



# **Biosynthesis of Hydroxy Citric Acid Mediated Zinc Nanoparticles and Its Antioxidant and Cytotoxic Activity**

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## **Authors' contributions**

*This work was carried out in collaboration among all authors. Idea and study was conceptualized by authors LA and SR collection of the literature and drafting the manuscript was by authors BS and LA. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/JPRI/2020/v32i2630845

### Editor(s):

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Complete Peer review History: <http://www.sdiarticle4.com/review-history/59850>

**Original Research Article**

**Received 20 June 2020**  
**Accepted 24 August 2020**  
**Published 05 November 2020**

## **ABSTRACT**

**Aim:** To evaluate biosynthesis of hydroxy citric acid mediated zinc nanoparticle and its antioxidant and cytotoxic activity

**Introduction:** Hydroxy citric acid is a chemical that is found in fruit rinds of *Garcinia cambogia*, *Garcinia indica*, and *Garcinia atroviridis*. Hydroxy citric acid is used to improve exercise performance and weight loss. Hydroxy citric acid can cause nausea, digestive tract discomfort, and headache when used short-term. Long-term safety is unknown. Zinc oxide nanoparticles are nanoparticles of zinc oxide that have diameters less than 100 nanometers. Zinc nanoparticles have

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a large surface area relative to their size and high catalytic activity. The exact physical and chemical properties of zinc oxide nanoparticles depend on the different ways they are synthesized. zinc oxide nanoparticles have also been shown to exhibit strong protein adsorption properties, which can be used to modulate cytotoxicity, metabolism or other cellular responses.

**Materials and Methods:** Evaluation of anti-oxidant was done by DPPH activity and evaluation of cytotoxic activity was done by brine shrimp lethality assay. Colour changes was noted in evaluation of cytotoxic activity

**Results:** The antioxidant activity was exhibited well at higher doses when compared to standard, but there was only 40% of cytotoxicity achieved which indicates its potential. Cytotoxic activity reveals that the drug showed a very good result at a concentration of 25  $\mu$ l it showed a 40% of lethality thereby showing that Hydroxycitric acid medicated zinc oxide nanoparticles shows a potent activity when compared to that with the standard.

**Conclusion:** The study concludes that hydroxy citric acid shows good antioxidant and cytotoxic activity.

**Keywords:** Antioxidant; cytotoxicity; DPPH; garcinia; hydroxy citric acid; zinc oxide.

## 1. INTRODUCTION

Hydroxycitric acid (HCA) is a derivative of citric acid that is found in a variety of tropical plants including *Garcinia cambogia* and *Hibiscus sabdariffa* [1]. There are four isomers, (+)- and (-)-hydroxycitric acid, and (+)- and (-)-allo-hydroxycitric acid. The (-)-hydroxycitric acid isomer is the one found in *Garcinia* [2]. Laboratory and animal studies of HCA have produced results that indicate a potential for modulation of lipid metabolism [3]. However, a clinical study has demonstrated that HCA has no effect in terms of weight loss or reduction of fat mass [4]. A meta-analysis published in 2010 revealed that gastrointestinal adverse effects were twice as likely for users of hydroxycitric acid. The use of HCA is contraindicated in patients suffering Colitis or Inflammatory Bowel Disease [5]. In a study in Zucker rats, which are genetically predisposed to obesity, *Garcinia cambogia* extract containing HCA showed that high doses led to significant suppression of epididymal fat accumulation, but also had high testicular toxicity [6]. However, this study has been criticized because of possible contamination of the HCA used and various design flaws [7]. Researchers at the University of Houston reported hydroxycitrate is capable of dissolving calcium oxalate crystals, a component of human kidney stones. Recent studies (2019) shows kidney stones are layered and the stones may form and dissolve by time. The researchers believe the effect could lead to the development of new drugs for human kidney stones [8,9]. HCA is usually marketed as a weight loss supplement either alone or in combination with other supplements. Some authors have suggested that HCA causes weight loss by competitively

inhibiting the enzyme adenosine triphosphatase-citrate-lyase [10]. *G. cambogia*'s suggested mechanism of action in obesity involves HCA-inhibiting lipogenesis, increase in lipid oxidation, and reduction of food intake [11] HCA competitively inhibits adenosine triphosphate-citrate (pro-3S)-lyase, an extra-mitochondrial enzyme that plays a role in fatty acid biosynthesis [12].

