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RESEARCH ARTICLE

Cytotoxic activity and Magnetic Behavior of green synthesized iron oxide nanoparticles on brain glioblastoma cells

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ABSTRACT

Iron oxide nanoparticles are one of the most applied metal nanoparticles with advantageous properties in biomedicine that can be cost-effectively and rapidly produced through green synthesizing methods. The purpose of this study was to study the toxicity of iron oxide nanoparticles (Fe_2O_3 NPs), which were green synthesized by Prosopis farcta extract, on brain glioblastoma cells (U87). Powder X-ray Diffraction (PXRD), Vibrating-Sample Magnetometer (VSM), Field Energy Scanning Electron Microscopy (FESEM), Energy-Dispersive Spectroscopy (EDX), and Raman technics were performed to evaluate the physicochemical properties of this product. According to results, the green synthesized Fe_2O_3 nanoparticles contained a spherical morphology in the size range of 20-45 nm with superparamagnetic features. Additionally, their cytotoxic activity was surveyed against U87 cells by MTT assay, and the outcomes indicated the lack of any cytotoxic activity until reaching the concentration of 500 µg/mL. Therefore, our synthesized Fe_2O_3 NPs can be proposed as a proper candidate for being applied in the drug delivery of cancer treatments.

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INTRODUCTION

In recent years, cancer has been recognized as one of the leading causes of death in the world [1]. Among the varying types, brain cancers are acknowledged as deadly diseases due to their late diagnosis, as well as limited conventional treatments that still remain as an unsolved problem [2, 3]. Related researches in this area identified most of these tumors to be glioblastoma, histiopathologically [4]. There is a very poor prognosis available for this cancer despite the many efforts [5], which highlights the necessity of discovering an efficient method for its timely diagnosis and treatment. Currently, the common treatments for this type of cancer include surgery followed by partial radiotherapy along with chemotherapy [6, 7].

Despite the numerous advancements in brain tumor surgery, it is impossible to remove all of the cancer cells for a variety of reasons. Consequently, the rapid growth of the remaining cancer cells in the tumor bed after surgery results in treatment failure and tumor recurrence [8]. The combination of radiotherapy and chemotherapy is used to

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annihilate the remaining cells, which is commonly faced with limiting factors such as the resistance of cancer cells to these treatments and the lack of inserting sufficient doses of chemotherapy drugs to these cells. Various methods were designed and proposed to eliminate the obstacle of radiation resistance and also increase the rate of drug delivery to cancer cells [9]. Considering the ongoing researches, the application of superparamagnetic nanoparticles can stand as an effective technique in this field [10-12]. These products can play an important role througout the treatment of cancer cells in the brain due to their effects on radiation sensitization and also the portability of various drugs [13].

In recent years, researchers tried many attempts to produce suitable nanodrugs for the treatment of cancer with the help of many physical and chemical methods provided by intermittent and therapeutic technologies [14]. Iron oxide is a significant candidate for the treatment of cancer due to its superparamagnetic properties and variable surface features. The synthesis of iron oxide in the presence of an oxidant leads to the production of several types of iron oxides, including magnetite and hematite, which were mainly exerted in medical research [15, 16]. Causing a reduction in drug resistance and drug dosage, as well as biocompatibility, biodegradability, and providing a greater efficiency in tumor diagnosis, targeting, and treatment are among the factors that prove the applicability of iron oxide magnetic nanoparticles in clinical application (Such as cell therapy, tissue repair, and drug conduction) [17].

The synthesizing physicochemical methods of nanoparticles are known to be relatively expensive and toxic with the risk of causing destructive effects on the environment [18]. In this regard, nanotechnology presented the valuable gift of green synthesis to the world, which can be conducted by natural and biodegradable agents, such as plant extracts as the reducing and trapping agents, in combination with metals such as iron [19, 20]. In comparison to the other mechanical strategies, this technology is safe, simple, non-toxic, and efficient. The required procedure for green synthesizing nanoparticles by the usage of plant materials is usually a single-step and effective reaction that lacks the need for surfactants and other capturing agents. Biologically active substances and compounds of plant extracts, including water-soluble active metabolites, can be applied in a single-step strategy to cause the reduction of metal ions into nanoparticles at room temperature [20].

