Volume 15, Issue 3, 152421 (1-19)

International Journal of Nano Dimension (IJND)

https://dx.doi.org/10.57647/j.ijnd.2024.1503.21

Bio-synthesis of copper oxide nanoparticles using beetle defensive gland extract: exploring diverse applications

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Original Research

Received:
19 February 2024
Revised:
14 March 2024
Accepted:
15 March 2024
Published online:
15 June 2024

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Abstract:

The present study systematically investigates a direct method for synthesising copper oxide nanoparticles (CuONP) utilising the defensive gland secretion of the beetle *Luprops tristis* Fabricius (*L. tristis*) using a greener method under microwave irradiation. By employing the unique biological approach of utilising the insect *L. tristis*, we successfully characterised the CuONP abbreviated as LCuONP through various analytical techniques, including UV-Vis spectrometry, FTIR analysis, SEM, TEM, Zeta potential analysis, and crystalline structure by XRD analysis. These LCuONP display crystalline, irregular spherical shapes with a rugged surface and have an average size of 15 nm. Moreover, we demonstrate that LCuONP-coated electrodes can effectively sense hydrogen peroxide without the need for enzymes, rendering them valuable biosensors. Additionally, LCuONP show dose-dependent antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, indicating their potential as powerful bacterial inhibitors. Notably, LCuONP induce chromosomal abnormalities in *Allium cepa* root tips, with higher concentrations leading to decreased mitotic indices and increased chromosomal aberrations. Furthermore, our investigation reveals the antioxidative properties of LCuONP and establishes their dose-dependent cytotoxicity against DLA cells. Overall, this study highlights the diverse applications of biosynthesised CuONP, including antimicrobial and antioxidant functions, as well as their potential in biosensing and cancer therapy. CuONP hold significant utility across various fields of life. Thus, synthesising CuONP from insect secretions presents a novel approach, transforming a nuisance pest into a valuable resource in a more environmentally sustainable, economically feasible, and non-toxic synthesis method.

Keywords: Allium cepa; Copper oxide nanoparticles; Differential pulse voltammetry; DPPH; Luprops tristis

1. Introduction

Copper oxide nanoparticles (CuONP), a crucial frontier in nanotechnology, possess distinct physical and chemical characteristics that make them essential in various applications [1]. These nanoparticles, typically ranging from 1 to 100 nm in size, exhibit exceptional optical, electronic, and catalytic properties different from those of larger materials. Their production and analysis have attracted global interest due to their potential in electronics, catalysis, medicine, and environmental cleanup. Recent studies have highlighted their distinct characteristics and broad usage in various scientific and industrial fields. [2–5]. Various synthesis techniques enable precise control over their dimensions, morphology, and surface characteristics, facilitating integration into electronics, catalysis, biomedical science, and environmental restoration [6, 7]. The substantial surface-to-volume ratio of nanoparticles enhances their reactivity, while quantum confinement phenomena on the nanoscale impart distinctive electronic, optical, and biological properties [8–10]. In electronics, they play crucial roles in advanced devices, while in environmental cleanup, they facilitate the breakdown of organic contaminants [11–14]. In medical science, they show promise as therapeutic agents, drug delivery carriers, and agents for medical imaging, particularly in cancer therapy [15, 16]. Recent research has focused on the use of

CuONP in cancer therapy, particularly in combination with other therapeutic agents or as photo thermal agents. CuONP have demonstrated the ability to induce cancer cell death through various mechanisms, including oxidative stress and apoptosis, while sparing healthy cells [17, 18]. The regulation of particle dimensions, structure, and crystal quality stands as one of the paramount considerations in the production process of these nanoparticles [19]. In order to achieve this, numerous synthesis methods have been developed; some of the most well-known ones include the sonochemical method, the sol-gel method, laser ablation, the electrochemical method, chemical precipitation, and surfactant-based techniques [20–24]. The biosynthesis of nanoparticles has gained prominence as an eco-friendly alternative to conventional chemical methods. In the case of CuONP, biological entities such as plants, microorganisms, and enzymes play a pivotal role in their synthesis. This environmentally friendly strategy not only offers a sustainable method for nanoparticle production but also introduces a range of bioactive compounds onto the nanoparticle surface, enhancing tremendous promise for a variety of uses [23–26].

Owing to their unique qualities and prospective uses, current work utilising the defensive gland secretion of beetle *L. tristis*. Because the secretions of defence glands from beetles in particular contain an extensive range of chemical elements, they have been recognised as a valuable source of bioactive chemicals. These secretions usually possess specific qualities, such as antibacterial, anticancer, and antioxidant activities, It makes them standout prospects for a range of scientific studies [27, 28]. The defence gland extract of *L. tristis* has demonstrated to be able to synthesise silver nanoparticles in recent investigations conducted in our lab [29]. The objective of this study is to synthesise CuONP using the defensive gland extract of the beetle *L. tristis*, referred to as LCuONP, in an environmentally conscious and economically viable manner. These nanoparticles boast a multitude of applications, spanning from their antimicrobial and antioxidant properties to their anti-mitotic capabilities, and even their potential in biosensing and cancer therapy. Notably, LCuONP exhibit remarkable dose-dependent antimicrobial activity against pathogens like *Staphylococcus aureus* and *Klebsiella pneumoniae*, suggesting their potency as formidable antibacterial agents. Moreover, LCuONP prompt chromosomal abnormalities in *Allium cepa* root tips, with heightened concentrations correlating with diminished mitotic indices and escalated chromosomal aberrations. Furthermore, our exploration unveils the antioxidative attributes of LCuONP and establishes their dose-dependent cytotoxicity against DLA cells, thus holding promise in cancer therapy. Additionally, LCuONP-coated electrodes possess the capability to detect exceedingly low concentrations of hydrogen peroxide, rendering them invaluable in the medical field. Reports exist on the synthesis of CuONP and their diverse applications across various organisms, such as bacteria, fungi, and plants [30]. However, synthesis from an insect source has not yet been documented. CuONP serve as indispensable assets across a spectrum of endeavors. Consequently, crafting them from insect secretions presents a novel and exquisite approach, transforming a pest into a precious resource through a synthesis method that is more ecologically sustainable, economically feasible, and devoid of toxicity.

2. Materials and Methods

2.1 Isolation of Defensive Gland Extract from L. tristis

The research specimen, *Luprops tristis* Fabricius, was procured from the college campus situated in Pattambi, Kerala, India (GPS coordinates: 10.809526, 76.199281). This member of the Coleopteran beetles, belonging to the Tenebrionidae family, is represented in Fig. 1 is a darkling

Figure 1. Experimental insect: *Luprops tristis*, a) A single insect, b,c,d) insects aggregated on the wall of a house.

beetle known for its preference for plant waste as its primary food source. Endemic to India, it boasts a length of approximately 8 mm with a striking ebony hue. Despite its benign nature towards humans, this unassuming creature harbours a potent defence mechanism in the form of a phenolic secretion capable of inducing skin irritation upon contact. Within this secretion lies a rich composition of phenol, flavonoids, alkaloids, and other bioactive compounds, prized for their adeptness as reducing and capping agents. Their tendency to congregate in large numbers within farmhouses and rooftops is notorious, often leading to disruptive living conditions. After careful manual selection, the specimens were housed in ventilated insect containers and transported to the laboratory for glandular extraction. Applying gentle pressure to the abdominal region facilitated the extrusion of defensive glands, with the resultant secretion meticulously collected into 300 µL capacity Eppendorf tubes filled with distilled water, ensuring impeccable purity free from any faecal contamination. These extracts subsequently formed the foundation for the synthesis of CuONP.

2.2 Production of Luprops tristis Mediated -copper oxide Nanoparticles (LCuONP)

To synthesise LCuONP, the gland extract from 30 *Luprops tristis* beetles was carefully introduced into 300 µL of distilled water. A 0.01 molar (M) solution of copper sulphate (obtained from NICE Chemicals PVT Ltd.) was meticulously prepared in deionized water. The reaction mixture was made by blending 300 µL of defensive gland extract (equivalent to 30 glands) with an equal amount of copper sulphate solution (0.01 M). Subsequently, the solution underwent precise microwave irradiation for duration of 15 minutes at a power level of 350 W. The discernible alteration in the solution's coloration was then meticulously observed.