Zinc oxide nanoparticles are nanoparticles of zinc oxide that have diameters less than 100 nanometers. They have a large surface area relative to their size and high catalytic activity. ZnO is a wide-bandgap semiconductor with an energy gap of 3.37 eV at room temperature [13]. ZnO nanoparticles are believed to be one of the three most produced nanomaterials, along with titanium dioxide nanoparticles and silicon dioxide nanoparticles [14]. The most common use of ZnO nanoparticles is in sunscreen. They are used because they reflect ultraviolet light but are small enough to be transparent to visible light [15]. They are also being investigated to kill harmful microorganisms in packaging [16] and in UV-protective materials such as textiles [17]. Many companies do not label products that contain nanoparticles, making it difficult to make statements about production and pervasiveness in consumer products [18]. Recent studies have shown that ZnO nanoparticles exhibit a high degree of cancer cell selectivity with the ability surpass the therapeutic indices of some commonly used chemotherapeutic agents in similar ex vivo studies [19]. ZnO nanoparticles have also been shown to exhibit strong protein adsorption properties, which can be used to modulate

cytotoxicity, metabolism or other cellular responses [20].

## 2. MATERIALS AND METHODS

### 2.1 Biosynthesis of Hydroxycitric Acid Mediated Zinc Nanoparticles

100 mg of hyaluronic acid is mixed and dissolved with 30 mM of 100 mL zinc sulphate solution and then it was kept in the orbital shaker for 24- 72hrs until the color change was observed [Fig. 1], which is the indication of nanoparticle synthesized.

### 2.2 Evaluation of Antioxidant Activity

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a stable free radical which reacts with compounds that can donate a hydrogen atom. It contains stable lipophilic free radical, nitrogen centered with purple color. DPPH acts as free radical which induces oxidation. The anti-oxidant can donate an electron to DPPH radical and change in absorbance at 517 nm will follow. There was a color change to pale yellow gradually. 2 ml of extract was added to five test tubes. 50% of the methanol solution (buffer), 0.1mm of DPPH solution was added to five test tubes in a different concentration ranging from 10-50 ul. The mixture was then incubated for 30 minutes in a dark place at room temperature. The absorbance value was spectrophotometrically analyzed at 517 nm. The blank used was methanol solution. Methanol solution mixed with 0.1 mM of DPPH solution was used as a control. Ascorbic acid was used as a standard. The IC<sub>50</sub> value (minimum inhibitory concentration) was calculated. Percentage of inhibition was estimated using the equation,

$$\% \text{ Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of control}}$$

Colour change was recorded before and after incubation.

### 2.3 Cytotoxicity Activity

Brine shrimp eggs were added to saline water in a hatching chamber. After 24 hours, exactly 10 hatched larvae (nauplii) were suspended in 6 wells containing 10 ml of saline water, each. Different concentrations being 5 µL, 10 µL, 15 µL, 20 µL and 25 µL of the nanoparticles synthesised was dispersed in each well with the last well as a control (without any nanoparticles). Post 24 hours, the number of surviving nauplii were counted and recorded.

## 3. RESULTS AND DISCUSSION

### 3.1 Antioxidant Activity

In this study the antioxidant activity was assessed in five different concentrations of reaction mixture from 10 µL, 20 µL, 30 µL, 40 µL and 50 µL. Antioxidant activity of different percentages of inhibition of oxidation such as 52%, 63%, 71%, 85% and 90%. Which plant extract mediated by copper nanoparticle at 50 µL of concentration exhibited a high antioxidant activity of 90%.

### 3.2 Cytotoxic Activity

Cytotoxic activity reveals that the drug showed a very good result at a concentration of 25ul it showed a 40% of lethality thereby showing that Hydroxycitric acid mediated Zinc Oxide nanoparticles shows a potent activity.

