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A New Synthesis of Copper Nanoparticles and Its Application as a Beta-Hematin Inhibitor

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Abstract: To prevent the development of drug resistance and unwanted side effects, nanomaterials have been studied for their potential to inhibit beta-hematin, an important protein for the survival of malaria parasites. The use of nanomaterials as a medication against parasites and mosquito vectors has recently shown promising drug therapeutic strategies. One of the newest areas of interest in nanotechnology and nanoscience is the environmentally friendly production of nanoparticles. Green synthesis to produce metal nanoparticles is the most important strategy to overcome the possible dangers of toxic chemicals for a safe and harmless environment. For this study, copper nanoparticles (CuNPs) were synthesized using Iraqi basil leaf extract, demonstrating its novelty in nanosciences. The formation of CuNPs can be seen visually as a color shift from green to brownish. UV-vis absorption spectra, Fourier transform infrared (FTIR), X-ray diffraction (XRD), energy dispersive X-ray (EDX), and scanning electron microscopy (SEM) were used to characterize the synthesized nanoparticles. The surface plasmon resonance property (SPR) of CuNPs is revealed by UV-vis analysis, which shows a distinctive absorption peak at 420–430 nm, whereas SEM reveals the spherical shape of CuNPs with sizes ranging from 30 to 50 nm.

Keywords: beta-hematin; CuNPs; malaria; nanoparticles

■ INTRODUCTION

Plasmodium spp. are parasites that are responsible for the illness known as malaria. *P. malaria*, *P. vivax*, *P. ovale*, and *P. knowlesi* are the four species within this genus that are responsible for causing malaria in humans. More than 120 species that make up this genus, *P. falciparum* and *P. vivax* are the ones responsible for the spread of malaria around the world [1-2].

Currently, chloroquine (CQ) is one of the 4-aminoquinoline derivatives indicated as an efficient drug to fight malaria, but the most dangerous parasite, *P. falciparum* has shown resistance to this type of drug. As a consequence, the development of new drugs is highly essential to illuminate or control malaria disease [3]. Mefloquine and halofantrine are chloroquine alternatives induced in the 1970s. Both have been used for 30 years before parasite resistance to *P. falciparum* strains

developed in these medications. Because of its small size and lipophilic properties, CQ is known to only impact parasites in the erythrocytic stage after diffusing across the erythrocyte and parasite membranes [4-5].

The primary hurdles in the battle against malaria are drug resistance and environmental and social concerns, thus making its eradication challenging for scientists. The control of parasite resistance is one of the main problems that researchers face in the twenty-first century. One way to solve the problem is to apply nanobiotechnology concepts to both vector control and patient therapy, for example, metallic nanoparticles (MNPs) [5-6].

A nanoparticle is a microscopic material typically ranging from 1 to 100 nm. It is synthesized by wet chemistry and only initially involves the formation of particles in solution, followed by drop casting and

solvent removal [6]. Nanoparticles are valuable in the consumer products, pharmaceutical, chemical, environmental, energy, agricultural, and communication sectors because of their significant thermal, chemical, optical, physical, and electrical properties [7-8].

MNPs, as one of the types of nanoparticles, have played an important role in the treatment of malaria, especially in the human infection stage as well as in targeting the mosquito vector. According to in-depth reviews by Rahman et al. [6] and Barabadi et al. [9], the majority of MNPs investigated for these uses are silver, gold, and palladium. Silver has shown strong antibacterial properties because of the release of Ag⁺ ions, which are expected to interact with phosphate and thiol groups found in DNA and proteins. The integrity of the cell wall is compromised as a result of this interaction, which also inhibits important enzymes, renders bacterial DNA and RNA inactive, and binds subcellular components [10]. Due to the special characteristics of its nanostructured form, silver nanoparticles (AgNPs)-based treatments for malaria against various phases of infection in both mosquito and human hosts are open new ways to use other MNPs in this field [11].