2.3 Characterisation

2.3.1 Structural Characterisation

The transformation of copper sulphate into copper oxide nanoparticles using the defensive gland secretion of *L. tristis* underwent comprehensive scrutiny. UV-Vis spectroscopy was employed to discern the interactions between compounds and the biosynthesised CuONP, which are vital for their stabilisation. FTIR spectroscopy was utilised to identify the specific compounds binding to the nanoparticles. The morphological characteristics and dimensions of the nanoparticles were meticulously elucidated through the application of transmission electron microscopy (TEM) and scanning electron microscopy (SEM). Furthermore, the stability of the nanoparticles, a critical parameter, was evaluated through a rigorous analysis of their zeta potential. Identification of the crystalline phase of the synthesised CuONPs was performed using advanced power X-ray diffractometer (XRD), D8 (Bruker, Germany).

2.4 Electrochemical Studies: Differential Pulse Voltammetry (DPV)

Biosynthesised CuONPs were utilised for examining hydrogen peroxide sensing. DPV measurements were conducted using an electrochemical workstation (Instrument Model DY2113, developed by Digi-Ivy). A three-electrode cell configuration was set up, comprising a platinum foil counter electrode, an Ag/AgCl reference electrode, and a working electrode coated with CuONP suspended in 10 ml of phosphate buffered saline. A potential within the range of 1.000 to -1.000 was applied during both the initial and final stages of the experiment. Hydrogen peroxide concentrations ranging from 10 µL to 60 µL were examined, and the resulting data were utilised to establish calibration linear relationships linking DPV current output with hydrogen peroxide concentration. The limit of detection (LOD) for the CuONP sensor was determined using the equation LOD = (3 × SD) / slope of the curve.

2.5 Biological Applications

2.5.1 Antibacterial Analysis

The dose-dependent antibacterial activity of LCuONP was assessed through a disc diffusion assay against both Gram-negative (*Klebsiella pneumoniae* ATCC 33591) and Gram-positive bacteria (*Staphylococcus aureus* ATCC 700603). To prepare the agar medium, 4 g of agar powder were dissolved in 100 ml of distilled water and subsequently poured into autoclaved Petri dishes. Pure microbial cultures were sub-cultured on nutrient agar and uniformly spread on individual plates. For the antibacterial assay, four distinct concentrations (5 µL, 10 µL, 15 µL, and 20 µL) of LCuONPs were employed. Filter paper discs (5 mm) were saturated with LCuONP, allowed to dry, and then positioned on the culture plate. Tetracycline served as the positive control. The plates were then positioned in a shaking incubator and left to incubate for 16 hours at 35°C. The bactericidal activity was determined by analysing the diameter of the inhibitory zone.

2.5.2 Antioxidant Analysis

The synthesised nanoparticles' antioxidant potential was evaluated through the DPPH (1, 1-diphenyl 2-picrylhydrazyl) method, which assesses free radical scavenging activity. A stock solution of DPPH was prepared by dissolving 3.9432 mg of DPPH in 100 mL of methanol, resulting in a concentration of 0.1 mM, and kept until usage at 4 °C. Subsequently, the DPPH solution (2 mL) was mixed with 1 mL of varying concentrations ranging from 20 to 100 µL of the synthesised nanoparticles. As a reference standard, ascorbic acid at a concentration of 100 µL was employed. A control mixture was comprised of 2 mL of the DPPH solution and 1 mL of distilled water. After that, the reaction mixture was added and allowed to incubate at room temperature in the absence of light for 30 minutes. The absorbance was measured spectrophotometrically at 517 nm. The antioxidant activity of the fabricated nanoparticles was calculated according to the percentage of DPPH radicals scavenged, as per the subsequent equation [31].

2.5.3 Chromosomal Aberration Assay

To investigate the influence of biosynthesised LCuONP on mitosis, freshly harvested *Allium cepa* (onion) bulbs were employed. Bulbs were chosen from a population with a

standard chromosomal count of 2n = 16, ensuring uniformity in bulb size. Experimental bulbs were immersed in LCuONPs at varying concentrations of 100 µL, 200 µL, 300 µL, 400 µL, and 500 µL, while the control bulbs underwent treatment with distilled water. Upon reaching a root length of 2-3 cm, chromosomal impact assessments were conducted. The squash technique was employed to create thin onion cell films after excising the roots from the primordial disc. Subsequently, nodules on roots were dyed with acetocarmine, with 1500 cells from the three most optimal preparations selected for analysis. Using a compound microscope of LEICA ICC50E, the cells were meticulously examined to discern any chromosomal aberrations. Next, these findings were contrasted with those of the control group.

2.5.4 Cytotoxicity Analysis

The evaluation of LCuONP cytotoxicity was conducted utilising Dalton's lymphoma ascites cells (DLA cells). These cells were extracted from the peritoneal cavity of mice with induced tumours, subsequently washed three times with normal saline. 0.1 mL of viable cell suspension was introduced to tubes holding different ratios of nanoparticles, and the volume was adjusted up to 1 mL with phosphate buffered saline (PBS). The control group contained equivalent concentrations of beetle gland extract that were incorporated. The test combinations were left to incubate for duration of 3 hours at 37 °C, after which 0.1 mL of 1% trypan blue were added. After a 3-minute interval, the enumeration of viable and non-viable cells was executed using a hemocytometer.

3. Results and Discussion

The biosynthesis of copper oxide nanoparticles (CuONP) encompasses several critical elements. Copper ions occur in different oxidation states, such as Cu(I), Cu(II), and a few Cu(III) ions. Whether utilising plant extracts, fungal extracts, algal extracts, or bacterial extracts, and controlling parameters like precursor concentration, pH, and temperature, the synthesis method maintains consistency for CuO, Cu_2O, and Cu_4O_3. These parameters play a substantial role in determining the nature of copper particles generated during the eco-friendly synthesis process [26, 32]. We selected CuONP for biosynthesis because of their distinctive characteristics, Copper, a vital mineral, naturally occurs in certain foods and can also be obtained through dietary supplements. It serves as a cofactor for various enzymes, referred to as cuproenzymes, which play roles in energy generation, iron regulation, activation of neuropeptides, formation of connective tissue, and synthesis of neurotransmitters [33]. Researchers worldwide are directing their attention towards copper-based nanoparticles, particularly CuONP, within the biomedical realm among various metal oxide nanoparticles. This focus is primarily due to their extensive bioactivity, safety, and cost-effectiveness [34]. In summary, the multifunctionality of copper oxide nanoparticles in the medical field highlights their potential for a range of therapeutic and diagnostic uses, positioning them as valuable assets in the progression of healthcare. Moreover, synthesising them from natural sources resonates with our objective of advancing environmentally sustainable and economically viable methodologies. When combined with the defensive secretion of Luprops tristis, we can further enhance the properties of CuONP. Despite the challenges posed by the beetle to human life in South India [35], we can repurpose its defensive secretion for beneficial applications.

During the present process, biomolecules are present in the defensive gland extract of the insect *Luprops tristis* Fig. 2, especially hydroquinones, reduce Cu^{2+} ions to the Cu^0 state

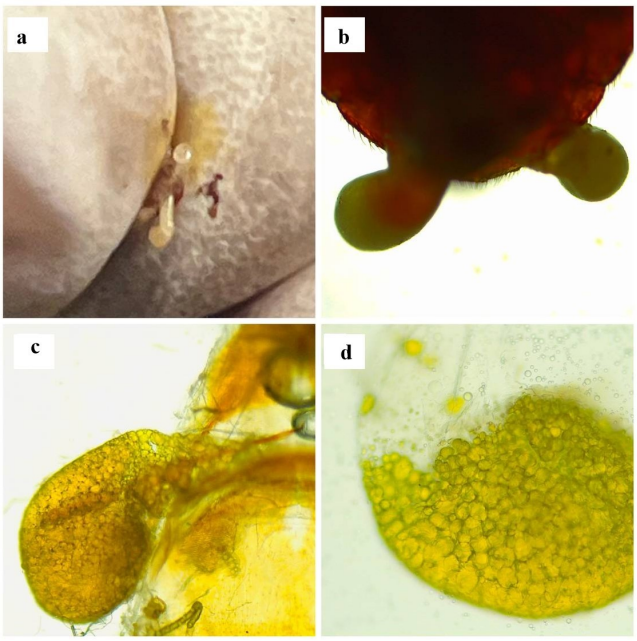

Figure 2. The defensive gland of *L. tristis*: Panel a) reveals the defensive gland exposed when the beetle is disturbed; panel b) provides a microscopic view of the glands, highlighting their intricate structure; panels c) and d) zoom in on a single gland lobe and the unique storage of its secretion in globule form.

