Cytotoxicity of Iron Nanoparticles Synthesized Using Dried Ginger

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Authors’ contributions

This work was carried out in collaboration among all authors. Author AB designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors MJ and SP managed the analyses of the study. Author SR managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Zingiber officinale (also called ginger) is commonly used for traditional treatment of various diseases and for culinary purposes. This plant species is a herbaceous and develops yearly pseudostems around one meter tall bearing limited leaf cutting edges. The synthesis of metal nanoparticles and nanocomposites is an emerging area of research and exploration in the field of material science for their unique size and shape and have reliant features that are different from the regular bulk structure. The aim of the present study was to synthesize iron nanoparticles using dried ginger and evaluate their cytotoxic effects against brine shrimp. The nanoparticles were
synthesized using dried ginger and characterized by UV-vis spectroscopy. The cytotoxicity assessment was carried out using brine shrimp lethality assay. The iron nanoparticles did not show any cytotoxicity to the brine shrimp. This eco-friendly synthesis of iron nanoparticles from dried ginger has proved to be convenient, inexpensive and can be safely used in wide range of medicine and dental fields.

**Keywords:** Cytotoxicity; dried ginger; iron; nanoparticles.

### 1. INTRODUCTION

Ginger (*Zingiber officinale*) is widely utilized for traditional treatment of various diseases [1]. Ginger belongs to the family Zingiberaceae, which also incorporates turmeric, cardamom and galangal. The related dicots in the class Asarum are regularly called wild ginger in light of their comparable taste. Ginger is used in traditional medicine for fever, cold, sore throat and applied as paste for headaches [2].

Nanotechnology is the subdivision of technology that deals with proportions of particles less than 100 nm. The synthesis of metal nanoparticles and nanocomposites is an emerging area of research and exploration in the field of material science for their unique size and shape and have reliant features that are different from the regular bulk structure [3].

Metal nanoparticles are extensively used in a wide range of applications including chemical and biological sensing, catalysis, energy, drug and gene delivery and electronics [4-10]. Nanoparticles have been intensively studied over the last decade due to their characteristics such as the physical, chemical, electronic and electrical properties [11].

Iron nanoparticles are highly reactive materials and are extensively used in magnetic recording media such as magnetic tapes. The nanoparticle-based catalyst showed six times better catalytic activity compared to conventional material. The biomedical applications include MRI (Magnetic Resonance Imaging) contrast enhancement and hyperthermia treatment [12,13].

With reference to our previous studies [14-22] and other available literature, no report has yet highlighted the synthesis of iron nanoparticles using dried ginger. Studies on cytotoxicity of plant mediated iron nanoparticles are less reported [23-25]. Brine shrimp lethality assay utilizing *Artemia salina* larvae is a more comprehensive and effective test more commonly employed to evaluate cytotoxicity of bioactive compounds [26,27]. The present study aimed to biosynthesize iron nanoparticles from dried ginger and to analyse its cytotoxic effect.

### 2. MATERIALS AND METHODS

First, 100 mL of distilled water was taken in a measuring cylinder and 1 g of dried ginger extract was added into it. In 50 mL of distilled water, 0.5 g of dried ginger extract was added. The prepared extract was measured using a weighing machine. The solution was mixed well and the color of the solution was observed and noted. Secondly, the extract was boiled in the heating mantle for about 5-8 minutes. In each of the steps, the change in the color of the solution was noted and observed. After the extract preparation, iron chloride anhydrous was added (molecular weight-162.2 g). Thirdly, in 60 mL of distilled water, 0.324 g of iron chloride powder was added along with 40 mL of dried ginger extract. The prepared solution was kept in the magnetic stirrer. A magnet was placed inside the solution. The iron nanoparticles thus obtained was purified by repeated centrifugation at 7000 rpm for 10 minutes. The pellet was collected in a cuvette and peak value was measured. After 1 hour, the solution was taken in a cuvette and the peak value was measured in a spectrometer and the color change of the solution was observed periodically.

#### 2.1 Characterization of the Iron Nanoparticles

The synthesized iron nanoparticles were optically characterized using UV-vis absorption spectroscopy. The reduction process to form iron nanoparticles is evident by identifying the characteristic wavelength of the color of the iron nanoparticles as the atoms and molecules undergo transition from ground state to the excited state and exhibit surface plasmon resonance peak positioned at a certain wavelength.
2.2 Cytotoxic Effect of Iron Nanoparticles

Cytotoxicity effect determines whether the bioactive compound is toxic to cells [28]. Brine shrimp lethality assay using the crustacean, *A. salina* is a reliable and convenient method to evaluate the cytotoxic effect of bioactive chemicals. Brine shrimp eggs were procured from Aquatic Remedies, Chennai. Artificial sea water was prepared in a hatching chamber by dissolving 36 g of iodine free salt into 1000 mL of distilled water. The hatching chamber was divided into dark area where shrimp eggs were added and light area with the lamp above. The brine shrimp hatch in two days to mature as larva. The hatched nauplii were used to evaluate the cytotoxic effect of dried ginger mediated iron nanoparticles. 10-12 mL of saline was added in a 6 well ELISA (Enzyme Linked Immunosorbent Assay) plate. Different concentrations of dried ginger mediated iron nanoparticles (5 µl, 10 µl, 15 µl, 20 µl, 25 µl) were added to each well. The artificial sea water up to 10 µl was taken in a well as control. 10 nauplii were added at each well and the number of live nauplii observed after 24 h incubation (Fig. 1).

