Antitumor impact of iron oxide nanoparticles in Ehrlich carcinoma-bearing mice

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ABSTRACT

Magnetic nanoparticles are promising approaches for cancer treatment. Iron oxide nanoparticles have been pre-formed by the co-precipitation method to investigate their antitumor activity on Ehrlich carcinoma in mice. The nanoparticles have been studied by UV spectrophotometer, transmission electron microscopy, dynamic light scattering, and zeta potential measurement. To determine the therapeutic impact of iron oxide nanoparticles in tumor-bearing mice, in vivo experiments were carried out. Fourier Transform Infra-red (FTIR) spectroscopy and DNA comet assay were used to estimate the toxicity of nanoparticles of iron oxide. The tissue was analyzed using FTIR spectral parameters such as the area beneath the peak, peak position, and peak intensity. The results showed that iron oxide nanoparticles significantly led to antitumor activity against Ehrlich carcinoma. There was a low shift in position and intensity at the amide I band (1650 cm\textsuperscript{-1}) in tissues of mice treated with iron oxide nanoparticles compared to the control group. Comet examination showed a decrease across all the parameters of the assay (tail moment, tail DNA percentage, and tail length) in the tumor + iron nanoparticles group, having values lower than the tumor group. According to the presented results, iron oxide nanoparticles have exhibited therapeutic efficacy against cancer.

1. Introduction

One of the most health problems is cancer despite tireless efforts to find drugs for it (Nagai & Kim, 2017; Naghavi, 2019; Torre et al., 2015). This is justified by the significant increase in cancer incidence and mortality rate from the end of the eighteenth century until today (Ferlay et al., 2014). Although there has been a steady and continuous increase in cancer rates, there has been a general decline in death rates in recent years (Miller et al., 2016). The decline in death rates can be easily linked to the continuing advances in the medical and pharmaceutical fields that have allowed a reduction in cancer deaths (Soneji et al., 2014). Chemotherapy and radiotherapy are two of the most common methods of treating cancer, but due to their severe side effects (Liu et al., 2015; Mohan et al., 2019; Stadtmauer et al., 2000), researchers are looking to other less harmful treatments.

Recently, nanoparticles are attracting special interest for their use in the treatment of tumors (Heath & Davis, 2008) especially magnetic nanoparticles, as they are most effective in suppressing tumors (Gu et al., 2019). Moreover, it has a low toxicity effect and high biocompatibility with normal human tissues (Fouad & Mohamed, 2011; Hartman et al., 2008). It is necessary to maintain the stability of the nanoparticles to reduce their aggregation, to be highly biocompatible, and to improve cellular uptake (Darwish, 2017). It can be done by coating the surface of the particle with organic layers such as oleic acid (Azzam & Zaki, 2016; Darwish et al., 2016). Also, it was reported that iron oxide nanoparticles can target cancer DNA gens (Kovacevic, 2012) which enhances proliferation and apoptosis of cancer cells (Hartmann et al., 1997).

Fourier Transform Infra-red (FTIR) spectroscopy is a well-known laboratory technique for studying and characterizing the structure of a wide range of compounds (Igci et al., 2017; Wilson et al., 2010), among which polypeptides and proteins (Arumugam et al., 2019; Elshemey et al., 2010; Kong & Yu, 2007). FTIR has been applied for optical tissue diagnosis; it has been utilized to characterize several cancer types (Bellisola & Sorio, 2012; Diem et al., 2002; Eckel et al., 2001; Fabian et al., 2006; Kendall et al., 2009; Lima et al., 2015; Maziak et al., 2007). FTIR investigations have been primarily performed on whole tissues without further extraction (Elshemey et al., 2016; Verdonck et al., 2016) which has the advantage of time-saving and simplicity.

Comet assay in cancer studies has been continuously applied for the determination of DNA damage due to its specificity. Its most common method involves detection at high pH to detect DNA single-strand breaks and double-strand breaks (Olive et al.,...
Comet assay can detect specific DNA lesions that can identify individual DNA lesions and monitor individual therapy responses for DNA repair.

In the current work, we have examined iron oxide nanoparticles coated by oleic acid to assess its antitumor impact using Ehrlich carcinoma bearing mice as a tumor model.

2. Materials and methods

2.1. Chemicals

Oleic acid (≥99%) and ammonium hydroxide (26% NH₃ in H₂O) were purchased from Sigma–Aldrich, Germany. Iron (III) chloride hexahydrate (FeCl₃ · 6H₂O) (≥99.99%), iron (II) chloride tetrahydrate (FeCl₂ · 4H₂O) (≥99.99%) were purchased from Sigma-Aldrich (USA).