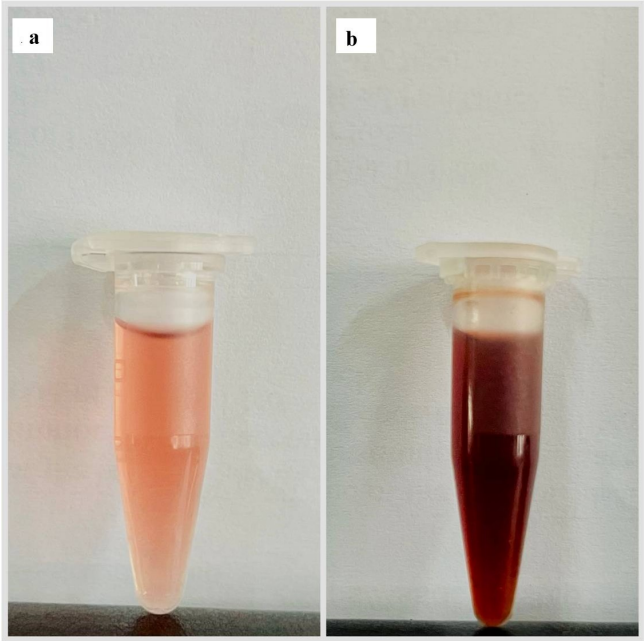

Figure 3. The figure illustrates the synthesis process of LCuONPs, showing the significant colour transition indicative of nanoparticle formation: Panel a) displays the initial mixture of defensive secretion and copper sulphate solution, while panel b) shows the notable colour change following microwave irradiation, marking the successful synthesis of nanoparticles.

while simultaneously oxidising them to form CuONP. Specific biomolecules within the sample extract act as an agent for capping and contribute towards the stabilisation of the resulting nanoparticles. Following the chemical characterization through techniques including UV-Vis spectroscopy and the use of fourier-transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), scanning electron microscopy (SEM), and zeta potential analysis, the study investigates the assessment of its biological applications encompassing hydrogen peroxide sensing, antibacterial analysis, antioxidant activity, chromosomal aberration or antimitotic activity, and anticancer activity.

3.1 UV-Visible Spectroscopy

The discernible alteration in colour of the reaction mixture, transitioning from purple to reddish brown as depicted in Fig. 3, subsequent to a 15-minute exposure to microwave irradiation, constituted the initial indication of LCuONP formation. Further confirmation of this observation was obtained using UV-visible spectroscopy. The production of CuONP was first confirmed optically and by means of UV-visible spectroscopy, which is a widely used technique to characterise synthesised metals and metal oxide nanoparticles. The reactive mixture's shift in colour because of the occurrence of surface plasmon resonance (Fig. 4) provides

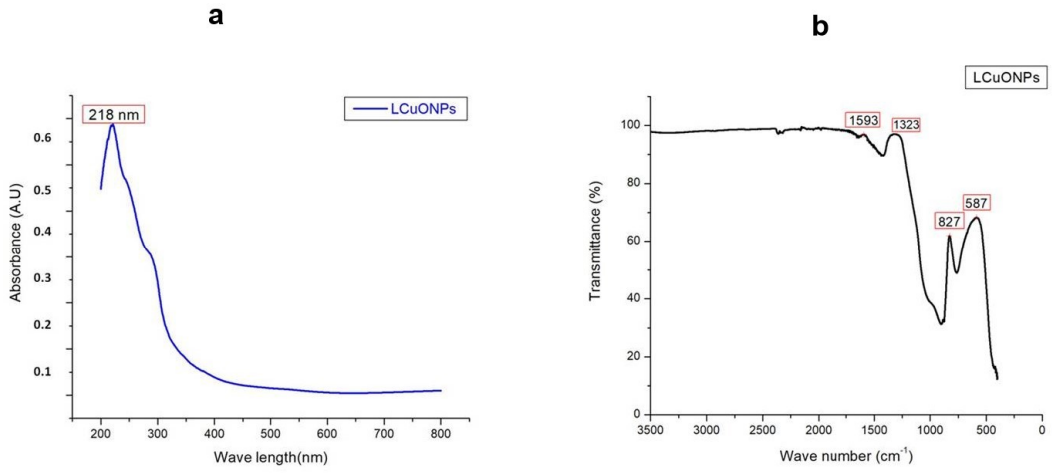

Figure 4. The figure presents the spectroscopic analysis of the study, with panel a) depicting the UV-visible spectrum of LCuONP, illustrating its optical properties, and panel b) showing the FTIR spectrum of LCuONP, highlighting the molecular vibrations and the presence of functional groups on the surface of nanoparticle.

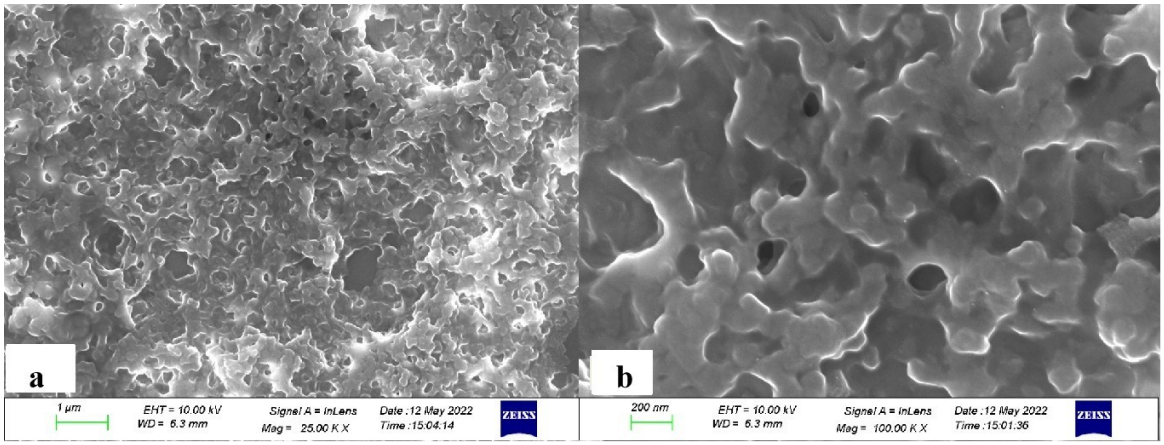

Figure 5. SEM images of LCuONP, providing detailed insights into their morphology: Panel a) at a scale of 1 µm reveals the general structure and aggregation of nanoparticles, while panel b) at a closer scale of 200 nm.

a helpful indicator that the mixture used in the reaction is producing LCuONP exhibited a prominent absorption peak at a wavelength (λ_{max}) of 218 nm, attributed to the surface plasmon resonance phenomenon. This phenomenon serves as a reliable indicator of LCuONP formation. The noteworthy broadening of the peak indicated a high degree of polydispersity among the particles, highlighting the evident interaction between the metallic copper and the biomolecules inherent to the gland extract. Research teams that have biosynthesised stable and economically viable CuONP from the foliage of *Ormocarpum cochinchinense* (*O. cochinchinense*) have confirmed the surface plasmon resonances of the nanosized copper oxide particles by observing the appearance of maximum absorbance at 200 nm [36].

3.2 FTIR Analysis of LCuONPs

The process of identifying functional groups involved in the bio-synthesised LCuONP was conducted through FT-IR spectroscopy. The recorded absorption peaks, delineated in Fig. 4, spanned a range from 4000 to 400 cm^{-1}. Especially, the spectrum exhibited prominent peaks at 1500 cm^{-1}, indicating the existence of metabolites in the vicinity of copper oxide nanoparticles that were synthesised. Our FTIR investigation affirmed that phenolic compounds assume a crucial role in stabilising copper oxide nanoparticles and exhibit enhanced affinity for interaction with them. Prior studies have hypothesised that specific bioactive constituents, including polyphenols, enzymes, and reducing sugars present in defensive gland extracts, might support the bio-reduction of copper oxide nanoparticles from copper ions, in addition to the stabilisation and encapsulation of metallic ions. The present work recorded the following peaks at 2361 (C-H stretch), 1829 (Carbonyl groups), 1324 (O-H in plane bend), 902 (C-H stretch), 827 (C-H stretch), 764 (aromatic out of plane ring bends), 587 (C-H) bend. According to Sharma et al.'s research, the development of CuO nanoparticles is indicated by the presence of discrete infrared absorption bands in the 400 – 600 cm^{-1} range. [37].