3. RESULTS AND DISCUSSION

3.1 Visual Observation

In the visual identification, there was color change at various intervals of the incubation time. The first color change was observed when the iron chloride is reduced to iron nanoparticles giving a light yellow color. After 2 hours of the incubation period there is change in the color from light yellow to reddish brown. After 24 hours the color was changed to brown. At the end of 36 hours the color of the solution became dark brown indicating the complete synthesis of the iron nanoparticles (Fig. 2).

![Cytotoxic effect observation](image1)

**Fig. 1.** Cytotoxic effect observation. (A) Different concentrations of iron nanoparticles synthesized from dried ginger. (B) ELISA plate wells with different concentrations of dried ginger mediated iron nanoparticles observed for presence or absence of live nauplii after 24 h incubation

![Color change in synthesis process](image2)

**Fig. 2.** Visual observation of color change in the synthesis process at various periods of incubation time. (A) Baseline; (B) After 24 h of incubation time; (C) After 36 h of incubation time
3.2 UV–vis Spectrophotometer Analysis

The UV- spectroscopy analysis depends on the arising color of the reaction mixture due to transition of ions from ground state to excited state. The reduction process of the solution was optically measured at different wavelength regions from 250 nm - 650 nm and was recorded at various time periods. In the present study, the UV–vis spectroscopy showed the surface plasmon resonance peak positioned at a wavelength of 450 nm (Fig. 3). This finding is inconsistent to a previously reported study [29] on the synthesis of iron nanoparticles from Mentha spicata leaf extract with absorption peak ranging from 360-430 nm.

3.3 Cytotoxic Effect of Iron Nanoparticles

The fatality of the nauplii was observed and the number of live nauplii after 24 h incubation was tabulated based on the concentration of dried ginger mediated iron nanoparticles added to each of the wells in the micro plates (Table 1). In the present study, the cytotoxicity of iron nanoparticles has been shown to be increasing at higher concentrations (Fig. 4). It was noted that the % cytotoxicity against A. salina was lowest at 5 µl and 10 µl concentrations (0% and 10%) respectively and moderate at 15 µl (30%) and 20 µl (20%) concentrations. The % cytotoxicity was highest (33%) at 25 µl concentration.

Iron nanoparticles can be synthesized by green approach [30-32]. Iron nanoparticles synthesized from various plant- based materials by reliable and simple methods are available in the literature. These nanoparticles can be synthesized via controlled bio-precipitation using extracts of different plant parts such as leaf extracts of garlic vine and oolong tea extract [30, 32]. The present study indicated that the biosynthesis of iron nanoparticles can be made possible from dried ginger and possess less cytotoxic effects. Kumar et al has studied the mortality rate of A. salina to detect the toxicity level of zero-valent iron nanoparticles and found 100% mortality at 100 µl concentration [25] suggesting increase in toxicity with increase in concentration which is in accordance with the present study. The iron oxide nanoparticles synthesized from Juglans regia have shown to be non-toxic on normal and cancerous cervical cell lines [23]. Iron nanoparticles synthesized from Punica granatum fruit peel extract showed potent anticancer activities against nasopharyngeal carcinoma and not toxic to normal cell lines [24]. Various biological activities like anti-inflammatory and anti-oxidant activities can be evaluated in future studies which would extend its inevitable role as therapeutic agent providing effective and valuable biomedical applications that is safe and economical.

Fig. 3. UV vis-absorption spectra analyses of iron nanoparticles synthesized using dried ginger recorded as function of time
Table 1. Brine shrimp lethality observed for different concentrations of iron nanoparticles

| Concentration | Day 1 | Day 2 |
|---------------|-------|-------|
| Test          |       |       |
| 5 µl          | 10    | 10    |
| 10 µl         | 10    | 9     |
| 15 µl         | 10    | 7     |
| 20 µl         | 10    | 8     |
| 25 µl         | 10    | 6     |
| Control       | 10    | 10    |

Fig. 4. Cytotoxic effect of dried ginger mediated Iron nanoparticles

4. CONCLUSION

Iron nanoparticles were successfully synthesized using green approach from dried ginger in the present study. The cytotoxic effects of the iron nanoparticles showed less cytotoxicity when used in lower concentrations. Hence these iron nanoparticles prepared from dried ginger are effective and safe when used at optimal concentrations in nanoformulations applied in the medical and dental fields. In the present study, the morphology of the synthesized nanoparticles and other biological activities were not reported. The future scope of the study would focus upon determining the various applications of the iron nanoparticles synthesized from dried ginger that can be utilized in dentistry and medical fields.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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