As a member of Leguminosea family and the subfamily of Mimosoideae, *Prosopis farcta* is native to the arid and semi-arid regions of America, Asia, and Africa. The medicinal properties of this plant include gastric ulcer, abortion, bloody diarrhea, rheumatism, laryngitis, heart pain, and shortness of breath. It is assumed that most of these properties are caused by the presence of Tannin, Tryptamin, Quercetin and Apigenin compounds in *P. farcta* [21]. Therefore, this study introduced the preparation of iron oxide nanoparticles by exerting the aqueous extract of *P. farcta*, and also evaluated the cytotoxic activity of synthesized nanoparticles on brain glioblastoma cells (U87).

MATERIALS AND METHOD

Extraction of Prosopis farcta

Distilled water was added to the powdered (ratio 1:10), and weighted bark plant of *P. farcta* to be shaken for 10 hours with a rate of 150 rpm. The obtained mixture was filtered by using a filter paper of Whatman No. 1. The prepared extract was stored in a refrigerator for the upcoming experiments.

Synthesis of Fe_2O_3 NPs

To begin the synthesis of nanoparticles, 20 mL of aqueous extract of *P. fracta* was added to Fe (III) chloride solution (1M) and stirred in 70 °C for 3 h. The pH of solution was adjusted to 11 by the application of NaOH solution (1M) and the obtained brown solution was dried at 80 °C. The resulting powder was calcined in a furnace at 400 and 600 °C for 2 h, separately. Finally, the brown powder of iron oxide nanoparticles (Fe_2O_3 NPs) was produced. The synthesized iron oxide nanoparticles at 400 and 600 °C were labeled as Fe-400 and Fe-600, respectively.

Characterization

The physicochemical properties of green synthesized Fe_2O_3 NPs were determined by the results of Powder X-ray Diffraction (PXRD, DAD4 Advance-Bruker model, Netherlands), Field Energy Scanning Electron Microscopy (FESEM, TESCAN model of MIRA3), and Fourier Transform Infrared spectroscopy (FT-IR, Bruker Tensor27) devises.

Cytotoxic performance

We examined the *in vitro* cytotoxicity activity of green synthesized Fe_2O_3 NPs on brain glioblastoma

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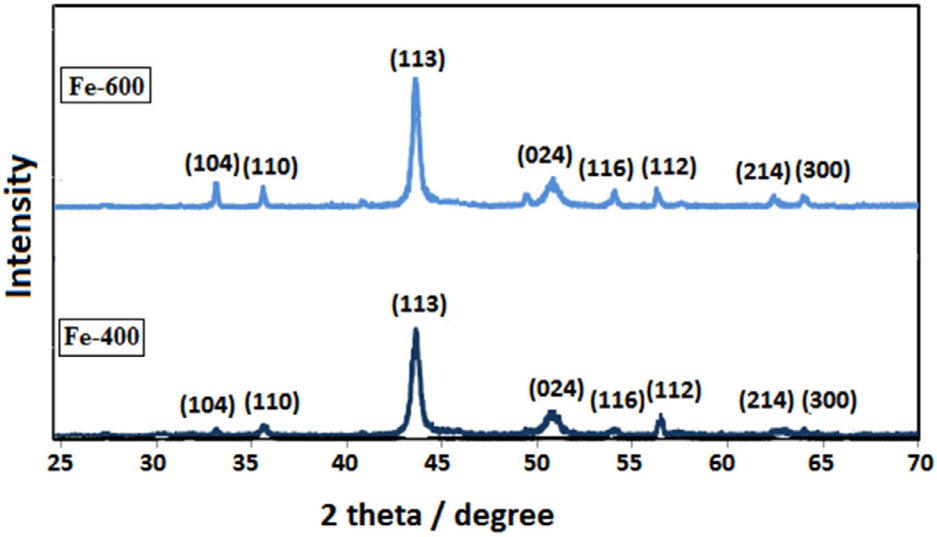

Fig. 1. PXRD pattern of green synthesized Fe_2O_3 NPs at 400 and 600 ˚C.

