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Green Synthesis of Nano $Zn_3(PO_4)_2$ Using *Curcuma* Extract and Their Effect on SK-OV-3 Human Ovarian Cancer Cell Lines

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Abstract. Zinc phosphate (ZnP) has many implementations in different areas. Nanoparticle materials synthesis utilizing plant extracts is an alternative to classic physical and chemical methods. The biological synthesis of nanoparticles is of significant importance due to its simplicity, eco-friendliness and extensive antimicrobial activity. In this study, the reported a new method for the synthesis of $Zn_3(PO_4)_2$ nanoparticles using *Curcuma* extract. The ZnP nanoparticles was characterized by X-ray diffraction (XRD) and scanning electron microscopy (SEM) with energy dispersive X-ray analysis (EDX) and show an average particle sizes of 42.83 nm and 117.05 nm as determined from XRD and SEM respectively. They were then evaluated on ovarian cancer cell lines at concentrations of (25, 50, 100, 200 and 400) µg/mL after 24 and 48 h with the viability percentages being 92.58%, 80.55%, 70.71%, 56.57%, 34.67% and 69.37%, 55.11%, 46.87%, 32.96%, 8.98 %, respectively. The obtained nanoparticles have half-maximal inhibitory concentrations (IC_{50}) of 233.4 and 69.75 g/mL after 24 and 48 h of incubation with SK-OV-3 cells, respectively. Therefore, as anticancer medications, zinc phosphate nanoparticles seem to have potential therapeutic benefits with the medication being secure and not harmful at all dosages.

Keywords: green synthesis, *Curcuma* extract, zinc-phosphate nanoparticles, ovarian cancer

Introduction

There are increased interest in nanomaterials due to their novel physical and chemical properties if compared to bulk one. These extraordinary properties have reflected a multitude of innovative applications in many fields such as medicine and pharma, the electronics industry, agriculture, catalysis, food industry and in the vital and medical scope with properties special treatment against cells of cancer (Jarullah *et al*., 2023; Sadeghi-Aghbash and Rahimnejad, 2022; Iqbal *et al*., 2019).

Green synthesis of nanoparticles employing plant extracts is considered an emerging area of research as it is potentially advantageous over chemical or microbial synthesis and it eliminates the elaborate process and can also meet large-scale production in addition to being low cost (Hassan *et al*., 2018; Nagajyothi *et al.*, 2017).

The problem of cancer remains a critical health problem and its treatment holds large promise. Depending on the kind and step of cancer, various therapies of patients are treated like chemotherapy, radiation therapy or together sometimes. Despite making up just around 5% of all occurrences of female cancer *i.e* cancer of ovarian is the most common cause of women's deaths (Midya *et al*., 2023; Siegel *et al*., 2013).

Nano ZnP utilized in different exceptional application such as drug delivery (Yuan *et al*., 2013) and lubricant additives reported by (Lv *et al*., 2013), circulating tumor cells detection (Guo *et al*., 2016), anti-corrosion pigment (Wan *et al*., 2017; Tamilselvi *et al*., 2015) and bones tissue regeneration (Kumar *et al*., 2017), toxic metals sensing and removal (Hsini *et al*., 2021; He *et al*., 2016), antibacterial as well as anticancer agents (Shakir and Hammadi, 2022; Vafaei *et al*., 2020; Horky *et al*., 2019). In light of the huge and versatile applications of ZnP nanoparticles, researchers developed many synthetic ways for it (Shathi *et al*., 2022).

The aim of the current study is the synthesis zinc phosphate nanoparticles utilizing *Curcuma* extract and to investigate their anticancer activity toward an ovarian cancer cell line.

Materials and Methods

High purity absolute ethanol, $Zn(NO_3)_2.6H_2O$, $(NH_4)_2HPO_4$ and deionized water employed in this study were obtained from. Multinational chemical companies

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whereas *Curcuma* powder purchased from the local Iraqi market.

Preparation of *Curcuma* extract. 100 g of dry *Curcuma* powder taken and 1000 mL of deionized water added to it at a ratio of (1:10). The mixture was place on a magnetic stirrer at a temperature of 45 °C with continuous stirring. After that, it was left with in a tight cover flask for 24 h in dark place at room temperature, then filtered first using several layers of medical gauze and then with filter paper. The filtrate was collect in glass bottles until used to prepare nanomaterials (Mahdie *et al*., 2011).