3.3 Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM)

The examination of nanoparticle morphology employed scanning electron microscopy for the study, as shown in Fig. 5. The SEM image provided a clear depiction of the significant density of LCuONP produced via the defensive gland extract of *L. tristis*. These LCuONP exhibited a predominantly spherical shape, characterised by a rugged surface

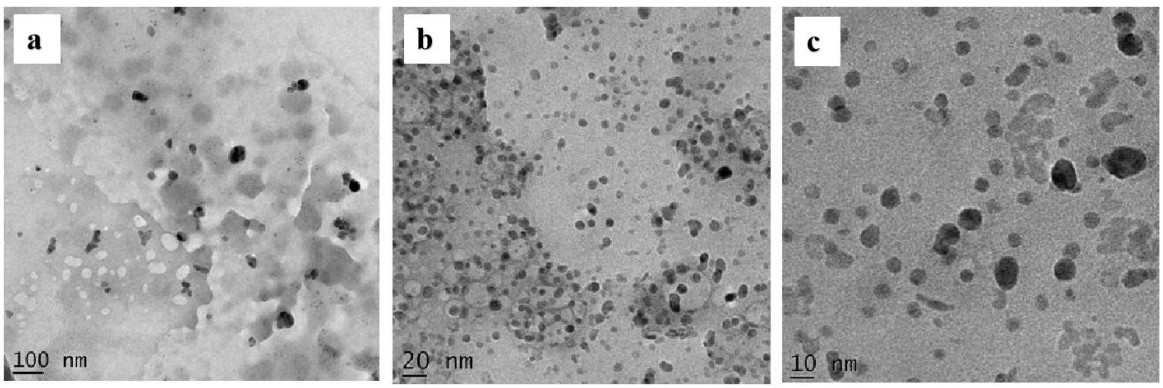

Figure 6. Transmission electron microscopy (TEM) images of LCuONP: Panel a) displays nanoparticles at 100 nm, showing the overall morphology; panels b) and c) represent higher magnification views at 20 nm and 10 nm, respectively, revealing detailed surface texture and particle size distribution of LCuONP.

texture and a uniform dispersion, as discernible in Fig. 6. Additionally, there was a propensity for individual nanoparticles to aggregate, culminating in the creation of larger nanoparticle structures. CuONP from *L. tristis* was credited with the presence of hydrogen bonding and electrostatic interactions among the bio-organic compounds. Size of the LCuONP was ranging from 15 ± 05 nm. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) images illustrate that the particles exhibit a uniform dispersion and possess a crystalline structure. An in-depth examination of the biosynthesized LCuONP was conducted through transmission electron microscopy (TEM), as depicted in Fig. 6. This examination revealed an average nanoparticle size of approximately 15 nm. These results are consistent with past studies conducted in the field [38].

3.4 Zeta Potential Analysis

To gauge the stability of the biosynthesised LCuONP, we employed a nanoparticle analyser (HORIBA SCIENTIFIC SZ 100). The results demonstrated substantial stability, with a zeta potential of -22 meV for LCuONP, as illustrated in Fig. 7. Our prepared LCuONP exhibited commendable stability, aligning with earlier research on the zeta potential. The negative value of the zeta potential underscores this stability, enhancing uptake through electrostatic interactions between the cationic nanoparticles and the cationic membrane. The elevated absolute zeta potential value indicates a robust repulsion between the particles, effectively inhibiting their aggregation [39, 40].

3.5 XRD Analysis of biosynthesised copperoxide nanoparticles

X-ray diffraction (XRD) analysis was employed to scrutinise the precise structural composition of copper oxide (CuONP). The synthesised CuONPs revealed a crystalline monoclinic structure as determined by x-ray powder diffraction. The XRD pattern, illustrated in Fig. 8, covered the 2θ range of 0° to 80°, revealing the polycrystalline nature of the synthesised CuONPs, specifically indicating the monoclinic tenorite phase of CuO structure. Notably, various small, distinct diffraction peaks corresponding to copper were observed at approximately $2\theta = 38^\circ$ and 50° , representing the (111) and (211) planes of the primitive structure of CuONP, respectively. The XRD spectrum was compared with the database of the Inorganic Crystal Structure Database (ICSD) and aligned well with previous literature findings [41, 42].

3.6 Electrochemical hydrogen peroxide sensing by differential pulse voltammetry

Hydrogen peroxide (H_2O_2) is a common intermediate in many biological reactions and an essential metric for tracking these bioprocesses, H_2O_2 measurement has garnered a lot of attention [43, 44]. The efficiency and low cost of electrochemistry in identifying H_2O_2 have been shown by previous research [45]. In this inquiry, a series of measurements pertaining to the hydrogen peroxide concentration (3M) within the range of 10 µL to 60 µL were conducted and depicted in Fig. 9-a. Additionally, calibration linear correlations between DPV current output and hydrogen peroxide concentration were established and illustrated in Fig. 9-b. The determined limit of detection was found to be 0.9 µL. The LCuONP synthesised within the scope of this present investigation hold potential for application in electrochemical hydrogen peroxide monitoring systems. The interaction of hydrogen peroxide molecules with the functionalised LCuONP may induce alterations in electrical conductivity or redox processes, which can be discerned and linked to hydrogen peroxide levels. Polyalizarin Yellow R nanocomposite and CuONP combined in a sensor for hydrogen peroxide detection by DPV show a LOD value of 0.03 µM [46]. A hydrogen peroxide analysis electrochemical sensor was created using copper-doped CuONP, and it was found that the detection limit was 0.23 µM [47]. Noteworthy is the fact that biosynthesised CuONP may exhibit greater biocompatibility compared to their chemically synthesised counterparts, thus warranting substantial attention

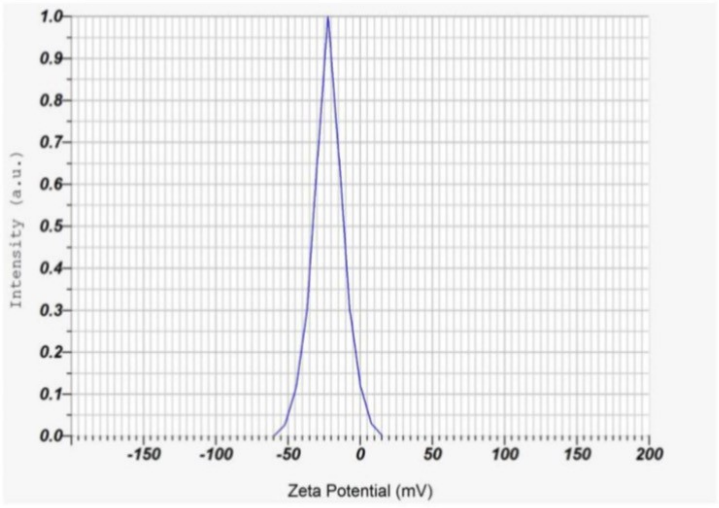

Figure 7. Zeta potential analysis of LCuONP: This figure presents the zeta potential measurement, illustrating the surface charge and stability of LCuONP within colloidal solutions.

