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Green Synthesis of Mn3O4 Nanoparticles using chia seeds extract, characterization, and cytotoxicity on the HL-60 cells

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Abstract

In medicine, nanoparticles are successfully replacing anticancer drugs (NPs). In this study, Mn3O4-NPs were made using the green chemistry Chia seed extract method, and they were then characterized using various methods, including FTIR, XRD, EDX, and SEM. HL-60 cells were tested against Mn3O4 nanoparticles at varied concentrations (20, 40, 80, and 160 µg/ml), with an average size of 35.27 nm in the XRD. After 24 hours, the killing rate was 4%, 14%, 24%, and 40% in that sequence. Moreover, the rates were (4%, 26%, 44%, and 40%) in that sequence after 48 hours. Half-maximal inhibitory concentrations (IC50) for Mn3O4 -NPs are 220.5 at 24 hours and 97.83 at 48 hours. Mn3O4 nanoparticles offer potential therapeutic advantages as anticancer drugs. The drug was safe at all concentrations (not harmful).

Keywords:

Mn3O4 nanoparticles, HL-60 cells, chia seeds

One of the main global health issues, cancer is a condition brought on by aberrant cell division [1]. Chemotherapy, radiation therapy, immunotherapy, surgery, targeted therapy, and hormone therapy are common forms of cancer therapy [2, 3]. Such treatments typically have severe side effects and a higher risk of recurrences. As a result, nanoparticles have lately been employed to get around the drawbacks of traditional therapy approaches [4]. Due to the remarkable penetration of nanoparticles into human tissue, nano-drug carrier systems have demonstrated advantages in cancer treatment [5]. Metal nanocomposites are multi-component substances of numerous phases, at least one of which is nanoscale in size [6]. Compared to their traditional counterparts, they exhibit a number of exceptional qualities. Several industries heavily utilize nanocomposites, including drug delivery [7] and recently bio-medical applications like antibacterial and cancer treatments [8]. Green nanotechnology offered tools for converting biological systems to environmentally friendly methods of synthesizing nanomaterial while avoiding any associated harm. Green techniques use natural sources and plant extract because so many hazardous chemical compounds are used in making these nanoparticles. Green nanotechnology can offer environmentally friendly nanoparticles that don't use toxic ingredients in their synthesis by merging green chemistry and engineering principles [9−11]. Due to its eco-friendliness, safety, and biocompatibility, green chemistry has gained popularity [12−14]. An increasing amount of interest has been shown in the extending types of plant extracts for producing metal oxide because using plant extracts to synthesize metal oxide nanoparticles has some advantages, such as not requiring any pollutants and toxins and being environmentally friendly. In recent years, numerous reviews on the green synthesis of nanoparticles have

confirmed the promising potential of these approaches for the large-scale manufacturing of metal oxide. [15, 16]. Due to their low cost, eco-friendliness, availability of manganese in various states, and natural abundance, manganese oxides are a mixed oxide material suitable for various applications including catalysis, electrochemistry, and medicine [17,18]. Manganese dioxide, dimanganese trioxide, and tri-manganese tetraoxide are products of the various oxidation states of manganese. Due to its unique structural and electrical characteristics with unique ion exchange, catalysis, molecule adsorption, and magnetic and electrochemical properties, Mn3O4 nanoparticles have drawn much attention. Manganese is recognized as a crucial component of metabolism and is well-regulated by biological systems. Mn3O4 nanoparticles have superior biological characteristics and minimal toxicity [19]. In the current work, we demonstrate a green Synthesis of Mn3O4 nanoparticles using chia seed extract and using manganese chloride salts as a precursor material by utilization of a precipitation method and study cytotoxicity on the HL-60 cells.

Experimental

The chemicals used in this study are Mncl2.4H2O, NH4OH, HCl, ethanol, and Deionized Water. It was from excellent international companies of high purity.

Green synthesis Mn3O4 nanoparticle

A of 50 g of chia seeds was taken, 500ml of deionized water was added and placed on the magnetic stirrer for half an hour at a temperature of 50 ℃, then left in a dark place for 24 hours [20]. Dissolve 0.5M of MnCl2.4H2O in 1M of hydrochloric acid prepared by mixing the acid with chia seed extract to form a solution and put the solution on the magnetic stirrer for 30 minutes. The number and the temperature of 40 °C pH was adjusted to 7 by dropper 2M from NH4OH solution and left on the magnetic stirrer for two hours, after which the filter was filtered. The residue was washed using ethanol once, and deionized water twice, and the residue was dried at a degree of 120 °C for 6 hours and then burned the precipitate at450 °C for 4 hours.

