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The Potential of Green-Synthesized Copper Oxide Nanoparticles From Coffee Aqueous Extract to Inhibit Testosterone Hormones

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Abstract

In this study, copper oxide nanoparticles (CuONPs) were produced at room temperature using the sol-gel method with coffee powder extract and copper sulphate pentahydrate ($CuSO_4.5H_2O$) as the copper source. Fourier transforms infrared spectroscopy (FT-IR), x-ray diffraction (XRD), ultraviolet-visible spectroscopy (UV-Vis), transmittance electron microscopy (TEM), and scanning electron microscopy were used to analyze synthesized copper oxide nanoparticles with a spherical particle shape (SEM). An absorption band at 281 nm can be seen in the UV-Vis absorption spectrum. The final product was identified as highly crystalline CuO with diameters ranging from 15 to 30 nm by XRD. The SEM images show a network of randomly oriented CuO NPs with an average size of 20 nm and thicknesses of about 8 nm. The effect of copper oxide nanoparticles on the amount of testosterone hormone in human male and female serum was investigated in this study. Copper oxide nanoparticles had an inhibitory effect on testosterone levels, according to the findings. Different concentrations of copper oxide nanoparticles have an influence on testosterone hormone binding to receptors and thus on its level in the blood, which affects biological processes that are dependent on hormone concentration, according to the findings.

Keywords: Testosterone, Nanoparticle, Copper oxide, Coffee, Hormone.

1. Introduction

Nanotechnology includes the characterization, manufacture, and manipulation of devices, structures, or materials with at least one dimension (or components with at least one dimension of about (1-100) nm in length) (1).Due to their unique physical and chemical properties, nanomaterials are widely used in a variety of sectors (2), (3). Copper oxide nanostructures have sparked a lot of attention due to their diverse range of uses, including superconductors (4), Catalytic(7), enormous magnet resistance materials(5),(6).

Optical, electrical, and gas sensors (8), solar energy conversion (9), and organic–inorganic nanostructure composites preparation (10). A p-type semiconductor, CuO has a band gap of 1.7 eV. (11). When incorporated into coatings, plastics, textiles, and other materials, it can also be used as an antimicrobial, anti-biotic, and anti-fungal agent (12). Methods for generating CuONPs include the sol–gel approach (13), alkoxide-based method (14), electrochemical techniques (15), precipitation pyrolysis (16), microwave irradiations (17), solid-state reaction method (18), and thermal degradation of the precursor (19). Plants such as neem (20), alfalfa (21), Cinnamomum camphor (22), tamarind (23), lemon grass (24) and coffee extract have recently been described for green synthesis of different nanoparticles. Testosterone ($C_{19}H_{28}O_2$) is a male sex hormone generated in the testes and required for the healthy growth and function of the male sex organs (see figure 1) (25). The maturation process of sperm. The testicular laying cells produce most of it (26). The adrenaline cortexes of both men

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and women produce approximately 0.5 milligrams every day (27).

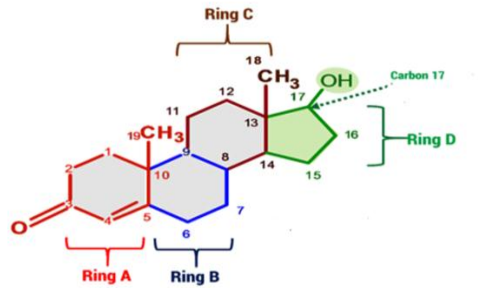


Fig (1). Testosterone hormone chemical structure.

During intrauterine development, testosterone influences brain structure and neuron differentiation, with long-term consequences for brain functions (28). Testosterone is required for the development of male reproductive tissues such as the testes and prostate, as well as secondary sexual traits such as increased muscle and bone mass and hair growth in male humans (29). Men's testosterone levels directly and indirectly (through dihydrotestosterone (DHT) in the prostate) stimulate genital development (30-32). Furthermore, testosterone has an impact on the health of both males and females, as well as the prevention of osteoporosis (33). Anomalies such as frailty and bone degradation can occur in males who have insufficient testosterone levels (34-35). The synthesis of copper oxide nanoparticles from coffee aqueous extract, water as a solvent, and copper sulfate pentahydrate as a copper source was carried out using the Sol-gel method, which is an environmentally acceptable and inexpensive method (36). The goal of this study is to see how different concentrations of manufactured copper oxide nanoparticles affect the amount of the sex hormone testosterone in male and female patients' serum.