2.2. Formulation of oleic acid-coated iron oxide nanoparticles

In one liter of distilled water, 54 g FeCl₃ · 6H₂O and 19 g FeCl₂ · 4H₂O were dissolved and heated to 70°C. To precipitate the black iron oxide magnetite, ammonium hydroxide (60 mL) was applied rapidly to the formulated solution. The suspension was stirred at 70°C for 30 minutes to vaporize any ammonium salts impurities. The nanoparticles were transferred to 0.5 L of dichloromethane and 10 mL of oleic acid, which serves as steric stabilizers, after cooling to room temperature.

2.3. UV spectrophotometry

The UV spectra of iron oxide nanoparticles were recorded using a UV Spectrophotometer (Shimadzu, Japan).

2.4. Transmission electron microscope (TEM)

The prepared iron oxide nanoparticles’ morphology and size were examined by TEM (JEM 1230; Joel, Japan).

2.5. Dynamic light scattering (DLS) and zeta potential measurement

The size and zeta potential of the formed iron oxide nanoparticles were measured by a Nano ZS90 Zetasizer (Malvern Panalytical, UK) at room temperature.

2.6. In vivo experiment

2.6.1. Treatment protocol

All animal strategies were approved by the Institutional Animal Care and Use Committee at Cairo University (CU-IACUC) (approval number: CUIF2078). Fifteen Swiss male albino mice were purchased from the National Institute of Cancer, Egypt, with a mean weight of 23 ± 3.5 g. Mice were divided randomly into three groups, five mice per group. Group one was control (negative control). Groups two and three were injected into their right thigh subcutaneously with the suspension of Ehrlich ascites. Tumors matured into a single solid mass fourteen days later, with sizes
of approximately 0.29 to 0.62 cm$^3$ in the mice. Mice in group two were injected longitudinally into the tumor with 10 µg/ml of saline (positive control). Group three was injected into the tumor with the same volume (10 µg/ml) of iron oxide nanoparticles. After injection, all mice were maintained at a constant temperature of 24°C for 24 h and then sacrificed. Mice of all experimental groups were sacrificed with overdose of an anesthetic at the end of the treatment period.

### 2.7. Fourier-transform infrared spectroscopy (FTIR)

FTIR spectra were registered for lyophilized tumor tissues. Tissues were crushed with KBr at a 1:100 ratio and are pressurized with a hydraulic press under a pressure of 15,000 pounds of pressure. The samples' pellets have been scanned over the spectrum of 400–4000 cm$^{-1}$ by type A FT/IR-4100 (A Basic Vector, Germany). All tissue samples underwent the same treatment, and thus, it is likely that the spectral variations observed are correlated with the corresponding variations in the various samples.

### 2.8. Processing of FTIR data

Control, tumor, and tumor + iron nanoparticles samples registered FTIR spectra were collected, the area was standardized, and the average spectrum was computed for each of the three samples. The difference between control, tumor, and tumor + iron nanoparticles samples was explored using the absorption peak at 1645 cm$^{-1}$ (owing to stretching vibration of C = O bond). To obtain quantitative values, FTIR characterization parameters such as peak position, peak intensity, and area under the peak were computed for the chosen peak. With the assistance of Microsoft Excel 365, the parameter of average area under the peak was computed from the spectral data using the trapezoidal integration technique.

### 2.9. Comet assay

A comet test is a sensitive technique for determining DNA damage (Rageh et al., 2020). The comet test was conducted to identify and compare DNA damage to
tumor tissues in the treated and control groups. For each sample, a fluorescence microscope with a magnification power x 400 was used to search for comets in the cells. Kinetic Imaging, Ltd. (Liverpool, UK) software analysis (comet 5 images) connected to a CCD camera has been used. The length of migrated DNA and the percentage of it were measured. Also, the tail moment and the olive moment have been determined.

2.10. Statistical analysis

All data measured have been computed as an average and standard deviation (STD). For a comparison of data
with the control group, one-way variance analysis (ANOVA) was used. $P < 0.05$ was shown to be significant.

3. Results and discussion

In this study, we synthesized iron oxide nanoparticles. The UV spectrum of iron oxide nanoparticles was shown in Figure 1(a). The formation of iron oxide nanoparticles can be confirmed by the presence of a strong absorption at 300 nm and a peak at 495 nm. The absorption bands were due to surface plasmon (Mahdavi et al., 2013; Rosli et al., 2018). TEM micrograph elucidates the spherical shape of the prepared iron oxide nanoparticles (Figure 1(b)).

DLS results displayed that iron oxide nanoparticles have a size distribution of $32.67 \pm 13.86$ nm signifying the monodispersity of the sample (Figure 2). The zeta potential of iron oxide nanoparticles was $+40.3 \pm 9.9$ mV.