Green synthesis of zinc phosphate nanoparticles. 0.5 M of $Zn(NO_3)_2$. $6H_2O$ solution was prepared by dissolving 7.42 g of zinc nitrate in 50 mL of *Curcuma* plant extract. 0.5 M of $(NH_4)_2HPO_4$ was prepared by dissolving 3.3 g of it in 50 mL of *Curcuma* plant extract as well. Drops of $(NH_4)_2HPO_4$ solution gradually added to the first solution and placed on the magnetic stirrer at a temperature of 45 °C. with continuous stirring until a light yellow precipitate appeared at pH 3. The precipitate washed twice with deionized water and once with absolute ethanol then dried at 100 °C for 12 h.

Characterization of zinc phosphate nanoparticles. Several techniques, such as X-ray diffraction (XRD), scanning electron microscopy (SEM) and EDX spectroscopy used to characterize the prepared nanoparticles.

Zinc phosphate MTT nanoparticles assay. In this experiment, 10 mg/mL of the MTT dye (3-[4,5-dimethylthiazole-2-yl]-2, 5-diphenyl tetrazolium bromide) was utilized. ZnP nanoparticle samples diluted in 0.2 % DMSO to produce (25, 50, 100, 200 and 400) µg/mL concentration gradients. In RPMI medium, a sample of 200 µL suspended cells (1×10^5 cells/well) was prepared. At 37 °C and 5% CO_2, the cells were cultivated for 24 h. Thus, cell cultured for 24 and 48 h after receiving 20 µL of ZnP NPs treatment. Each sample received the MTT reagent, which incubated for 5 h at 37 °C and then at 570 nm, the absorbance was measured (Taghavi *et al*., 2016).

Zinc phosphate nanoparticle hemolysis assay. ZnP nanoparticles at varied dosages (50, 100, 200 and 400) µg/mL screened using the hemolysis assay to find toxic or non-toxic substances. After being remove from the lab and placed in an EDTA tube, the blood sample seen under a microscope at a magnification of 100. After the plasma layer eliminated, the centrifuge cycle repeated for 10 min, washing the cells with 1 mL of PBS each time. After the blood cells washed several times, the blood cell suspension created by mixing 1 mL with 9 mL PBS. The antagonist introduced to each tube in volumes of 1200 µL at progressively higher concentrations and 300 µL of the cell suspension total of 1.5 mL. The (+) option after centrifugation demonstrates the compound's toxicity when mixed with blood components. Because the blood components did not mix after centrifugation, the (-) option denotes that the substance was not toxic (Zhi *et al*., 2020).

Results and Discussion

Utilizing X-ray diffraction to characterize zinc phosphate nanoparticles. X-ray diffraction was used to identify the crystal structure of $Zn_3(PO_4)_2$ synthesized using the green synthesis technique, as illustrated in Fig. 1 which seem to agrees well with card number 00-021-1489 in the ICDD. The Debye-Scherer equation used then to determine the average crystal size that appears to be 42.83 nm and falls within the range of nanoparticles. In addition, the diffraction peaks broadening refers to the crystal size being small.

Zinc phosphate nanoparticle characterization with energy dispersive X-rays. Using energy dispersive X-ray EDX, the composition of the produced $Zn_3(PO_4)_2$ nanoparticles ascertained. The findings revealed the presence of zinc (54.2%), oxygen (23.3%) and phosphorous (22.5%), as shown in Fig. 2. From the appeared results, the phosphorous concentrations were low referring to the substance being of high purity.

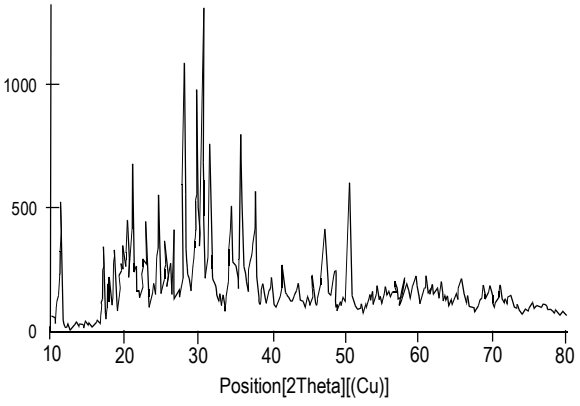

Fig. 1. X-ray diffraction spectrum of ZnP nanoparticles.

Zinc phosphate nanoparticle characterization using SEM. Figure 3 shows the shape and structural characteristics of the $Zn_3(PO_4)_2$ nanoparticles. The created nanoparticles were in the nanometer range and SEM image showed that although most electrostatically bound together, some substantially separated from one another. These nanoparticles are about 117.05 nm in size.