Characterization methods

The Mn3O4 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).

MTT test for Mn3O4 nanoparticles

For this experiment, MTT dye (3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide) was employed at a concentration of 10 mg/ml. To obtain concentration gradients of 20, 40, 80 and 160 µg/ml, samples of Mn3O4 nanoparticles were dissolved in 0.2% DMSO. In the RPMI medium, a sample of 200 µl suspended cells (1 104 cells/well) was dispersed. The cells were grown for 24 hours under 5% CO2 at 37 °C. After being treated with 20 µl of Mn3O4 -NPs, the cell cultures were subsequently incubated for an additional 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 [21].

Hemolysis test for nanoparticles of Mn3O4

The hemolysis assay screened for Mn3O4 at various levels (50, 200, and 500 µg/ml) to identify harmful or non-toxic compounds. The blood sample was retrieved from the lab and put in an (EDTA) tube before being viewed 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 rinsed with PBS after the plasma layer had been removed, 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 Specified 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 medicine was not hazardous because the blood components did not mix after centrifugation [22].

Results and discussion

Characterization of Mn3O4 nanoparticles by FTIR

The characterization of Mn_3O_4 nanoparticles by FT-IR spectrum is shown in Figure.1 It was observed that an average band appeared at a frequency of 632cm^{-1} due to the stretching of the beam Mn-O and the emergence of a broad band at a frequency of 3364. cm^{-1}, we return to stretching O-H consistent with the literature [23]

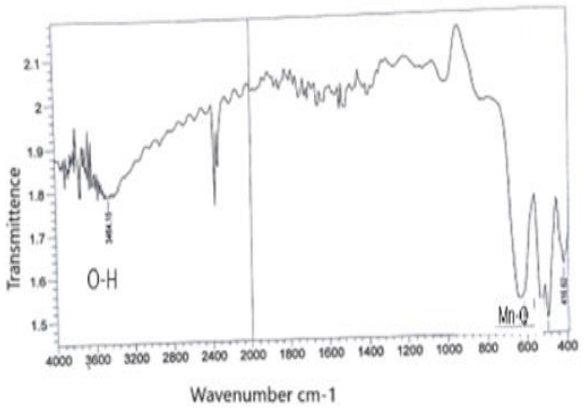

Fig. 1: Characterization of Mn3O4 nanoparticles by FTIR

Characterization of Mn3O4 by X-ray diffraction

The crystalline structure and phase purity of nanoscale Mn3O4 are characterized by X-ray diffraction, as shown in Figure .2. Data from ICCD International Center for X-Ray Diffraction [Card no: 96-101-1263] The average crystal size was calculated as 35.27 nm, and the crystalline form was found to be tetragonal [24].

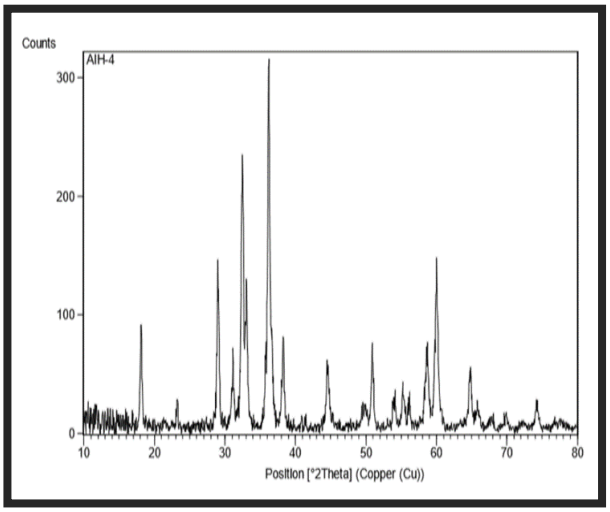

Figure (2) XRD spectrum for Mn3O4 nanoparticles of green chemistry

Characterization of Mn3O4 nanoparticles by EDX

The percentage of elements present in the nanoscale Mn3O4 by energy-dispersive X-rays is shown in Figure .3, as the results showed the presence of manganese (62.6%), oxygen (28.6%) and carbon (8.8%).