Figure 3 showed the average FTIR spectra of control, tumor, and tumor + iron nanoparticles tissue samples. Notably, the tumor sample demonstrated different spectra compare to both control and tumor + iron nanoparticles samples, the amide II (1540–1570 cm$^{-1}$) and amide I (1600–1700 cm$^{-1}$) bands have the uppermost absorption peaks, which was confirmed by data in (Ziegler et al., 2018, 2014). Surprisingly, the three samples were separated at the absorption peaks around 2925 cm$^{-1}$ and 2850 cm$^{-1}$. These peaks were due to saturated lipids and the side chain of proteins (Zohdi et al., 2015). Noteworthy, the tumor sample has the lowest peak values.

3.1. Characterization of tissue FTIR

Tissue characterization statistical significance was calculated using parameters generated from the absorption peak at 1650 cm$^{-1}$. Figure 4(a–c) introduced the characterization parameters which distinguished between control, tumor, and tumor + iron nanoparticles samples. The parameters were peak position at 1645 cm$^{-1}$, the peak strength at 1645 cm$^{-1}$, and the area under peak (AUP) at 1633–1720 cm$^{-1}$ band. These parameters were a reliable light spot because they can differentiate between samples significantly.

The peak position of the amide I of the tumor sample has been shifted to 1652 cm$^{-1}$ compared to 1648 cm$^{-1}$ for the control and 1650 cm$^{-1}$ for the tumor + iron nanoparticles (Figure 4(a)). Figure 4(b,c) showed that the tumor samples have the lowest values of both peak intensity and the AUP. The values of the tumor

Figure 5. Parameters of comet assay in control, tumor, and tumor + iron nanoparticles groups. The data are presented as mean ± standard deviation ($n = 5$).

Figure 6. Comet images of a) control group; b) tumor + iron nanoparticles group; and c) tumor group.
samples were 0.00022 and 0.02 respectively, while the control samples have a peak intensity of 0.00026 and AUP of 0.0229, and the tumor + iron nanoparticles samples have a peak intensity of 0.00024 and AUP of 0.0217. This confirmed that FTIR parameters can differentiate tumor samples from control and tumor + iron nanoparticles ones, which proved the inhibitory effect of iron oxide nanoparticles on cancer in the tumor + iron nanoparticles mice group.

Figure 5 represents comet assay to evaluate the effectiveness of iron oxide nanoparticles in damaging the tumor tissue DNA. Comet examination displays a rise in the comet parameters (tail moment, tail DNA percentage, and tail length) in tumor and tumor + iron nanoparticles groups compared to the control group. Briefly, the tumor + iron nanoparticles group showed significantly lower values of the comet parameters than the tumor group. The group of mice treated with iron oxide nanoparticles showed tail moment, tail DNA percentage, and a tail length of 15.13, 76.66, and 15.35, respectively. While mice groups that had tumors only showed tail moment, tail DNA percentage, and a tail length of 17.09, 96.80, and 25.22, respectively. The comet images in Figure 6 reinforced these findings. DNA damage was observed in many cancer types (Hanahan & Weinberg, 2011; Vodicka et al., 2019), the comet assay was widely used to evaluate the effectiveness of iron oxide nanoparticle treatment on cellular DNA in tumor tissues. The findings suggest that iron oxide nanoparticles can be used to treat tumors. These findings corroborated previous research on the use of iron oxide nanoparticles to treat tumors (Li et al., 2017; Stolyar et al., 2021). Iron oxide nanoparticles prevented cancer growth and development by promoting apoptosis and inhibition of cell proliferation (Li et al., 2017). The positively charged iron oxide nanoparticles bound onto the cell surface, as they have opposite signs of zeta potential (+40.3 ± 9.9 mV) compared to negative phospholipid head groups on cell membranes (Negoda et al., 2013). Therefore, the cytotoxic mechanism includes the disruption of the cell membrane, which led to necrotic cells, and the disruption of the mitochondrial membrane, which led to apoptosis.

4. Conclusion

Iron oxide nanoparticles with a mean diameter of 32.67 and a zeta potential of + 40.3 ± 9.9 mV promote tumor therapy in vivo, as these nanoparticles damage the DNA of cancer cells and affect the cell membrane to prevent cancer growth and development.

Authors’ contributions
Amr Abd-Elghany and Ebtesam Mohammed conceived, designed the research, and analyzed the data, conducted experiments and contributed new reagents or analytical tools, and wrote the manuscript. The authors have read and approved the manuscript.

Compliance with ethical standards
Conflict of interest The authors declare that they have no conflict of interest.

Disclosure statement
No potential conflict of interest was reported by the author(s).

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