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In vitro myco-synthesized copper oxide nanoparticles: A promising antiviral agent with antioxidant, anti-inflammatory, and anti-cancer activity

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ABSTRACT

The most suitable approach for manufacturing different metal and metal oxide nanoparticles is to use fungi. The purpose of this work is to examine how fungi that have been isolated from soil produce CuO-nanoparticles (CuO-NPs). It has been demonstrated that *Aspergillus niger* (*A. niger*), which was obtained from soil and recognized using both conventional and molecular methods, is capable of producing CuO- nanoparticles. UV, XRD, FITR, and TEM have all been used to characterize these nanoparticles. The antioxidant potential of synthesized CuO-nanoparticles was examined by the process of DPPH scavenging. Red blood cells' membrane stabilization technique was used to investigate the anti-inflammatory properties. The MTT procedure was utilized to explore the anticancer activity. The antiviral was identified by the decrease in the ability of Coxsackie-B, Adenovirus-22, and Adenovirus-40 to infect Vero Cells. CuO nanoparticles have a spherical form, a peak at 345 nm, and diameters in a range from 4 to 18 nm. Using the DPPH technique, the biosynthesized CuO-NPs were found to have antioxidant action, with an IC_{50} value of 27.93±2.4 µg/mL. The anti-inflammatory proportion of the biosynthesized CuO-NPs was 98.6% at 1000 µg/mL and dropped to 48.9% at 100 µg/mL. The biosynthesized CuO-NPs exhibited anticancer action versus MCF-7, PANC1, and Caco2 cells, with IC_{50} values of 82.02 ± 1.04 µg/mL, 91.45 ± 1.89, and 151.45 ± 0.89, respectively. Ascending concentrations of CuO-NPs enhanced their antiviral effect (%) against the Co-B4, Adeno-22 and Adeno-40 viruses. The myco-synthesized CuO-NPs showed encouraging results and have the potential to be utilized in a range of therapeutic uses.

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Introduction

Nanotechnology is a cutting-edge discipline of study and construction that is transforming to several industries through utilizing the special properties possessed by the produced substances at the nanoscale (Abdel-Azeem et al. 2020, Srivastava et al. 2021, Bouafia et al. 2023, Abdel-Azeem et al. 2020). One of the latest and greatest advances in this sector is the creation and utilization of nanomaterials, which have a wide range of potential uses in technical and medical devices due to their enlarged

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surface dimension, rigidity, and conduction (Gu et al. 2023).

Copper oxide (CuO) nanoparticles are potent antibacterial, catalyzing, consistent and protective particles that have demonstrated intriguing behavior in several biomedical fields (Grigore et al. 2016). Naturally occurring CuO nanoparticles (CuO-NPS) are inexpensive materials. To produce CuO-NPs with the proper dimension and shape, manufacturing techniques' manipulation is paramount in this process (Gawande et al. 2016). There are numerous physical and chemical techniques for preparing nanoparticles. CuO-NPs are prepared biologically by extracting various plant components, microorganisms, and algae, as documented in scientific research (Shamsuddin & Nordin, 2019; Naz et al. 2020).

In contrast to physical and chemical approaches that need costly and dangerous chemical substances, biogenic production is easy, inexpensive, and environmentally friendly. It also produces environmentally friendly and biocompatible nanoparticles (Javed et al. 2021, Sharaf et al. 2024). It has been reported that CuO-NPs can be synthesized with bacterial extracts (Khodair et al. 2019). The myco- synthesis of CuO-NPs utilizing various fungal extracts has also been done in limited studies (Consolo et al. 2020; Mani et al. 2021). Furthermore, utilizing several algal species reported on the production of CuO-NPs produced by algae (Bhattacharya et al. 2019; Araya-Castro et al. 2021; Taherzadeh et al. 2021).

CuO-NPs have great antibacterial capabilities (Alshawwa et al. 202) and are employed as possible disinfecting agents towards nosocomial diseases, which makes them useful for an extensive variety of nano-medical applications (Baek & An, 2011; Perreault.et al. 2012; Ostaszewska et al. 2015) Besides, (Grigore et al. 2016) state that they are used in surgical dressings because of their potent bactericidal properties towards various bacterial strains. CuO NPs have also been shown to have fungicidal action against a small number of fungal strains. CuO NPs reported to have extensive application as biological sensors in the detection of naturally occurring precursors in the body. Furthermore, they may play a crucial role in the therapy of various cancer forms as anticancer drugs and employed as efficient nano-carriers (Chevallet et al. 2017; Naz et al. 2018).

In this work CuO-NPs were myco-produced using isolated *Aspergillus niger*. The myco-synthesized CuO NPs were characterized via UV–visible spectroscopy, FT-IR, XRD, TEM. Moreover, the antioxidant, anti-inflammatory, anticancer, as well antiviral properties of the prepared CuO-NPs were examined. Furthermore, cytotoxicity of myco-synthesized CuO nanoparticles was detected by examining their impact on different levels towards Vero cells.