cells (U87) by the usage of MTT assay. Briefly, a certain number of cells (10^4) were aliquot onto each well of a 96-well microplate and incubated in a humidified atmosphere of 5% CO_2 and, 95% air at 37 ˚C to reach the confluence of about 70-90%. Then, 150 µL of nanoparticles were added to each well subsequent to being incubated at 37 ˚C in a serum containing media for 24 h. In the following, the medium was removed and the wells were washed twice for 2-3 min with 150 µL of phosphate buffer saline. 25 µL of MTT (3-(4,5 Dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium, Sigma-Aldrich, USA) stock solution was transferred into each well to perform an incubation process in a humidified atmosphere of 5% CO_2 and 95% air for 4 h at 37 ˚C. 100 µL of DMSO was added to each well in order to dissolve the produced formazan. As the last step, the adsorption of each sample was recorded by the usage of ELISA reader (Model 50, Bio-Rad Corp, Hercules, CA) at a wavelength of 570 nm and also, the percentage of cell viability (survival) was calculated through the following formula:

Cell viability (%) = [100 × (sample abs)/ (control abs)].

RESULTS AND DISCUSSION

PXRD analysis

Fig. 1 exhibits the PXRD pattern of green synthesized Fe_2O_3 NPs by the aqueous extract of *P. fracta*. Accordingly, the observance of peaks with indexes of (104), (110), (113), (024), (116), (112), (214) and (300) lines indicated the formation of Fe_2O_3 NPs [22]. The crystalline size of Fe-400 and Fe-600 was calculated by the application of Debye-Scherrer equation ($D=0.89\lambda/\beta\cos\theta$; where D refers to the crystal size of particle, λ would be the X-ray wavelength used in the test, β stands for the Full width at half maximum in radians, and θ represents the Angle of diffraction) [23], which was obtained to be 18 and 29 nm for Fe-400 and Fe-600, respectively. Apparently, heightening the applied calcination temperature results in increasing the crystalline size of particles.

FESEM and EDX analysis

The morphology and size of green synthesized Fe_2O_3 NPs by *P. faracta* aqueous solution were identified through the outcomes of FESEM analysis. Fig. 2 demonstrates the spherical morphology of green synthesized Fe-400 and Fe-600, which were observed in a size of 20-30 nm and 35-45 nm, respectively. As it is displayed, the sizes of particles were increased as a result of heightening the applied calcination temperature and therefore, this parameter can function as a growth agent of particles. The elements of green synthesized Fe_2O_3 NPs was determined by the exertion of EDX analysis and the obtained EDX graph is presented in Fig. 3, which displays the peaks of Fe and O elements at both temperatures.

FT-IR analysis

The FT-IR spectra of green synthesized Fe_2O_3 NPs was examined in the range of 400–4000 (Fig. 4).

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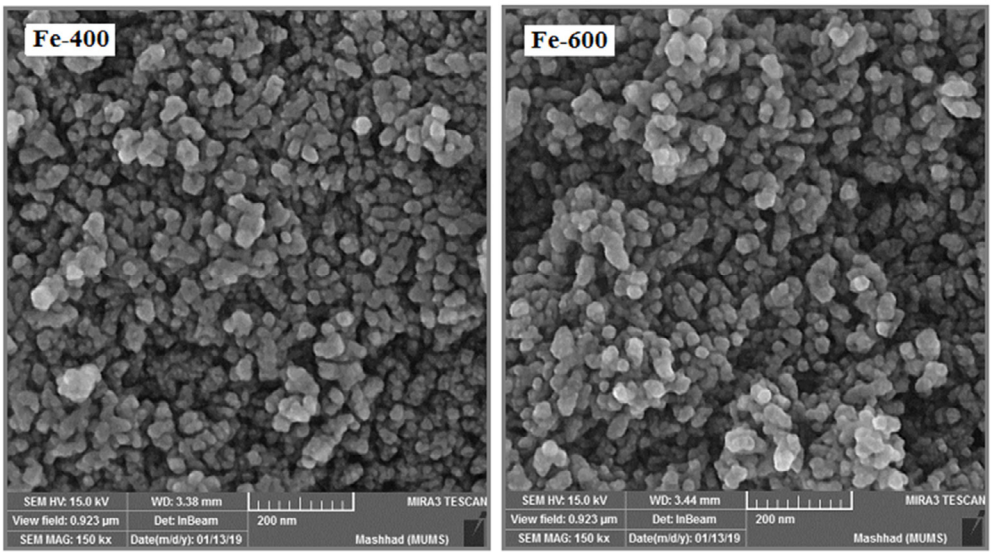

Fig. 2. FESEM images of green synthesized Fe_2O_3 NPs at 400 and 600 ˚C.