Materials and Methods

Preparation of coffee powder aqueous extract

A local market in Baghdad, Iraq, provided the coffee powder. 2.5 g fine coffee powder was combined with 100 ml distilled water in a 250 mL glass beaker to prepare a coffee aqueous extract. The mixture was boiled for 15 minutes at 40 °C. After cooling to ambient temperature, the mixture was centrifuged for 5 minutes at 6,000 rpm and filtered with Whatman No. 1 filter paper. The aqueous extract was finely powdered and kept at 5°C for future investigations.

Synthesis of CuO Nanoparticles using Coffee Extract

In a perfect reaction, 2.5 g of copper sulphate pentahydrate ($CuSO_4.5H_2O$) was dissolved in 15 mL of distilled water in a 250 mL conical flask and magnetically swirled at room temperature for 5 minutes in a typical reaction mixture. Following that, 30 mL of coffee aqueous extract was added to the copper ion solution, and the resulting mixture was refluxed in a sand bath for six hours at 70 °C with constant stirring. The mixture was then dried for four hours at 40 °C, generating a black powder as shown in figure (2), showing the creation of copper oxide nanoparticles.

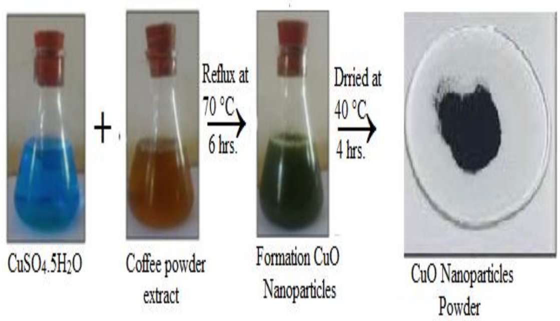


Fig (2). CuONPs Powder Preparation.

Characterization Techniques

A Hitachi S-4500 scanning electron microscope was used to examine produced copper oxide nanoparticles. Powder X-ray diffraction (XRD) was carried out using a Shimadzu XRD-6000 X-ray diffractometer with CuKa radiation λ = 1.5405 Å over a wide range of Bragg angles (3o $\leq 2\theta \leq$ 80o). A Shimadzu FTIR-Prestige-21 spectrophotometer was used to perform FT-IR spectroscopic observations.. Using a Schimadzu 1601 spectrophotometer in the wavelength range of 200 to 700 nm with a resolution of 1 nm, the UV-Vis spectra of copper oxide nanoparticles was recorded as a function of reaction time by diluting 0.1 ml of the sample with 2 mL of deionized water.

Patients and methods

Copper oxide nanoparticles and Enzyme Linked Immune Sorbant Assay (ELISA) Kits for testosterone were employed in this investigation (Germany) (Elabscience specializes in immunodiagnostic technology for life science community). Its operation is based on hormone specimens and hormone conjugated in coated wells competing for binding. The inhibition percentages of testosterone binding between antigen and antibody were calculated by comparing hormone concentrations with CuONPs (20 μL of CuONPs was added to the sample assay) to hormone concentrations without CuONPs (20 µL of distilled water was added to the sample assay) under the same conditions, using equations (16): -

% inhibition = 100 – Activity in the presence of nanoparticles / Activity in the absence of nanoparticles x 100.

The effect of CuONPs concentration on testosterone binding to receptors was examined experimentally. Serial concentrations of CuONPs were prepared by diluting stock solutions with distilled water, and hormone concentration was determined under the same conditions.