In fact, the production of MNPs from plant extracts has evolved into a method that is both affordable and kind to the environment. This method utilizes a variety of reducing and capping agents to create stable MNPs. Benelli et al. [12] provided an in-depth description of how biosynthesis of MNPs from plant extracts introduces the enormous potential of such nanosystems to the fight against the vectors of a wide variety of infectious diseases, such as malaria.

Because plants contain a wide variety of bioactive substances, various parts of plants or entire plants have been used in green synthesis to produce copper nanoparticles (CuNPs). Examples of plant species extract that are efficiently used to create CuNPs are *Punica granatum* peel [13], *Zingiber officinale* stem [14], *Citrus medica* Linn. (Idilimbu) juice [15], *Ziziphus spina christi* (L.) Willd – fruit [16], *Asparagus adscendens* Roxb. root and leaf [17], *Eclipta prostrata* leaf [18], *Ginkgo biloba* Linn leaf [19], *Plantago asiatica* leaf [20], *Thymus vulgaris* L [21], black tea leaf [22], *Terminalia catappa* leaf [23], *Azadirachta indica* leaf [24] and *Artemisia* leaf [25].

The present study aims to synthesize CuNPs using extracts from Iraqi basil leaves. The potential inhibitory action of CuNPs against beta-hematin proteins was also investigated for the first time in an attempt to show a similar activity with the well-known applications of AgNPs.

■ EXPERIMENTAL SECTION

Materials

Copper sulphate, hemin chloride, sodium hydroxide, acetate buffer, and CQ were purchased from Sigma-Aldrich with high purity.

Instrumentation

UV-vis spectrophotometer 80 UV-vis spectrophotometer (Biotech engineering management Co. Ltd., UK), centrifuge Laboratory Retirezle (Centrifuge Laboratory Retirezle), magnetic stirrer VF2 (Funkentstört–Germany), heater/hot plate (IKA, Germany), scanning electron microscope (SEM, Zeiss, Germany), and Fourier transform Infrared spectroscopy (FTIR Perkin Elmer Instrument Japan) were used in this work.

Procedure

Green synthesis

Preparation of aqueous extracts. Iraqi basil leaves were collected from the Babylon governance and left to dry for 4 d at room temperature. Then, 100 mL of deionized water was added to 20 g of desiccated. The mixture was boiled at 100 °C for 35 min, filtered with filter paper, and stored at 4 °C for up to a week.

Preparation of CuNPs. CuNPs were synthesized following the published procedure by Al-Khafaji et al. [25], with a minor modification. Briefly, 100 mL solution of plant extract was slowly added with continuous stirring into 200 mL of 0.01 mM copper sulfate solution. The mixture was then centrifuged for 15 min at 8000 rpm after being incubated at room temperature for 24 h in a dark place. Changing color from green to brown gives an indication of the formation of CuNPs. A Millipore filter (0.5 M) was used to purify the residue. As a final point, nanoparticle pellets were gathered and left to dry at 60 °C to produce a brownish precipitate, which

was then stored for the following day's work. After purification, CuNPs were characterized using UV-vis spectroscopy, FTIR, XRD, SEM, and EDX.

Beta-hematin formation

A fresh heme stock solution was made by dissolving 16.3 mg hemin chloride in 0.2 M NaOH. The stock solution was centrifuged for 15 min at 7 g to remove the remaining hemin crystal. The concentration was 58,400 mM in 0.1 M NaOH based on the absorption at 385 nm [26]. A double-beam spectrophotometer was used to capture all absorption spectra. The formation of beta-hematin (BH) was carried out in accordance with the published technique, with minor modifications [27-28]. In brief, heme chloride (10 mL) was heated in an acetate buffer (0.56 M, pH 5) in the absence of CQ or CuNPs at 70 or 80 °C with a variety of CQ concentrations (10–50 µM) and CuNPs (10–50 µg/mL) concentrations separately. An amount of 1 mL of heme solution was withdrawn at the designated periods, and BH formation was calculated.