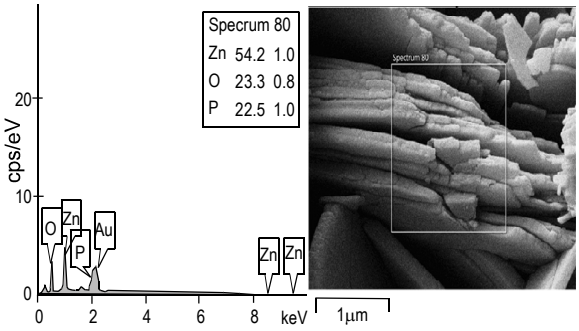

Fig. 2. Energy-dispersive X-rays of ZnP nanoparticle.

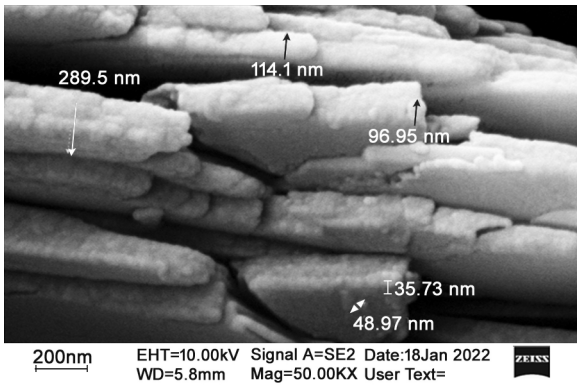

Fig. 3. SEM image of ZnP nanoparticle.

Inhibition of ZnP nanoparticle for SK-OV-3 human ovarian cancer cells. The viability percentage of SK-OV-3 cells after adding $Zn_3(PO_4)_2$ nanoparticles prepared by the green chemistry method for 24 h at a concentration of (25, 50, 100, 200 and 400) µg/mL were 92.58%, 80.55%, 70.71%, 56.57% and 34.67%, respectively, while after 48 h were 69.37%, 55.11%, 46.87%, 32.96% and 8.98 % respectively. Whereas the killing cell percentage of SK-OV-3 cells after 24 h at a concentration of (25, 50, 100, 200, 400) µg/mL were 7.42%, 19.40%, 29.29%, 43.43% and 65.33%, while after 48 h were 30.63%, 44.89%, 53.13%, 67.04% and 91.02% as shown in Table 1. This means a decrease in viability percentage and an increase in the killing of SK-OV-3 ovarian cancer cell lines with increased concentration of zinc phosphate nanoparticles at the time of exposure of 24 h and 48 h as in Fig. 4(ab). After 24 and 48 h of incubation with SK-OV-3 cells, the half-maximal inhibitory concentration (IC_{50}) of ZnP nanoparticles was assessed using a normalized response, where the IC_{50} values were 233.4 and 69.75 µg/mL, respectively see Fig. 5(a and b). The test analysis in Fig. 6 of the ZnP nanoparticle's cytotoxicity showed that it was harmless (non-toxic) at all doses.

Numerous investigations have also revealed that experimental techniques, cellular absorption efficiency, concentrations, exposure time and nanoparticle size may influence the degree to which Zn NP-mediated cell death occurs (Wang *et al*., 2011). Zinc nanoparticles can kill tumor cells, but they have little to no effect on normal cells in terms of cell death, according to recent studies in the related literature (Taghavi *et al*., 2016). Numerous studies also examined zinc's ability to withstand oxidative stress and produce ROS when combined with nanoparticles (Asare *et al*., 2012; Foldbjerg *et al*., 2009). Reports were also included in the literature. It proven that zinc ions could cause ROS-induced cytotoxicity. In light of this, Horky *et al*. (2019)

Table 1. Effect of $Zn_3(PO_4)$ nanoparticles on viability percentage and killing cell percentage SK-OV-3 cells line at 24 and 48 h

Concentration (µg/mL)	Viability% SK-OV-3 (24 h)	Killing cell% SK-OV-3 (24 h)	Viability% SK-OV-3 (48 h)	Killing cell% SK-OV-3 (48 h)
25	92.58	7.42	69.37	30.63
50	80.55	19.40	55.11	44.89
100	70.71	29.29	46.87	53.13
200	56.57	43.43	32.96	67.04
400	34.67	65.33	8.98	91.02