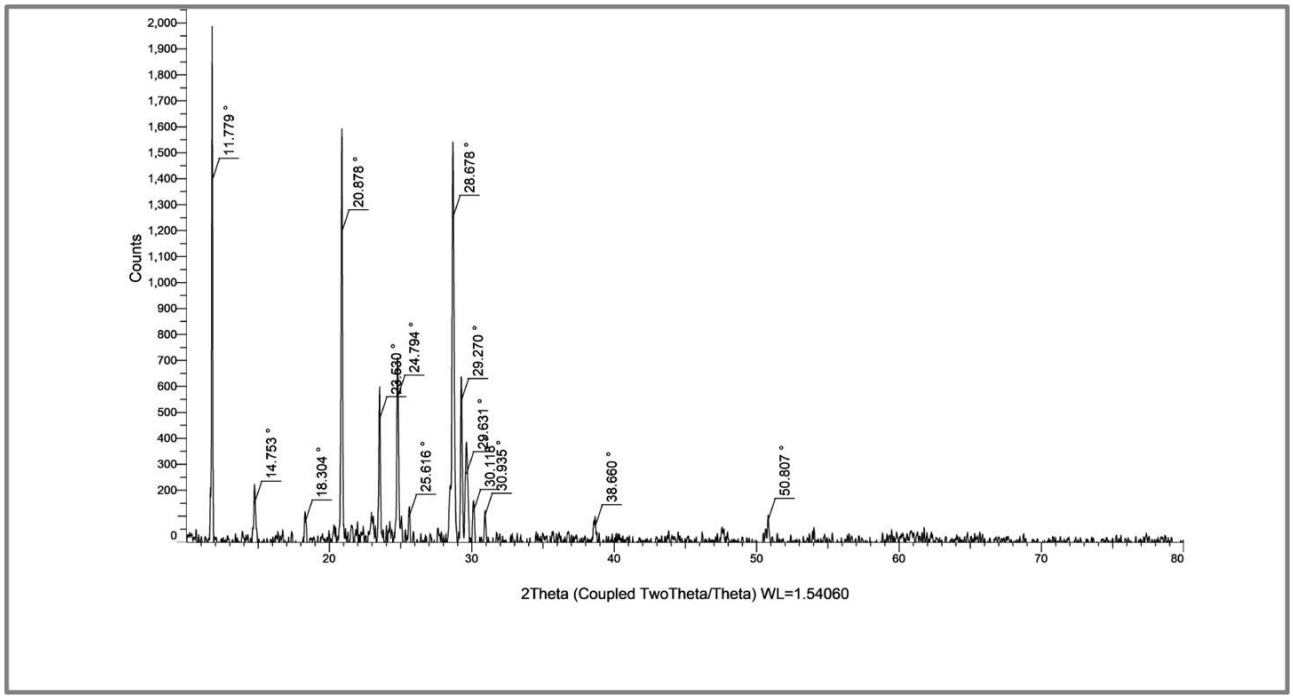

Figure 8. X-ray diffraction (XRD) analysis showcases the crystalline structure of copper oxide nanoparticles, highlighting their distinct monoclinic phase patterns and crystallite sizes.

as a result of their potential to enhance the sensitivity and specificity of hydrogen peroxide sensors. This current study corroborates findings from previous research endeavours, thereby contributing to the existing body of knowledge in this field. Metal oxides offer desirable attributes for sensor fabrication, including thermal stability, resistance to irradiation, and the propensity to form diverse nanostructures. Additionally, their environmentally friendly nature is a key factor driving interest in developing nanosensors with these materials. Utilisng metal oxides like CuO, which possess electro-catalytic properties, is an excellent approach to fulfilling this objective [48].

3.7 Antibacterial Assessment

Within the present investigation, disc diffusion test was employed to scrutinise the antibacterial attributes. The impact

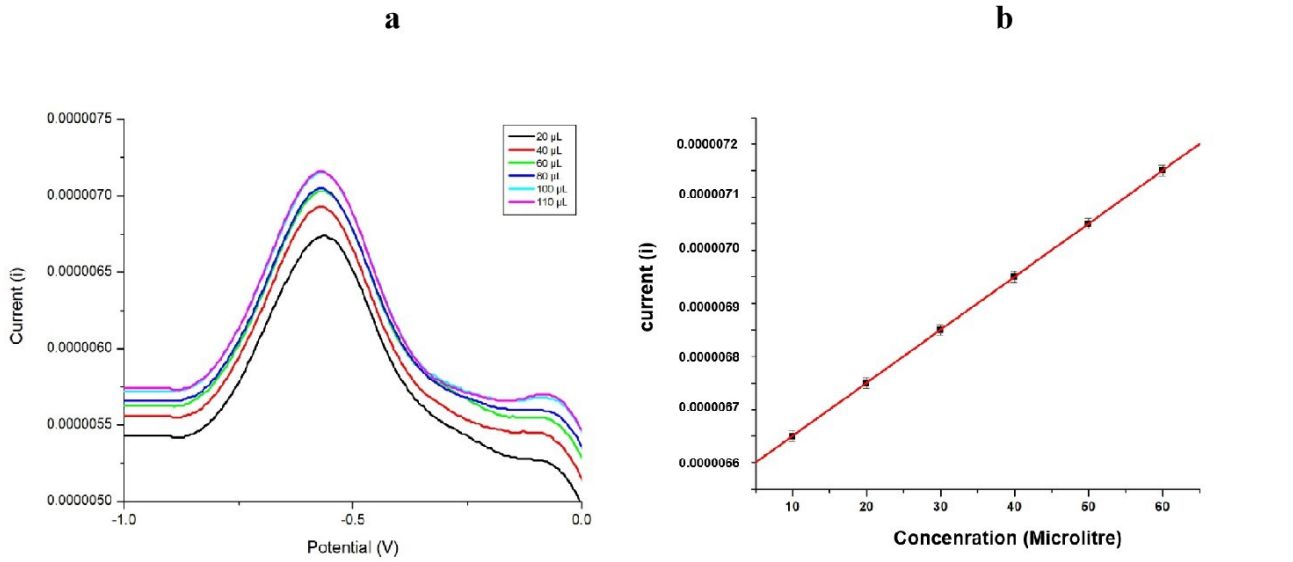

Figure 9. Hydrogen peroxide sensing via differential pulse voltammetry: a) depicts the DPV measurements of an electrode coated with LCuONP across varying concentrations of hydrogen peroxide. Figure b) presents the calibration curve, correlating the DPV current output with the concentration of H2O2, to demonstrate the sensitivity and detection limit of the LCuONP-based sensor.

of LCuONP on the proliferation of both Gram-negative bacteria (Klebsiella pneumoniae) and Gram-positive bacteria (Staphylococcus aureus) was evaluated. Control plates impregnated with solvent (20 µL) demonstrated a zone of inhibition (ZOI) measuring 6.33 mm. However, when filter paper soaked in varying concentrations of LCuONP (5 µL, 10 µL, 15 µL and 20 µL) was utilised, the inhibitory zones' diameters exhibited a spectrum of 6.66 ± 0.57, 9.66 ± 0.57, 8.33 ± 0.57 mm, 11.12 ± 0.57 mm, respectively, for K. pneumonia, as illustrated in Fig. 10. When it comes to S. aureus, the ZOI for the control (20 µL) was 10 mm, while filter papers saturated with 5 µL, 10 µL, 15 µL, and 20 µL of LCuONP displayed inhibition zone diameters of 10.33 ± 0.57 mm, 12.66 ± 0.57 mm, 14.66 ± 0.57 mm, 17.33 ± 0.57 mm, respectively. This pattern signifies a progressive augmentation in the inhibition zone's diameter with an escalating concentration of LCuONP, as portrayed in Fig. 10. CuONP find diverse scientific applications, they demonstrate remarkable efficacy against various pathogenic microbes. When present in high concentrations, CuONP induce the process that results in cell lysis in bacteria by producing reactive oxygen species. Additionally, CuONP exhibit anticancer and antifungal properties. Their antimicrobial activity makes them valuable for food preservation and agricultural purposes, providing protection against different pathogenic fungi and bacteria [49] . Copper-containing nanoparticles have a larger surface area and unique crystal structures compared to other copper molecular materials. In distinct ways, they can affect different biological components of microbial cells, resulting in increased antibacterial activity because they dissolve more quickly in solutions, releasing more metal ions. They work against microorganisms by producing reactive oxygen species (ROS), rupturing membranes and cell walls, and reacting with DNA and proteins [50–52].