The absorbance of each sample was measured at 400 and 700 nm after heating the treated and untreated heme solution separately to verify BH formation. As a result, heme fractions were determined after being converted to BH using the following Eq. (1) [5].

$$\text{Fractions} = \frac{(A_{400} - A_{700})_{\text{control}} - (A_{400} - A_{700})_{\text{sample}}}{(A_{400} - A_{700})_{\text{control}}} \quad (1)$$

RESULTS AND DISCUSSION

Phenols and flavonoids are the most important phytochemicals found in various parts of the plant such as the shoot, leaf, stem, flower, root, and fruit. These phenolic compounds have hydroxyl and carbonyl groups that give them powerful antioxidant properties [29]. The green synthesis technique increased the stability of MNPs, preventing aggregation and allowing the adsorption of phytochemicals on the surface of the MNPs to increase the reaction rate of MNPs [30]. Different characterization techniques can be used to analyze the physical and chemical properties of MNPs, such as size distribution, agglomeration degree, surface charges, surface area, and surface chemistry of MNPs [31].

UV-vis Spectrophotometry

The absorbance spectrum using UV-vis was recorded in the range 420–430 nm, as shown in Fig. 1. CuNPs' surface plasmon resonance is the primary contributor to this absorbance band. A similar outcome was reported when *Artemisia* leaf extract was used [25].

FTIR Spectral Analysis

Peaks at 3342, 3331, 2928, 2372, 1643, 1627, 1429, 1261, 1082, 740, 621, and 534 cm^{-1} have been shown by the FTIR spectroscopy chart of CuNPs in Fig. S1. From those indicated peaks, the broad absorption peaks of CuNPs detected at 3342–3300 cm^{-1} represent the higher concentration of alcohols, with O–H and N–H stretches of amines. The intense peaks at 2929 and 2890 cm^{-1} correspond to asymmetric C–H stretching of the $-\text{CH}_2$ and $-\text{CH}_3$ groups, respectively. It is also believed that the amine and carboxylate group existing in the leaf extract of *Hagenia abyssinica* is responsible for binding proteins with the surface of Cu, thus leading to the high stability of biosynthesized nanoparticles [32].

A peak at 1643 cm^{-1} indicated the existence of C=O, while a peak at 1625 cm^{-1} corresponded to C=C. The indicated peak by 1429 cm^{-1} shows C–N stretching amide. A moderate peak at 1261 cm^{-1} corresponds to C–O stretching of phenolic compounds. C–O–C stretching displays 1082 cm^{-1} . Additionally, CuNPs spectrum displays a broad peak centered at 534 cm^{-1} , which is assumed to be evidence of Cu's interaction with extracted biomolecules [25].

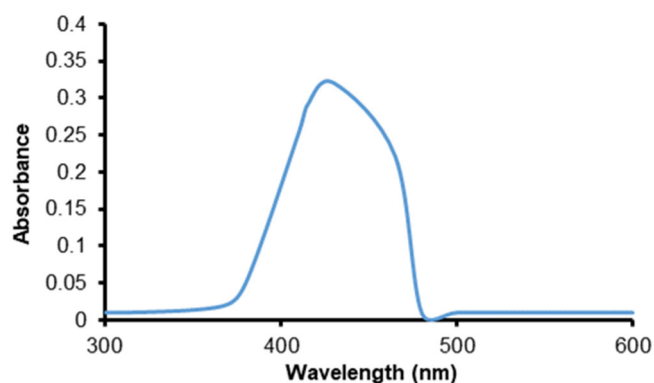


Fig 1. UV-vis spectrum of CuNPs

SEM, XRD, and EDX

As can be seen in Fig. 2(a), the size plus shape of synthesized CuNPs is represented via SEM micrographs. The SEM images revealed that the particles were nonhomogeneous according to their shape and size. Nanoparticles had a spherical shape with a typical size of CuNPs in the range of 30–50 nm, and no aggregation. Fig. 2(b) depicts the EDX spectrum for nano-copper. The peaks of the main elements Cu, C, and O were definitely recognized. No other peaks were presented, thus proving evidence of the purity of MNPs Fig. 2(c).