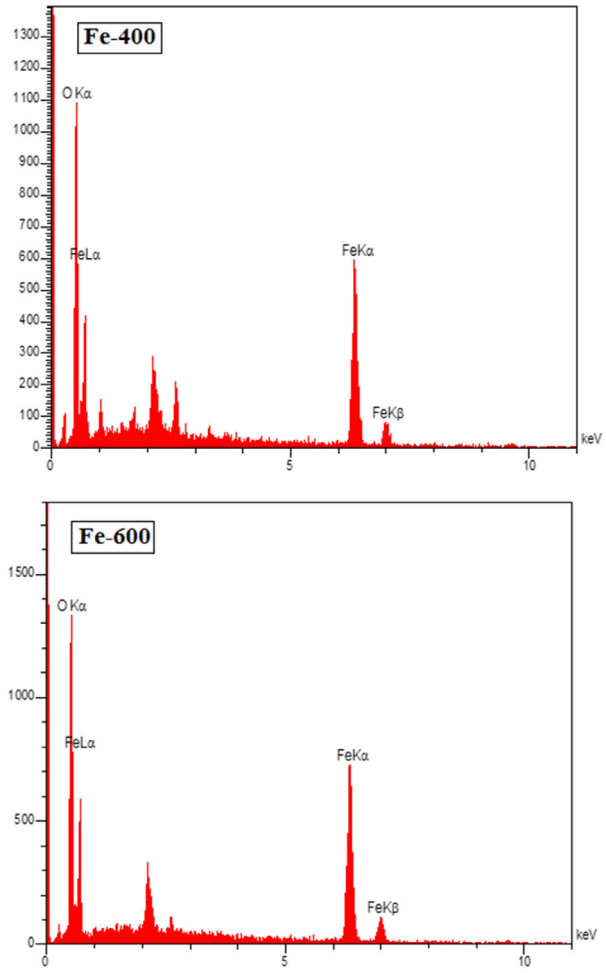

Fig. 3. EDX graph of green synthesized Fe_2O_3 NPs at 400 and 600 ˚C.

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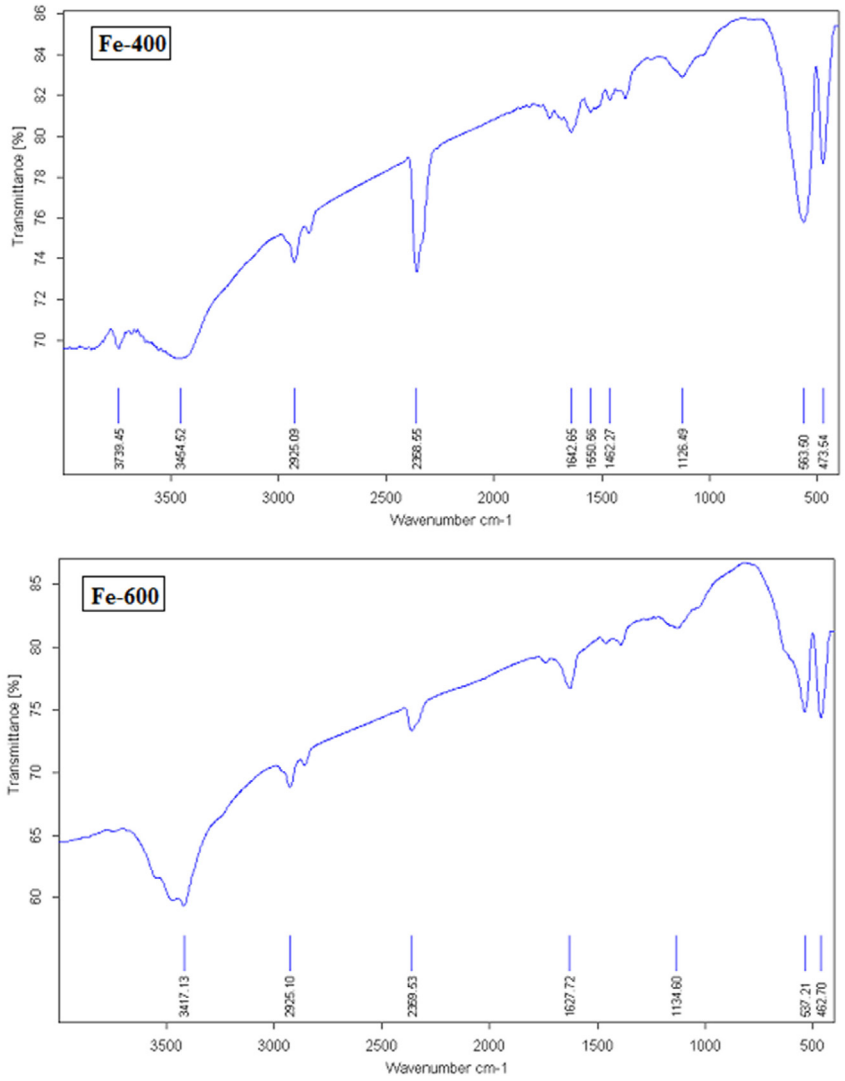