Metal and metal oxide nanoparticles have drawn interest in the last few decades as potential treatments for viral and bacterial illnesses [53–57]. Nanoparticle-based antibiotics and drugs are appealing due to their reduced toxicity, eco-friendliness, and potential disease-fighting properties. Notably, CuONP demonstrate a strong antibacterial effect against a range of infections [58]. Their vast surface area, compact stature, biocompatibility, and reactivity contribute to efficient bacterial cell eradication. Bio-fabricated CuONP are effective against bacterial strains that are both gram-positive and gram-negative. Green-synthesized Cu and CuONP have demonstrated the impact of antimicrobial agents on Pseudomonas aeruginosa, *Clostridium difficile*, *Staphylococcus aureus*, and *Escherichia coli*, etc [59]. Additionally, CuONP derived from *Gloriosa superba* leaf extract inhibit gram-positive bacteria (*Staphylococcus aureus*) and gram-negative bacteria (*Klebsiella aerogenes*) [49]. The antibacterial efficacy of LCuONP exhibits a dose-dependent characteristic; an elevation in the concentration of LCuONP results in an augmented antimicrobial effect, particularly towards *S. aureus* when compared with *K. pneumonia* (Fig. 11). CuONP cause the build-up of ROS, or reactive oxygen species, disrupting the cell membrane's integrity and directly contributing to cellular toxicity [60]. The toxicity induced by CuONP in bacterial cells also leads to the degradation of mitochondria, ribosomes, and various protein channels within the cell membrane of bacteria. However, the precise mechanism underlying the antibacterial action of CuONP remains an on-going area of research.

3.8 Antioxidant activity

The DPPH assay, widely recognised for its efficacy in assessing nanoparticles' ability to neutralise free radicals, has been extensively utilised in evaluating antioxidant potential. Various concentrations of LCuONP (20 µL, 40 µL, 60 µL, 80 µL, 100 µL) were used in this research, and every sample was combined with DPPH to yield 2 ml solutions. Ascorbic acid, employed as the standard, was made soluble in purified water as the solvent. Subsequent to a 30-minute incubation period under conditions of darkness, the solution underwent UV spectroscopic examination. At 517 nm, the

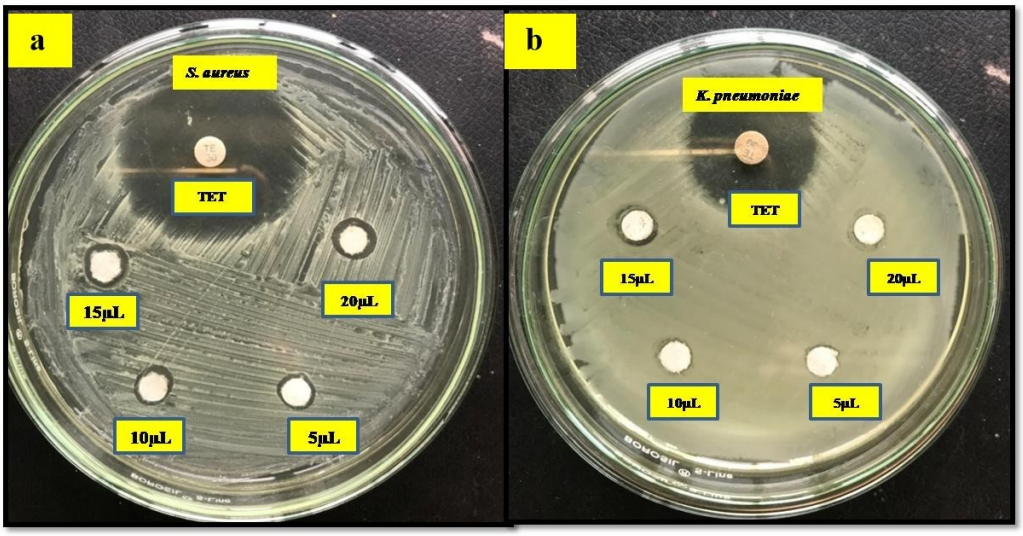

Figure 10. Antibacterial study using a disc diffusion assay of LCuONP, where panel a) illustrates the inhibitory effect of LCuONP on *Staphylococcus aureus* and panel b) demonstrates its impact on *Klebsiella pneumoniae*.

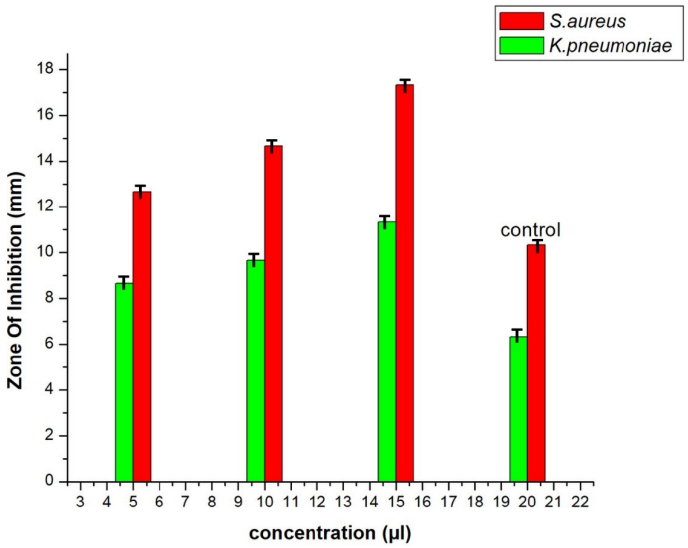

Figure 11. Comparison of the antimicrobial activity of LCuONPs against *S. aureus* and *K. pneumoniae*, showing the differential effectiveness of the nanoparticles.

maximum electron absorption caused by DPPH free radicals was observed. Concentration-dependent scavenging behaviour of LCuONP has been meticulously detailed in Fig. 12. It was found that 74 µL of sample concentration was required to produce 50% scavenging activity (EC_{50} value). Notably, with an escalation in the concentration of LCuONP, the colour of the DPPH solution transitioned from a deep violet hue to a pale yellow shade. UV spectroscopic analysis substantiated the amplified antioxidant efficacy of LCuONP at higher concentrations, as delineated in the graph. These observations support the findings reported in diverse studies within the realm of bio-inspired synthesis of CuONP. One of the fundamental areas of inquiry in the field of nanoscience and technology pertains to the evaluation of the antioxidant potential exhibited by nanomaterials [61, 62]. Antioxidants play a crucial role in influencing the functioning of various biological systems. Within biological frameworks, free radicals are produced as a consequence of the interaction between biomolecules and molecular oxygen [63, 64]. Numerous investigators have scrutinised the antioxidant capabilities of a wide array of both natural and synthetic compounds [65]. The 2, 2-diphenyl-1-picrylhydrazyl (DPPH) scavenging assay is widely recognised as the predominant approach for in-

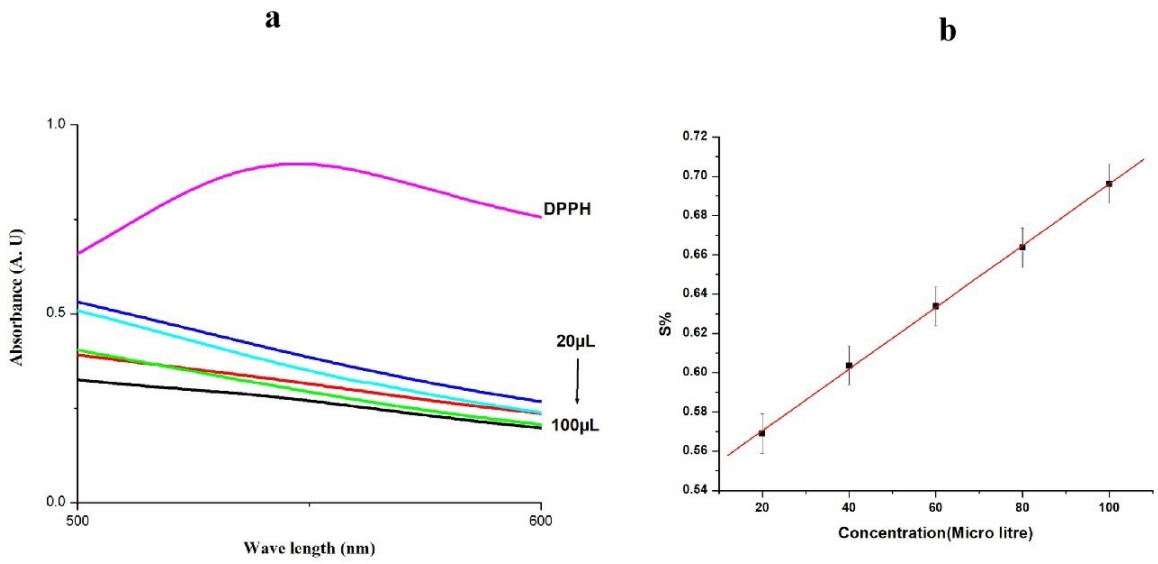

Figure 12. The antioxidant activity of LCuONP determined by the DPPH assay, with panel a) depicting the scavenging activity at various concentrations of LCuONP, highlighting its dose-dependent efficacy. Panel b) presents the calibration curve, correlating the concentration of LCuONP with the percentage of DPPH radical scavenging.