Furthermore, very few copper atoms on CuNPs surface are expected to be oxidized, yielding small amounts of copper oxide (CuO and Cu₂O). The biomolecules of plant extract have surface hydroxyl groups that convert copper ions to CuNPs. Confirm this with patterns from XRD analysis of CuNPs, which are shown in Fig. 2(c). The observing peaks in patterns, 44.62°, and 64.41° correspond to (200), (220), while a pattern of 37.51 corresponds to (111). The data have a big

agreement with previous work [25].

The Activity of CuNPs as a BH Inhibitor

In vitro tests, the colorimetric method was used to confirm whether or not CuNPs can work as a BH inhibitor and matched with a mechanism that Chinappi et al. [33] suggested the colorimetric technique provided good evidence, like the growth rate of heme crystal, besides identifying clear differences in BH formation with and without CQ and CuNPs. The temperature effect on the rate of BH formation was investigated before using CuNPs as heme crystallization inhibitors compared to commercial CQ (Fig. 3). After every 30 s, the absorbance of each sample at 400 and 700 nm was recorded with a double-beam spectrophotometer. The best incubation time, which is indicated when BH in every sample starts crystallization, nucleation time, and growth rate of heme crystals, have been investigated. As shown in Fig. 3, after 24 h incubation at room temperature, the results showed the most significant inhibition in the formation of BH when CuNPs were used