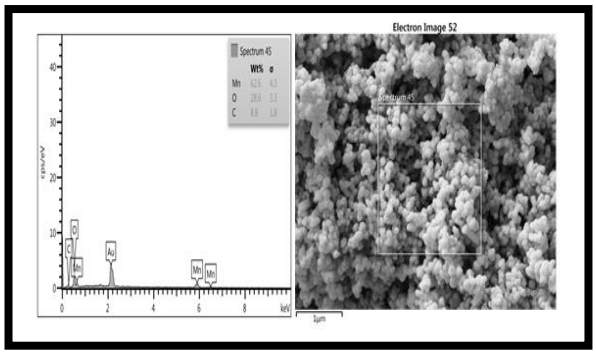

Figure .3 Energy dispersive X-ray spectrum of Mn3O4 nanoparticles

Characterization of Mn3O4 nanoparticles by SEM

As seen in Figure 4, the morphological and structural compositions of Mn3O4 nanoparticles were determined using a scanning electron microscope (SEM). These particles have an average diameter of roughly 80.61 nm

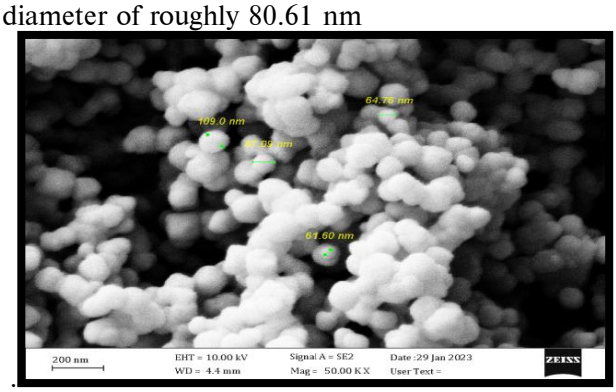

Figure .4 Scanning electron microscopy of Mn3O4 nanoparticles

Mn3O4 nanoparticles' impact on HL-60 cells.

Figure 5 demonstrates the vitality of HL-60 cells after 24 hours of treatment with Mn3O4 NPs at various doses (20-160 µg/ml) compared to Blank. The killing rate was 4% at a concentration of 20µg/ml and 14% at a concentration of 40µg/ml. There is a correlation between the percentage of inhibition or killing and the increase in concentration, with the killing rate being 24% at concentrations of 80µg/ml and 40% at concentrations of 160µg/ml. However, at a 20 g/ ml

dosage, the death rate was just 4% following a 48-hour incubation period. It was 26% at a concentration of 40 g/ml, 44% at an 80 g/ml concentration, and 65% at a 160 g/ml concentration; Figure 6 illustrates a relationship between time and the percentage of inhibition or killing.

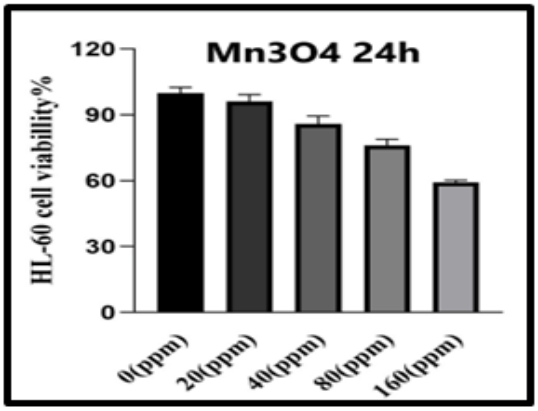

Fig. 5: Inhibition of Mn3O4 Psfor HL-60 cells in 24 h

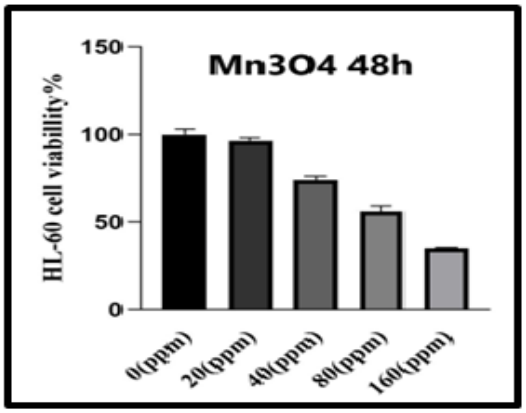

Fig. 6: Inhibition of Mn3O4 Psfor HL-60 cells in 48 h

Figure 7 illustrates the results, which indicated an IC50 value of 220.5 in 24 hours and 97.83 in 48 hours.