vestigating the antioxidant attributes of materials. This investigation ascertained the antioxidant efficacy of copper oxide nanoparticles synthesised employing an extract of *L. tristis* utilizing the DPPH radical scavenging assay. Within the DPPH methodology, antioxidants present in the sample effectuate the reduction of the nitrogen radical DPPH, which is stable and leads to a diminution in absorbance registered at 515 nm in wavelength. Substances capable of yielding oxygen atoms facilitate the conversion of DPPH to its reduced form, resulting in the loss of the characteristic violet hue in the solution. CuONP biosynthesised with an extract of *Galeopsidis herba* had a parameter value of 4.12 $\mu g\,mL^{-1}$. This outcome signifies that the CuONP obtained exhibit a notable degree of antioxidant efficacy [66]. The IC_{50} value for the green synthesis of CuONP using *Malus domestica* leaf extract was determined to be 24.73 $\mu g\,mL^{-1}$ [67].

3.9 Chromosomal Aberration Assay

The employment of biosynthesised CuONPs in chromosomal aberration studies provided compelling evidence of phytotoxicity induction, leading to the manifestation of chromosomal abnormalities represented in Fig. 13. This observation was substantiated by conspicuous findings illustrating a dose-dependent modulation in the proliferation of root cells subsequent to exposure to diverse concentrations of LCuONPs, ranging from 100 µL to 500 µL. The current inquiry has unveiled discernible chromosomal aberrations encompassing chromosome bridges, anaphase stickiness, vagrant chromosomes, fractured chromosomes, and lag chromosomes. The genotoxic potential of CuONP was quantified as a percentage relative to both control and experimental conditions. Analysis of the data indicated that the Mitotic Index (MI) within the control group remained within the anticipated range; conversely, in the experimental group, the percentage of chromosomal abnormalities displayed a gradual escalation, accompanied by a diminishing % MI (Fig. 14). Noteworthy is the fact that with an increase in LCuONP concentration, the lowest MI of 3.54 was noted at 500 µL, concomitant with the highest aberration percentage. According to the present investigation, onion bulbs exposed to varying nanoparticle concentrations may have harboured nanoparticles that, upon adherence to onion roots and infiltration of tissues, triggered the generation of reactive oxygen species (ROS), consequently perturbing redox equilibrium and inciting genotoxic and mito-depressive effects. The present study shows a gradual reduction in % MI with increasing concentrations of LCuONP. These outcomes are consistent with Nagaonkar's findings [68]. Furthermore, a noteworthy elevation in chromosomal irregularities was observed in roots treated with CuONP (2000 $\mu g\,mL^{-1}$), including occurrences of chromosome bridges (2 ± 0.2), metaphase stickiness (1.9 ± 0.05), anaphase stickiness (1.24 ± 0.1), vagrant chromosomes (1.24 ± 0.1), fractured chromosomes (1.7 ± 0.1), and lagging chromosomes (1.8 ± 0.3) as in [69].

4. Anticancer Study

Presently, cancer stands as the most hazardous and prevalent ailment, contributing significantly to global mortality rates. As of now, there are no definitive pharmaceutical interventions commercially accessible for cancer treatment. The conventional modalities of treatment, namely radiotherapy and chemotherapy, while frequently employed, entail substantial side effects and are linked to significant monetary expenses. Current investigations are concentrated on identifying a non-toxic biological drug as a viable alternative. In this particular situation, the advent of nanotechnology has demonstrated substantial promise in the effective management of various forms of cancer. Biologically synthesised CuONP have demonstrated noteworthy efficacy in experiments conducted on select cancer cell lines in humans. Utilising dry black beans for the green synthesis of CuNPs, observations revealed that these nanoparticles exhibited inhibiting effects on cervical cancer in humans and displayed cytotoxicity against the HeLa cell line through the generation of reactive oxygen species (ROS) [70].
Furthermore, investigations revealed that biosynthesised

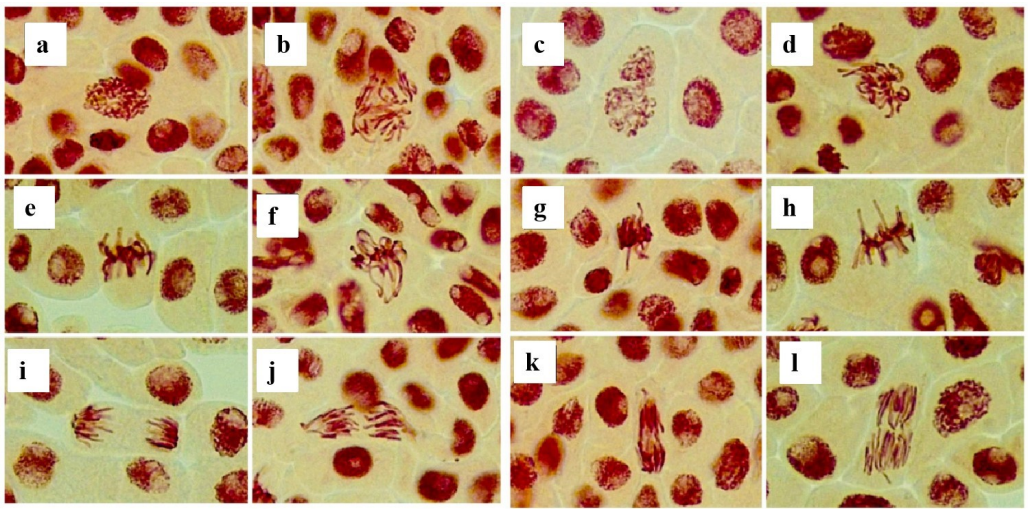

Figure 13. Chromosomal aberration assay; Effect of LCuONP on mitosis. a) Normal prophase, b,c,d) Abnormal prophase, e) Normal metaphase, f,g,h) Sticky and laggard metaphase, i) Normal anaphase, j,k,l) Anaphase bridges.

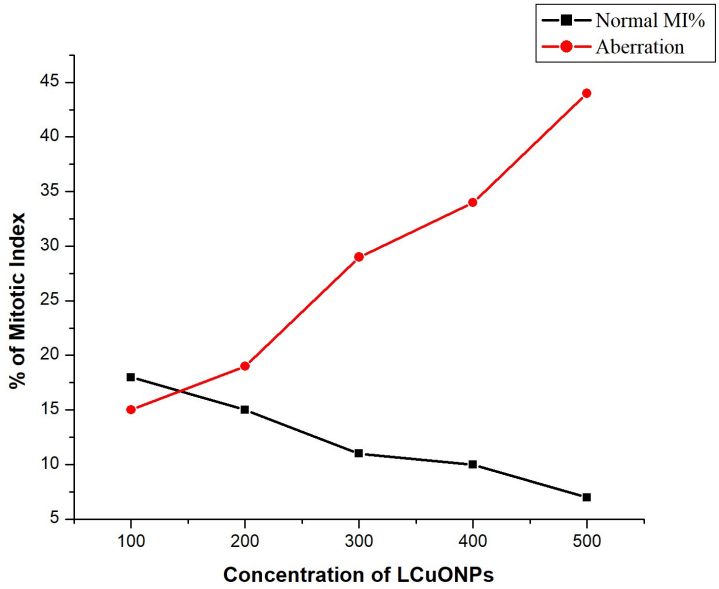

Figure 14. Comparison of increase in chromosomal aberration and decrease in mitotic index (MI).