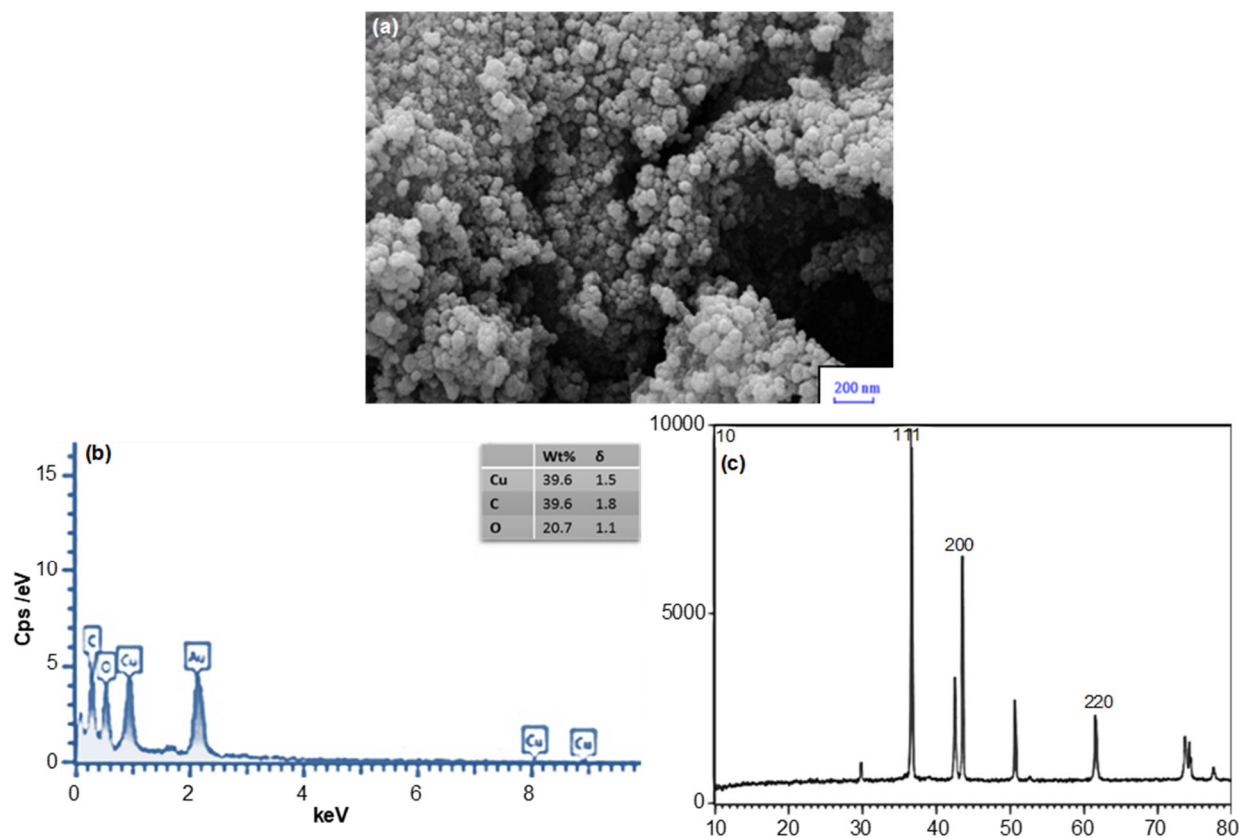


Fig 2. (a) SEM, (b) EDX, and (c) XRD analysis of CuNPs

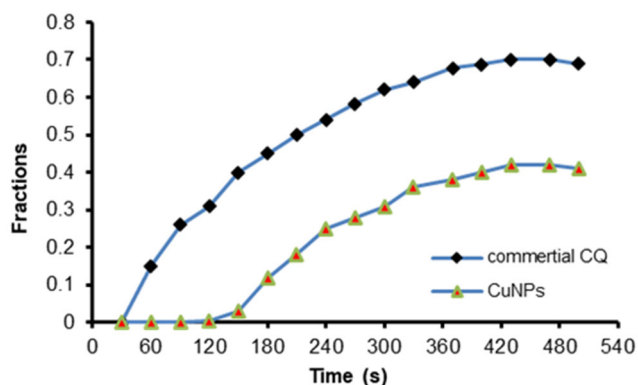


Fig 3. Time affects BH formation after adding CQ and CuNPs

as an inhibitor in comparison with commercial CQ, which was used in a range of concentration of 10–50 μM (Fig. 4(b)). The activity of CuNPs was determined at different concentrations (10–50 $\mu\text{g/mL}$). Results displayed a significant effect with % inhibition of 100% and 85% at 50 and 10 $\mu\text{g/mL}$, respectively.

To find out the difference between CQ and the synthetic CuNPs as an inhibitor for BH formation, a range

of concentrations was tested separately after each addition to haemin-chloride, and the rate growth of BH was evaluated by spectrophotometry. Overall, outcomes revealed that CuNPs inhibited BH formation more effectively than commercial CQ Fig. 4(b).

Changes in pH may alter the double-layer properties of CuNPs, increasing the chances of flocculation or coagulation, and may become membrane impermeable. This causes a lack in the formation of BH, a delay in the time of crystallization, which is required for the parasite to survive. This may be the reason for MNPs activity [34].

According to the crystalline shape of BH as observed from SEM analysis. Antimalarial activities of CuNPs were observed as a BH inhibitor strongly and indicated that MNPs might be shown to have a motivating effect against *P. falciparum* sensitive strain and CQ-resistance strain to be great non-artificial, antimalarial drugs Fig. 5.

The shape of untreated BH was observed to be a long, thin tapered crystal with changeable size, thus