Fig. 4. FT-IR graph of green synthesized Fe_2O_3 NPs at 400 and 600 ˚C.

The detected absorption band at 3400 cm^{-1} signified the strong stretching vibration of OH groups of adsorbed H_2O on nanoparticles surface, while the absorption band at 1600 cm^{-1} allocated to the stretching vibration of C-H groups. The recorded absorption bands at the points of 563 and 473 cm^{-1} for synthesized Fe-400, as well as the peaks at 537 and 462 cm^{-1} peaks for synthesized Fe-600, were related to the vibrational bands of O-Fe-O and Fe-O, respectively. Apparently, the growth of particles caused a decrease in the intensity of vibrational bands of O-Fe [24].

VSM analysis

The magnetic properties of green synthesized iron oxide nanoparticles were studied by the means of VSM analysis. The green synthesized Fe_2O_3 NPs were observed to be hematite. The Ms values of synthesized Fe-400 and Fe-600 were 15 emu/g and 1.4 emu/g, respectively (Fig. 5). According to the gathered data, the magnetic properties of NPs were decreased as a result of heightening the applied calcination temperature. Next to the displayed superparamagnetic behavior by synthesized Fe-400, increasing the calcination temperature and particle size caused a decrease in the superparamagnetic behavior of synthesized Fe-600. The outcomes were indicative of a direct relationship between the Ms content of Fe_2O_3 NPs with their particle size and particle shape anisotropy.

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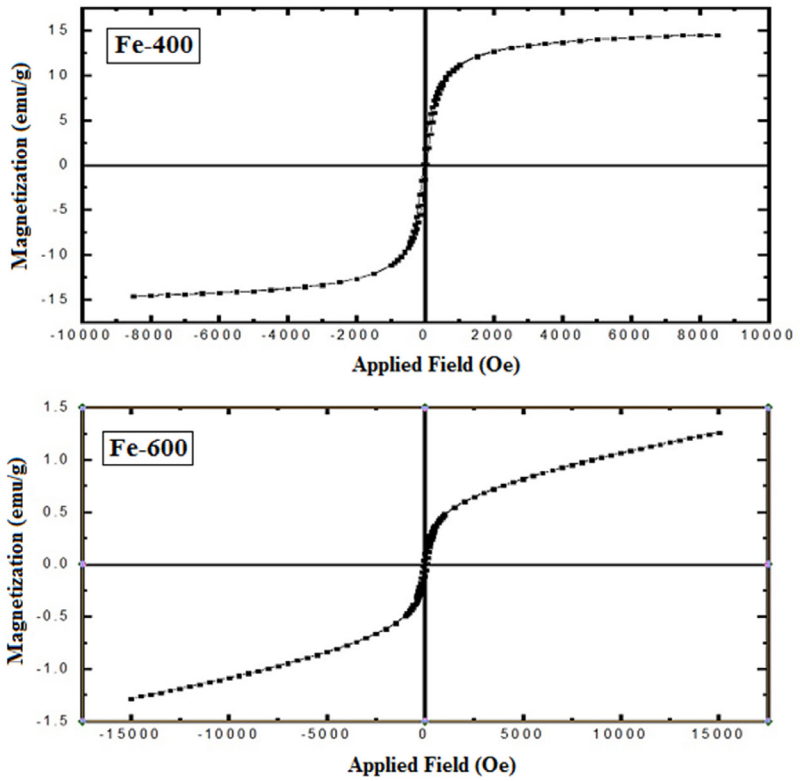

