP nanoparticle compound using the chemical precipitation method revealed that the product was safe (non-toxic) at all doses.

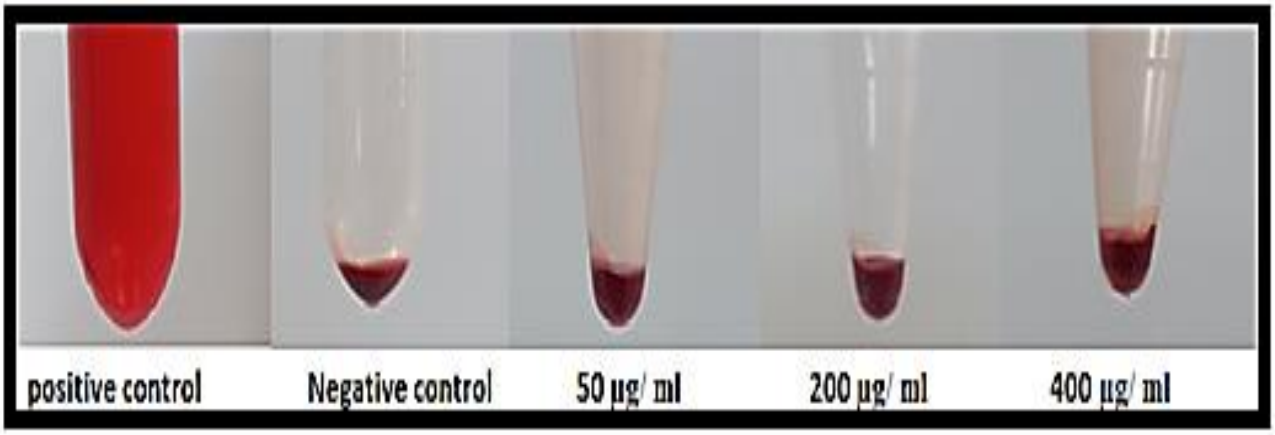

Fig. 7: Hemolysis test for ZnP nanoparticle

Numerous studies have also suggested that the extent to which Zn NP-mediated cell death may be influenced by experimental methods, cellular absorption efficiency, concentrations, exposure period, and nanoparticle size [19, 20]. According to recent studies in the related literature, zinc nanoparticles can destroy tumour cells while having little to no effect on normal cells in terms of cell death [21]. Identification of Apoptotic Assessments It is notable that several apoptosis regulators can be blocked or cause mitochondrial integrity during apoptosis since mitochondria can be considered critical signaling hubs within the apoptotic pathway. The primary function in mitochondrial-mediated apoptosis can also be played by anti-apoptotic proteins (Bcl-2) and Bcl-2 family proteins (Bak, Bax) [22, 23]. Additionally, numerous investigations looked into zinc's capacity for oxidative stress followed by the generation of ROS using nanoparticles. development [24, 25]. The literature also included reports. Demonstrated that zinc ions could trigger the cytotoxicity produced by ROS. Considering this, Horky et al. noticed an increase after exposure to antioxidants, rat liver, kidney, and blood delivery of several phosphate-based zinc nanoparticle formulations at a 2000 mg Zn/kg diet [26].

Conclusions

ZnPNPs showed encouraging cytotoxicity. Against cancerous leukaemia cells. Treated cells could potentially activate apoptotic pathways. Additionally, ZnPNPs increased the expression of the p53 gene, a tumour suppressor, at the mRNA level. Overall, manufactured new ZnPNPs could perhaps be used as an important landmark for undertaking an additional study on cancer treatment. So, the current study's findings provide hope for stopping the spread of leukaemia cancer. In contrast to chemotherapy drugs.

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