Sample

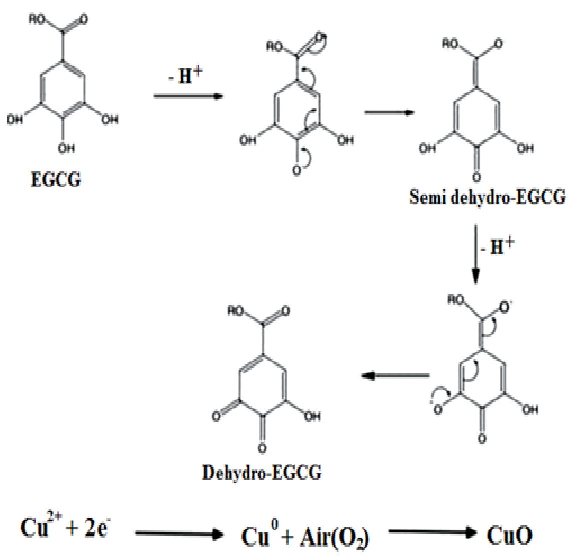
This study includes a total of 120 samples. Blood samples were obtained at the AL-Imamein AL-Kadhimaein Medical City Educational Hospital in Baghdad, Iraq, between December 2020 and May 2021. According to their clinical diagnosis, the 80 abnormal testosterone patients are separated into two groups: (40) males and (40) females (group A). A total of 40 healthy controls (group B) were enrolled in the study, whose ages were matched from 20 to 40 year. The control group was chosen from patients who are clearly stable, meaning they are neither hypertensive, diabetic, or have a family history of other chronic conditions. The quantitative assessment of testosterone hormone in human serum was carried out using a testosterone hormone immunoassay (sandwich method). Anti-testosterone hormone antibodies were immobilized in the solid phase of the well, and testosterone from the patient serum was bound. Five millilitres of venous blood were drawn, transferred to a plane tube, and allowed to coagulate at room temperature before being centrifuged at 1.174 xg for five minutes. The serum was then separated and kept at -20°C until the experiment. Within 10 minutes, each sample's optical density (OD) was evaluated using a Microplate reader set to 450 nm.

Statistical Analysis

SPSS version 17.0 for Windows was used for all stati -stical analyses in the experiments (Statistical Package for Social Science, Inc., Chicago, IL, USA). The mean and standard deviation of parameters were calculated using descriptive analysis (36).

Results and discussion

CuO nanoparticles were reduced and stabilized with coffee extract. Scheme 1 illustrates the proposed technique (37,38). This provides compelling evidence for the role of polyphenols in fast production as well as the stability of metallic nanoparticles in aquatic environments. It's possible that the well-dispersed copper nanoparticles were made by reducing copper ions with coffee extracts containing epigallocatechin gallate (EGCG), a reducing and capping agent. EGCG is a polar molecule that is very water soluble. During interactions, EGCG acted as a stable (electron + proton) donor, as shown in Scheme 1. Through oxidation, it was first transformed into the radical ion "semihydro-EGCG," and subsequently into dehydro-EGCG. Dehydro EGCG and EGCG combined to form a redox system that was capable of reducing Cu^{+2} to Cu^{0} (11). In order to produce a complex compound, the lone pair electrons in the polar groups of EGCG can occupy two sp orbits of the copper ion. Copper ions were added to the EGCG, and subsequently Cu(0) nanoparticles were created by reducing Cu^{+2} inside the nanoscopic templates. Small copper nanoparticles developed quickly in the presence of nanoscopic templates. The other explanation could be the dispersion impact of EGCG's oxidation product on copper nanoparticles once the reduction reaction is complete. Through oxidation, EGCG becomes dehydro — EGCG.



Scheme (1) The proposed mechanism for the formation of CuONPs.

Characterization of CuO nanoparticles

UV–Vis spectroscopy

The most extensively utilized technique for structural characterisation of nanoparticles is UV–Vis spectroscopy. Copper oxide nanoparticles' absorbance was measured in the 130-700 nm range. Figure 3 shows an example of typical excitation absorption at 281 nm (3).