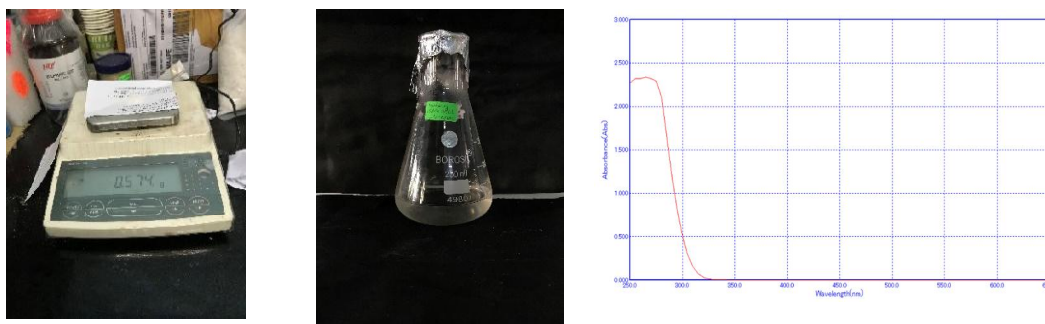


Fig. 1. Image showing the biosynthesis of hydroxy citric acid mediated zinc nanoparticles

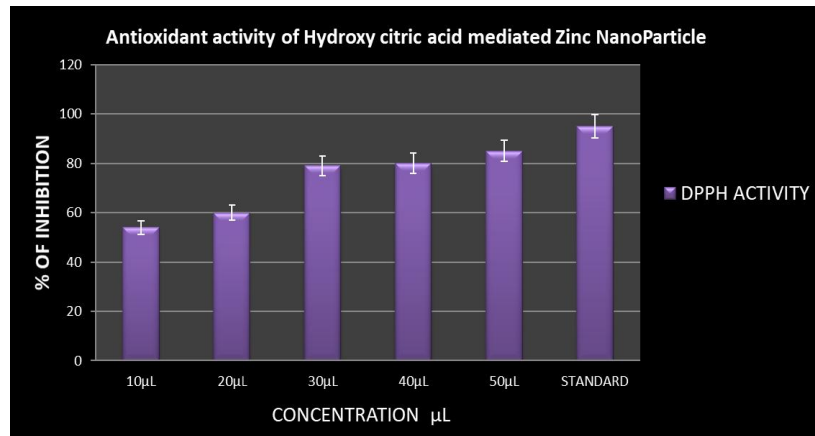


Fig. 2. Graph showing the antioxidant activity of hydroxy citric acid mediated zinc nanoparticles

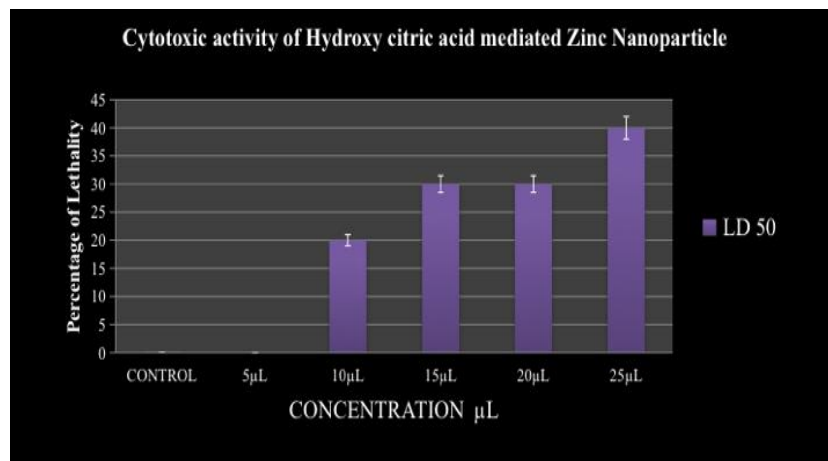


Fig. 3. Graph showing cytotoxic activity of hydroxy citric acid mediated zinc nanoparticles

#### 4. CONCLUSION

The study shows that at 40 microliters , it shows good antioxidant effect and at 20 microlitres it shows good cytotoxicity. So if the concentrations are increased further, it shows much better results. The study concludes that hydroxy citric acid shows good antioxidant and cytotoxic activity and it can be evaluated for further studies.

#### CONSENT

It is not applicable.

#### ETHICAL APPROVAL

It is not applicable.

#### ACKNOWLEDGEMENT

The authors are thankful to Saveetha Dental College for providing a platform to express our knowledge and for the support to conduct the study.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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