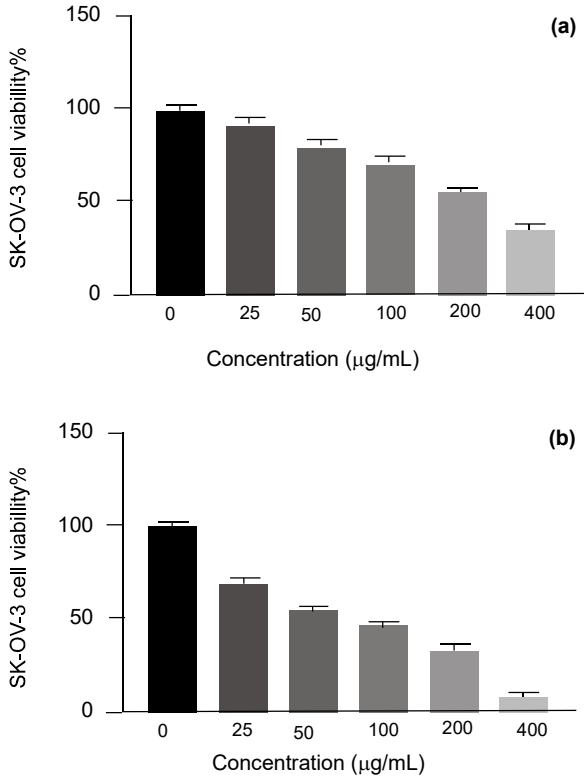

Fig. 4. Inhibition of ZnP nanoparticle for SK-OV-3 at **(a)** 24 h and **(b)** 48 h.

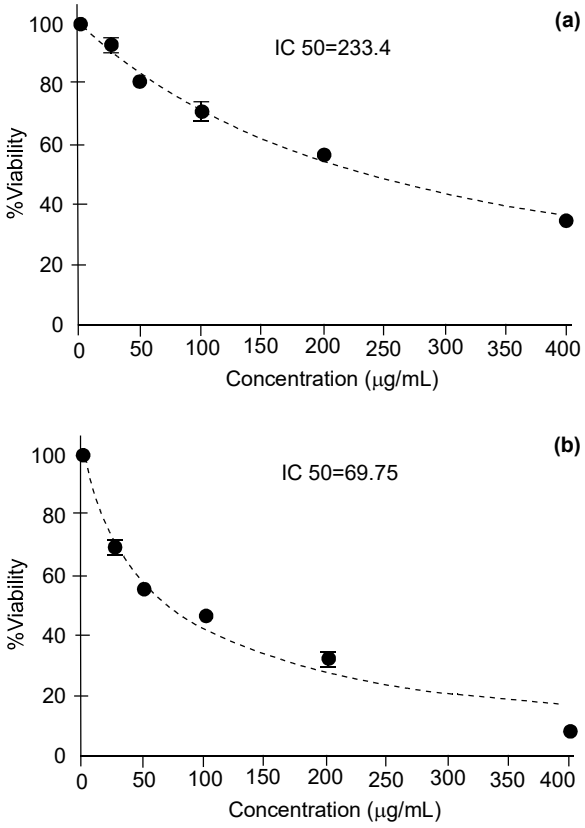

Fig. 5. IC_{50} of ZnP nanoparticle for SK-OV-3 at **(a)** 24 h and **(b)** 48 h.

reported a rise following the administration of multiple phosphate-based zinc nanoparticle formulations at 2000 mg/Kg diet, rat liver, kidney and blood.

Conclusion

Zinc phosphate nanoparticles successfully synthesized by the new green chemistry method using *Curcuma* extract and it has demonstrated positive cytotoxicity at different concentrations against malignant ovarian cancer cells. The results indicated that the killing of ovarian cancer cell lines increased with increased concentration of zinc phosphate nanoparticles at the time of exposure at 24 h and 48 h whereas the viability% shows otherwise. The results also showed that half-maximal inhibitory concentration (IC_{50}) of $Zn_3(PO_4)_2$ nanoparticles decreases with time increases when comparing results in 24 and 48 h. This indicates that Zinc phosphate nanoparticles have more anti-cancer activity than toxicity. Potentially, treated cells might open apoptotic pathways. Furthermore, ZnP-NPs boosted the mRNA expression of the tumor suppressor gene (p53). Overall, newly produced ZnP-NPs could be a

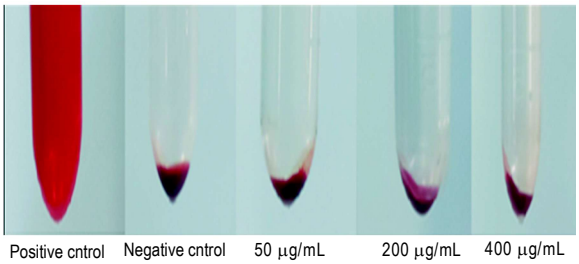

Fig. 6. Hemolysis test for ZnP nanoparticle.

significant step toward conducting future research on cancer treatment. In contrast to chemotherapy medications, the current study's findings offer hope for ovarian cancer prevention and control.

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Conflict of Interest. The authors declare that they have no conflict of interest.

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