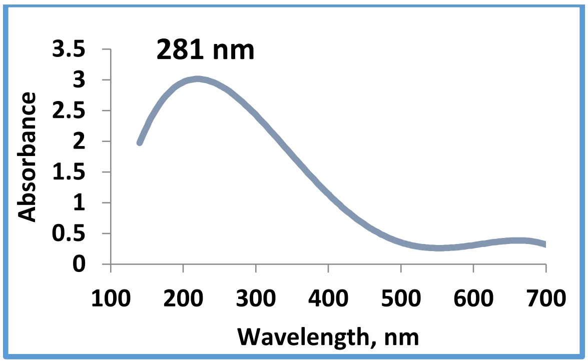


Fig. (3) UV–Vis spectroscopy of CuONPs using coffee powder extract.

Fourier Transforms Infrared Spectroscopy (FTIR)

The FTIR spectrum shown in Figure (4) shows strong and wide absorption bands at 479,512, 613,752, 1,071, 1,380, 1,603, 1630, and 3,422 cm-1. 476,512,613 Cu-O stretching vibrations The peak at 1,071 cm-1 indicated the presence of C–O stretching frequency, while the peak at 1,382 cm-1 indicated the presence of the germinal methyl group. The peak at 1,603 cm-1 demonstrated the presence of flavones adsorbed on the surface of CuO nanoparticles1630 bends the vibration of water molecules, suggesting the unreacted ketone group This indicates that water soluble organic coffee moieties were used to synthesize CuO. A broad absorption band at 3,422cm-1 was also discovered in the FTIR spectra, which was primarily for -OH.

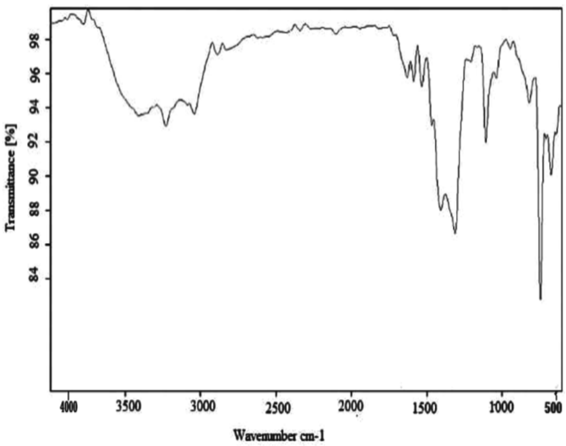


Fig.(4)FTIR spectrum of CuO NPs prepared from coffee powder extract.

X-ray diffraction (XRD) X-ray diffraction (XRD) studies

The X-ray diffraction (XRD) pattern of synthesized CuO nanoparticles is illustrated in Figure (5). The peak position with 2θ values of 32.5°, 35.4°, 38.7°, 46.2°, 48.7°, 53.4°, 58.3°, 61.5°, 66.2 and 68.1° correspond to (110), (002), (200), (112), (202), (020), (202), (113), (022) and (220) planes, respectively, indicating the spherical structure of the synthesized CuO NPs. The XRD pattern indicates the high phase purity. The average crystallite size of the CuO NPs as determined by Debye. The absence of impurity peaks in the formula Scherrer's a: **D = kλ / β cos θ** Where D is the particle size (nm), k is a constant equal to 0.94, is the wave length of X-ray radiation (1.5406 A), is the peak's full-width at half maximum (FWHM) (in radians), and 2 is the Bragg angle (degree) (38). The average crystallite size was discovered to be between 45-65.7. nm.