CuONP possess the capability to impede the growth of A549 adenocarcinomic human alveolar epithelial cells. Elevated concentrations of CuONP induce cellular toxicity and provoke DNA damage in A549 lung cells [71]. Additionally, biologically synthesised chitosan/copper oxide nanocomposites, employing the bioflavonoid rutin, exhibited notable antiproliferative efficacy when evaluated against the human lung cancer cell line [72]. In a separate study, treatment of HeLa cells with CuONP esulted in oxidative stress-mediated mitochondrial degradation. Normal control cells exhibited a typical mitochondrial structure, while treated cancer cells displayed a condensed and clumped mitochondrial structure, eventually resulting in apoptosis of the cancer cells [70]. Numerous nanoparticles have been examined for their pharmacological and biochemical traits, which include antioxidant and anti-inflammatory properties. These characteristics could potentially contribute to their anticarcinogenic and antimutagenic effects. Presently, biosynthesised nanoparticles play a crucial role in the treatment of various maladies, encompassing cancer. The present investigation guarantees the efficacy of biologically produced CuONP as an anticancer agent, utilizing Dalton's lymphoma ascites (DLA) cell lines in an in vitro setting. The CuONP demonstrate dose-dependent cytotoxicity against DLA cells. The assay reveals a progressive escalation in cellular death commensurate with the concentration of LCuONP (Fig. 15 and Table. 1). The potential for CuONP to enhance anticancer effects might stem from their ability to modulate different classes of histone deacetylases (HDACs) [88]. The ways in which CuONP can induce toxicity in cells encompass various mechanisms, such as the generation of reactive oxygen species [74], the release of copper ions, coordination effects, disruption of cellular homeostasis, autophagy, inflammation, surface modifications, dissolution, nanoparticle dosage, and exposure routes [89]. As previously noted antioxidants play a crucial role in preventing diseases caused by the effects of free radicals and are pivotal in the prevention and treatment of cancer. Antioxidants comprise a diverse array of molecules that have an interaction with free radicals, rendering them inert. By annihilating free radicals, antioxidants have the capacity to forestall and treat cancer. Consequently, the present investigation affirms that biologically synthesised LCuONP possess comprehensive anti-cancer properties. This antitumor attribute of copper oxide, as demonstrated in numerous antecedent research endeavours, is consistent with the findings of the current study. The biological activities of CuONP synthesised from different sources according to previous studies are given in Table. 2.

5. Conclusion

In the current global scenario characterised by heightened awareness of environmental issues and pollution, it is imperative to reduce the usage of hazardous compounds in diverse technical domains focused on application-driven research. Within this context, it is evident that employing CuONP as a non-hazardous alternative holds great significance in crucial research sectors such as drug delivery systems, addressing various health concerns, bolstering plant defence mechanisms, and within the textile industry, among others. Nevertheless, the synthesis process assumes

Table 1. Anticancer activity of LCuONP on DLA cells.

Concentration of LCuONP	Percentage of cell death
10	15.1 ± 1.34
20	20.8 ± 1.33
50	32.0 ± 1.01
100	54.9 ± 4.75
200	75.9 ± 1.78

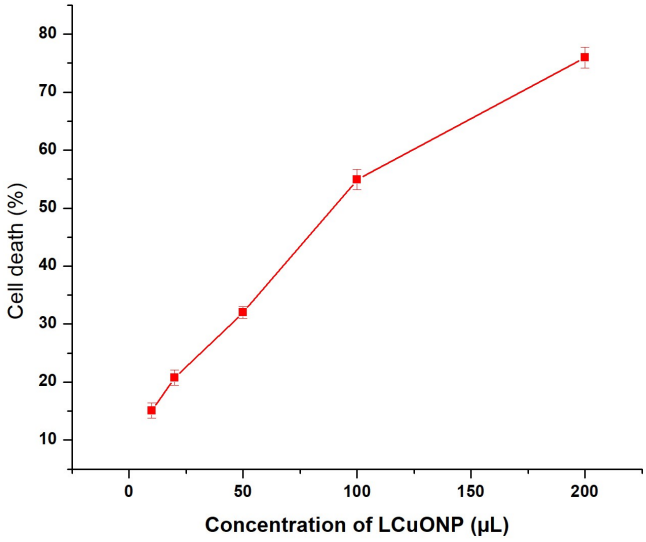

Figure 15. Anticancer study: This graph illustrates the impact of Luprops tristis-mediated copper oxide nanoparticles (LCuONP) on Dalton's Lymphoma Ascites (DLA) cells, demonstrating their efficacy in inhibiting cancer cell proliferation.

paramount importance, given that detrimental substances are employed in both physical and chemical synthesis of CuONP.

In contrast, the biological technique emerges as an eco-friendly, cost-efficient, reliable, stable, energy-conserving, and straightforward procedure. Nonetheless, to further advance the biomedical applications of CuONP, additional research endeavours should be undertaken to explore means of mitigating the toxicity associated with CuONP while concurrently preserving and augmenting their biological efficacy. In summary, this investigation sought to establish a direct methodology for the eco-friendly, cost-effective, and non-toxic synthesis of CuONP, employing the insect *Luprops tristis* as a distinctive biological approach. The successful biosynthesis of CuONP was rigorously confirmed through an array of analytical techniques, encompassing UV-Vis spectrometry, FTIR analysis, SEM, TEM, and zeta potential and XRD analysis. These nanoparticles exhibited crystalline structure, irregular spherical morphology with a rugged surface, demonstrating an average size of 15 nm. Moreover, this study showcased the enzyme-free glucose sensing capabilities of LCuONP-coated electrodes as biosensors, along with their dose-dependent antimicrobial efficacy against *S. aureus* and *K. pneumoniae*, bring out

Table 2. Biological application of biosynthesised CuONPs- comparison of previous studies.

Biological source used for reduction of copper	**Biomedical application**	**Reference**
Syzygium alternifolium	Anti cancer activity on Human breast cancer cell lines Antifungal effect against *T. harzianum*	[73]
Phaseolus vulgaris	Anti cancer effect on Breast, colon, liver and prostate cancer cells	[74]
Trichoderma asperellum	Anticancer effect on Human lung carcinoma A549 cancer cells	[75]
Lactobacillus casei Subsp. Casei	Anticancer effect on Human colorectal and gastric cancer cell lines (HT-29 and AGS)	[76]
Microalgae	Anticancer effect on MCF-7 breast cell line	[77]
Brown alga	Anticancer effect on Human breast MCF-7 cell line	[78]
Citrus medica Linn.	Antifungal effect on *Fusarium culmorum*, *F. oxysporum*, *F. graminearum*	[79]
Penicillium chrysogenum	Effect on *Fusarium oxysporum*, *Alternaria solani*, *Aspergillus niger*	[80]
Oxalis corniculata L	Effect on *Fusarium oxysporum*, *Alternaria alternate*, *Pythium ultimum*, *Aspergillus*	[81]
Saccharum officinarum	Effect on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*	[82]
Syzygium aromaticum	Effect on *Staphylococcus*, *Escherichia coli*, *Pseudomonas*, *Bacillus subtilis*	[83]
Solanum lycopersicum	Effect on *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*	[84]
Proteus mirabilis	Effect on *Pseudomonas aeruginosa*, *Salmonella typhi*, *Escherichia coli*, *C. perfringens*, *B. cereus*, *Staphylococcus aureus*, *E. faecalis*	[85]
Bifurcaria bifurcata	Effect on *Enterobacter aerogenes*, *Staphylococcus aureus*	[86]
Aloe vera	Effect on *Aeromonas hydrophila*, *Pseudomonas fluorescens*	[87]

their potential as potent bacterial inhibitors.
Significantly, the nanoparticles also elicited chromosomal aberrations in the root tips of *Allium cepa*, with escalating nanoparticle concentrations correlating to diminished mitotic indices and heightened chromosomal aberrations. Additionally, this investigation unveiled the antioxidative potential of LCuONP and elucidated their dose-dependent cytotoxicity against DLA cells. This comprehensive scrutiny underscores the versatility of biosynthesised CuONP, manifesting in their diverse applications, including antibacterial and antioxidant attributes, as well as their potential utility in biosensing and cancer therapeutics. Therefore, deriving copper oxide from insect secretions offers a unique and refined method, turning a nuisance into a valuable asset using a synthesis process that is environmentally friendly, economically viable, and free from harmful substances.

Acknowledgments
The first author, Sabira O., gratefully acknowledges the financial assistance from the Council of Scientific and Industrial Research, New Delhi, India, which was instrumental in the successful completion of this research article. We extend our sincere thanks to STIC CUSAT Cochin, India for TEM analysis, CSIF - University of Calicut, India for SEM analysis, and Amala Cancer Research Centre, Thrissur, India for anticancer study.

Authors Contributions
All authors have contributed equally to prepare the paper.

Availability of Data and Materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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