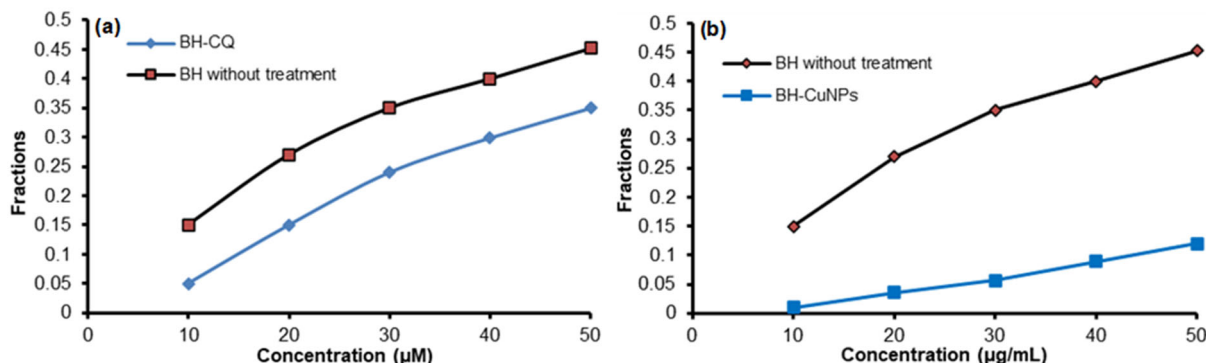


Fig 4. The effect of (a) CQ and (b) CuNPs on BH formation

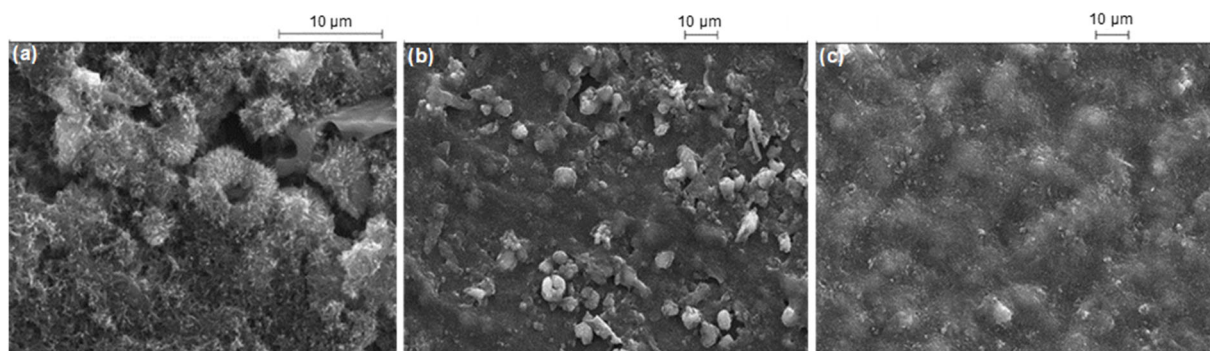


Fig 5. SEM image of beta-hematin crystals (a) BH formation with no treatment, (b) BH formation within 20 μM CQ, (c) BH formation within 20 $\mu\text{g/mL}$ CuNPs

providing a big agreement with the previous report [35]. Crystals become thicker and their arrangement was changed after using CQ, whereas Fig. 5(b) compared to untreated ones. While there is a significant change in heme crystalline structure after using CuNPs as inhibitors, they appeared less organized, forming unclear crystals Fig. 5(c).

■ CONCLUSION

The green CuNPs were effectively synthesized, and phytoconstituents such as polyphenols and tannins that have an important role as capping and reducing agents during the development of the CuNPs have been investigated. The synthesis of CuNPs has verified absorbance and reflectance spectra in the UV-vis spectrum. The existence of a capping agent on the CuNPs surface was confirmed by FTIR spectra. The XRD pattern and EDX spectrum both verified the composition and crystallized form of CuNPs. The spherical form and average particle size of 35.2 nm of the CuNPs determined by the SEM were sufficient proof of their nanomorphology. This research looked into the effectiveness of basil plant extract-derived CuNPs against beta-hematin formation as an antimalarial agent. In comparison to commercial CQ, the results show that CuNPs can substantially inhibit heme crystallization, which is necessary for the malaria parasite to survive.

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■ AUTHOR CONTRIBUTIONS

Rana Abd Al-Aly Khamees Al-Refaia conducted the experiment and wrote the manuscript, Eman Alrikabi revised the manuscript, Rafaela Vasiliadou did proofread the manuscript, Ahmed Ali Alkarimi had interpretation of XRD and SEM analysis.

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