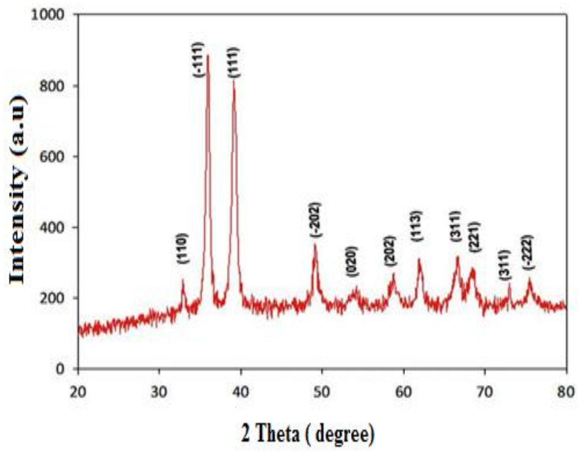


Fig. (5) XRD patterns of CuONPs dried at 300°C.

Transmittance Electron Microscopy

The TEM image of CuONPs revealed that the particles are nearly spherical in shape, with a slight thickness difference. A histogram revealed that the typical particle size was 25-45 nm, as illustrated in figure (6).

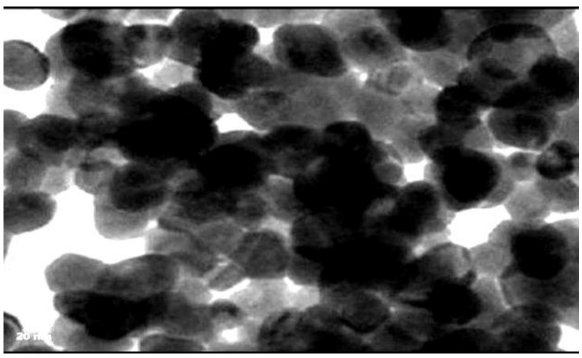


Fig. (6) TEM image of CuONPs.

Scanning Electron Microscopy

SEM scans of synthesized copper oxide nanoparticles illustrated in figure (7) with particle sizes ranging from (55.7- 120) nm were obtained.

The EDS method was used to analyse the subject. The energy dispersive spectra of the samples obtained from SEM-EDX analysis demonstrate that the sample produced using the above method has pure CuO phase. The findings revealed that the reaction product was made up of high purity copper oxide nanoparticles.

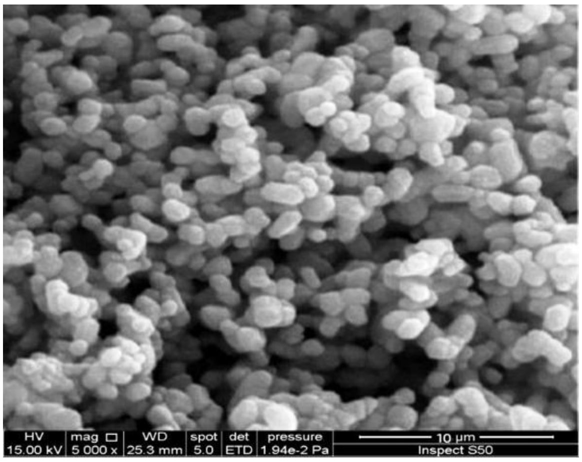


Fig. (7) SEM image of CuONPs.

Biological effect of CuONPs on Testosterone hormone level

Table (1) shows the mean levels acquired of BMI in sera of healthy and patient female and male. It is obvious from the results that for healthy female and male, they are within normal range. Meanwhile, patient's female and male have high level of testosterone hormone.

Table 1: Comparison between Control and Patients groups in Body Mass Index-BMI for Female and Male.

Group	Mean ± SD of BMI (kg/m^2)	
	Female	Male
Control	22.06 ±0.37	22.13 ±0.34
Patients	24.80 ±0.27	25.13 ±0.38
T-test	0.929 **	1.194 **
P-value	0.0001	0.0001
**** (P≤0.01).**		

The activation or inhibition percentage of various concentrations of synthesized copper oxide nanoparticles for testosterone level was studied on sera of female and male patients. The results presented in Table (2) and figure (8) indicated in general inhibition effect on testosterone hormone level for female patients by four concentrations of CuONPs while a little activation effect occurs only at 0.02 M of nanoparticles. On the other hand for male patients, testosterone level was activated at all studied CuONPs concentrations.

Table 2: Effect of CuO Nanoparticles concentrations on Testosterone Hormone level in Female and Male Patient.