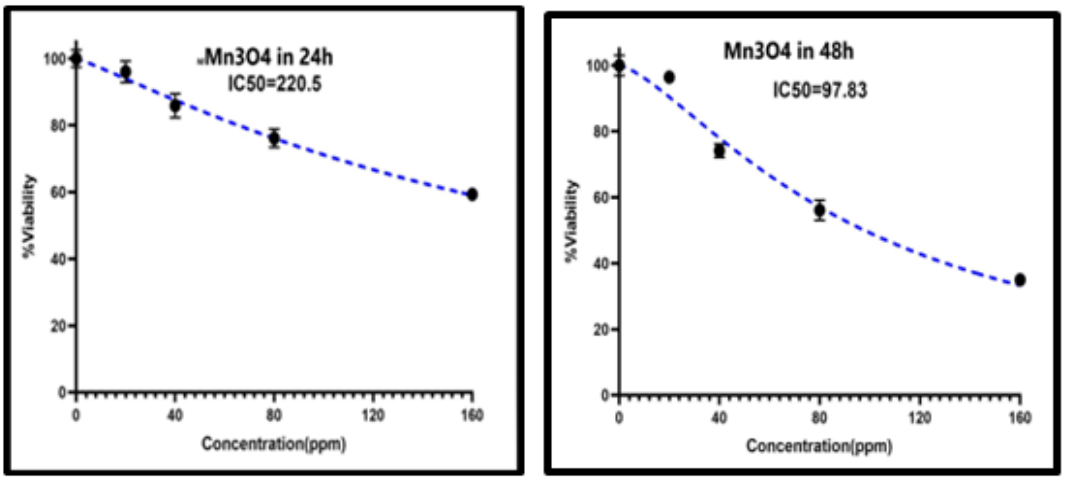

Figure 7. IC50 value for Mn3O4NPs at 24 and 48 hours

The cytotoxicity of the Mn3O4 nanoparticles compound (shown in Fig. 8) was examined, and the findings indicated that the compound was safe (non-toxic) at all doses.

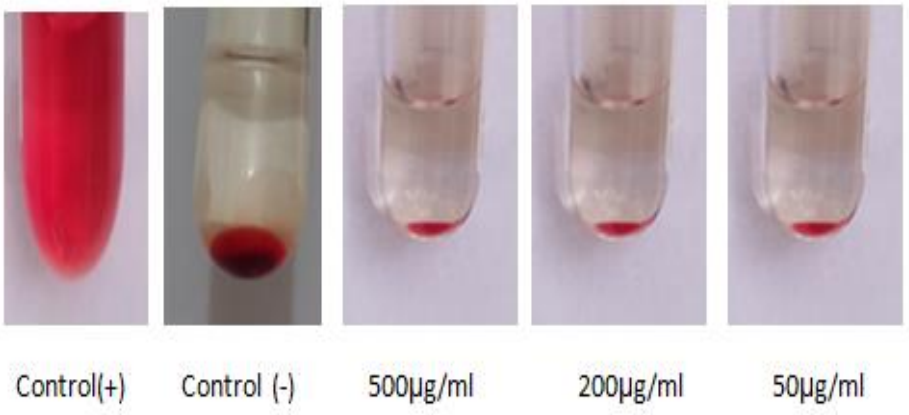

Fig. 8: cytotoxicity test for Mn3O4 nanoparticles

Nanoparticles to treat leukaemia have attracted much interest in recent years. Metal nanoparticle synthesis and modification based on the form, size, and target accumulation are necessary to build a practical nanotechnology approach. Among the nanoparticles with promise in contemporary nanobiotechnology are Mn3O4 particles for their antioxidant, antibiofilm, antibacterial, and anticancer properties [25,26]. According to earlier research, the NPs have demonstrated practical anticancer effects against various cancer cells, including colon, cervical, leukaemia, breast, and neuroblastoma [27]. They do this by increasing the amount of intracellular ROS, disrupting the mitochondrial membrane, and inducing programmed cell death against cancer cell lines.

Conclusions

The current work covered the green chemistry of producing Mn3O4 nanoparticles by co-precipitating Chia seed extract. Mn3O4 nanoparticles' structural properties were examined using FTIR, XRD, and SEM. ZnCO3 nanoparticles have been proven in experiments to be effective leukaemia reducers and may have therapeutic effects as anticancer agents. Increasing the amount of ROS inside cells, rupturing the mitochondrial membrane, and inducing programmed cell death against them.

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