Fig. 5. VSM image of green synthesized Fe_2O_3 NPs at 400 and 600 ˚C.

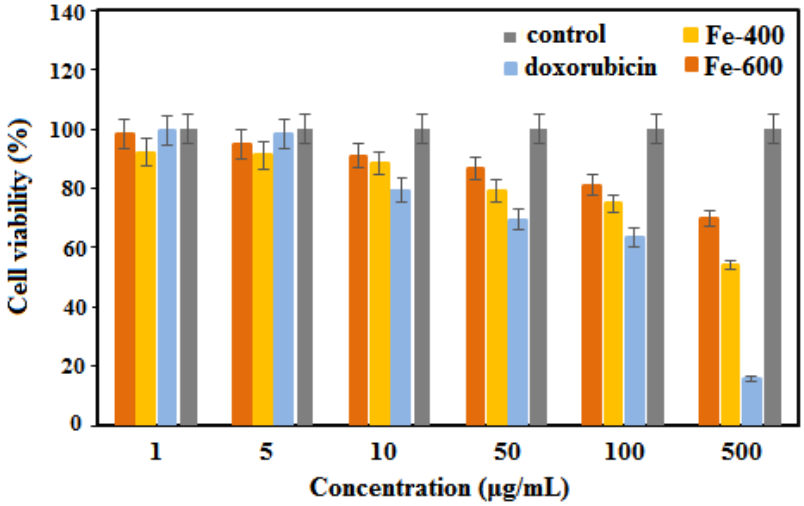

Fig. 6. Cytotoxic activity of green synthesized Fe_2O_3 NPs on U87 cell line at 24 h of incubation time.

Cytotoxic activity

We evaluated the cytotoxic effect of green synthesized Fe_2O_3 NPs on U87 cell line through the conduction of MTT assay. This test was performed on nanoparticles with a concentration range of 1-500 µg/mL with 24 h of treatment time (Fig. 6). According to results, the cytotoxic effects of green synthesized Fe_2O_3 NPs showed a dependency on concentration and particle size, while lacking any activity at concentrations lower than 500

μg/mL. The application of doxorubicin in a concentration of 500 μg/mL led to the inducement of high cytotoxic effects. According to the study of Ankamwar *et al* on different cancer cell lines, the toxicity of iron oxide nanoparticles was indicated to be dependent on nanoparticles concentration, while reporting the lack of observing any cytotoxic effects in concentrations lower than 100 μg/mL [25]. Due to their non-toxic and high magnetic properties, the application of green synthesized nanoparticles can be suggested for drug delivery and cancer treatment.

CONCLUSION

In this study, green synthesized Fe_2O_3 NPs was performance by using *P. fracta* extract at 400 and 600 °C of calcination temperatures. It demonstrate that *P. fracta* extract is able to reduction and stability of particles. The green synthesized nanoparticles was characterized using variety analytic methods. The results shows that particle have spherical shape with particle size 25-30 nm and 35-45 nm for Fe-400 and Fe-600, respectively. The results of VSM analysis presented that green synthesized nanoparticles have superparamagnetic property. The cytotoxicity performance of green synthesized Fe_2O_3 NPs presented no toxicity against U87 cell line. Therefore, green synthesized Fe_2O_3 NPs was suggested as a proper candidate to utilize in the field of drug delivery agent to cancer treatment.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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