Conc. CuONPs/ M	Conc.Testosterone/ ng ml^{-1} for male	Conc.Testosterone/ ng ml^{-1} for female
0.00	2.60	1.30
0.01	2.97	0.90
0.02	3.00	0.92
0.04	2.96	0.94
0.06	2.61	1.30
0.08	2.50	1.32

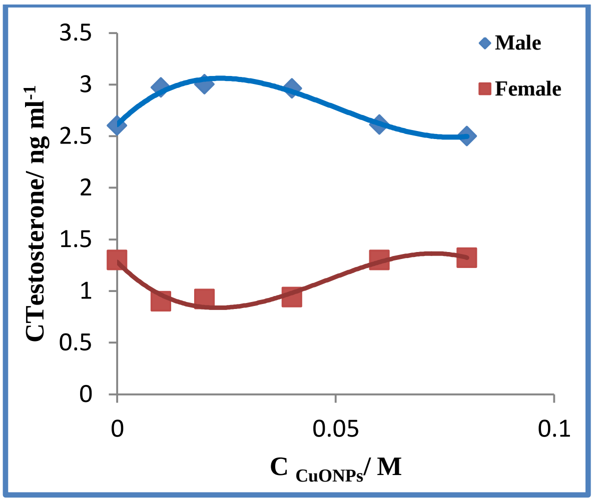


Figure (8) Effect of CuONPs concentrations on Testosterone hormone level for male and Female patients.

To the best of our knowledge, the present study is the first one to look at the effects of copper oxide nanoparticles on sex hormone binding to its receptors in human serum. Other studies focus on nanoparticles' effect on male reproductive function in mice and cell lines. In a recent work, after the delivery of polyethylene glycol -NH2 capped AuNPs to Imprinting Control Region (ICR) mice, elevated testosterone levels were observed with no changes in luteinizing hormone or follicle stimulating hormone values (17). According to Ivo Iavicoli *et al.*, the majority of the negative effects of nanoparticles on male reproductive function are caused by changes in testicular structure, spermatogenesis impairment, and changes in the biosynthetic and catabolic pathways of testosterone. (9). The surface chemistry of nanoparticles seems to be plays an important role in inducing hormonal alterations (9). Larson determined in living ovarian tissue that gold nanoparticles affected production of progesterone, a sex steroid hormone that affects the production of estrogen and testosterone. These effects may be due to the ability of these nanoparticles to bind to sulphur containing serum proteins as well as forming hydrophobic interactions (18). In this case, some of the signalling proteins which interact with receptors can be bound to AuNPs (19). Sivaraj et al., reported in their study that protein adsorption to the nanoparticle surface can mediate the uptake of the nanomaterial via receptor-mediated endocytosis (20). Shankar *et al*., demonstrated that many different plasma proteins adsorb on nanoparticles spontaneously, and that the surface chemistry of the nanoparticles in growth media/plasma is not the same as the originally synthesized materials (21). Instead, the nanoparticles adopt the physiochemical properties of the adsorbed protein shell (22) (23). This means that the concentration of receptors present in serum has an important role in nanoparticle impact on sex hormone binding to its receptor.

Conclusions

A pristine hexagonal structure of black powder was generated after only 4 hours of heat treatment at 40 °C. This method of introducing and adapting green chemistry ideas to the synthesis of nanoparticles is intriguing. The method also has the advantage of being simple to synthesize in a typical setting and reasonably inexpensive, allowing for a more realistic scale-up and deployment of the manufacturing process. This study found that varied concentrations of CuO nanoparticles caused variable variations in testosterone hormone levels in both male and females. It increased in males with low testosterone levels in higher dose NPs, but considerably decreased in lower dose NPs. When high NPs were used in a dose-dependent manner, it resulted in a decrease in high testosterone levels in females. However, when a lesser amount is administered, there is a considerable rise. Testosterone levels are likewise affected by nanoparticles in a dose-dependent manner.

Conflicts of interest

There are no conflicts to declare.

Acknowledgments

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