Material and Methods

Isolation, purification of Microorganisms

The specimens of the soil were obtained at a depth of around 10 cm from cultured land. Every soil specimen (1 g) was submerged in 100 ml of distilled water. A one ml of soil suspensions ranging from 10^{-1} to 10^{-6} concentrations of the different specimens were put on dishes containing Czapex-Dox Agar medium (Ullah et al. 2017) and complemented with 0.5% chloramphenicol to inhibit the development of bacteria (Ratna et al., 2015). Every specific dilution was tested on three separate sets of plates. After that, the diches were then left at 27±2°C for seven consecutive days, or till the colonies started to appear.

Hyphae tips or collections of cells were re-inoculated onto fresh plates to purify the fungal species. Under the microscope and visually, the purified isolates were examined. According to Al-Enazi et al. (2018), only pure isolates were grown on Czapex-Dox Agar medium slants and stored at -4°C for future studies. The capability of the pure fungal isolates to produce metal oxide nanoparticles was screened, and the most efficient cultures were chosen based on their capacity to produce nanoparticles.

Classical and Molecular identification of the fungus

The chosen fungal strain, known as Mekky1502, was first identified conventionally using phenotypic and microscopic inspection in accordance with standard steps (Diba et al. 2007), and then it was identified molecularly using ITS sequence evaluation. According to White et al. (1990), primers for ITS1 (5¨ CTTGGTCATTTAGAGGAAGTAA-3´) and ITS4 (5¨ TCCTCCGCTTATT GATATGC 3´) were used to amplify and sequence the ITS gene. As previously pointed out, the PCR process and sequencing technique were successful. Those deposited in GenBank with the ClustalX 1.8 program (http://www.clustal.org/clustal2) to compare their ITS gene sequences. Additionally, the neighbor-joining approach (MEGA v6.1) program was used to create the phylogeny tree, and the bootstrap evaluation (1000 repetitions) was used to assess the tree's credibility (Fouda et al. 2022).

Preparation of metal oxide nanoparticles

The fungus strain FU.1 was introduced into 100 milliliters of PD broth medium and left to develop for five days at 26 ± 2°C. After the duration of incubation, any remaining medium ingredient washed out three times with deionized water and the seeded PD broth media (Thermo-fisher, USA) was spun up at 9,950 rpm for five minutes to acquire the fungal biomass. Then, for 24 hours in the dark

and with constant movement (150 rpm), 10.0 g of the harvested fungal biomass was combined with 100.0 mL of deionized water. In order to use the supernatant—a fungus biomass filtrate—as a biological catalyst for the manufacture of CuO-NPs, the entire mixture was spun up after that.

The following steps were used to achieve the manufacturing of CuO-NPs employing the fungi filtrates: To achieve a 5 mM level, 100 µg of $Cu(CH3COO)_2.H_2O$ (98%, Sigma, USA) was added to in 5 mL dis. H_2O and then combined with 95 mL of microbial filtrate. The finished combination was agitated at 100 rpm for one hour, then 1 N NaOH was added dropwise to get the pH down to 7.9 (Consolo et al. 2020). As a positive control, the fungi filtrate devoid of metal precursor was employed concurrently. CuO-NP production is indicated by the color shift of the biomass filtrate from colorless to green in color. The formed NPs were recovered by spinning at 9,950 rpm for 12.0 minutes, followed by three rounds of washing in deionized water and two hours of burning at 205°C (Nassar et al. 2023).

Characterization of myco-synthesized CuO nanoparticles UV- spectrophotometer

The intensity of the green color that formed prior to combining fungal biomass filtrate with metal was tested using UV-Vis spectrophotometer (Geneway, USA) to detect the absorbance at a wavelength in the range of 0–600 nm. A cuvette was stuffed with 2.0 mL of prepared nanoparticles and detect their absorbance at regular intervals time examine the maximal surface plasmon resonance (Peddi et al. 2021).

FT-IR examination

The functional groups in biosynthesized CuO-NPs were investigated by FT-IR (Thermo-fisher, USA). A 10 mg of synthesized CuO-NPs was combined with potassium bromide (KBr, ≥ 98.0%), and compressed to create a disc. The disc was then scanned at wavenumbers between 400 and 4000 Cm-1 (Rabiee et al. 2020).

TEM testing

Transmission electron microscopy (JEOL, 1010, Japan) was applied to evaluate the morphological traits, such as sizes and forms, of the fungal-mediated production of CuO-NPs. Ultra-sonication was used to disperse the CuO-NPs powder in MiliQ H_2O, and then a few drops of this solution were put onto a carbon grid. Prior to checking, the remaining solution was removed from the filled grid by touching it with paper towels (Fouda et al., 2022).

XRD analysis

Using the technique of X-ray diffraction (DW-XRD-2660A, China) coupled to Cu-Kα as an X-ray source (λ = 1.53 Å) at 40 KV and 30 mA, consequently, the structure and stage of the myco-synthesized CuO-NPs was investigated. The X-ray scan was accomplished between two Theta values, ranging from 10° to 80°. Using Debye-Scherrer's equation and XRD analysis, the mean crystallite diameter of CuO-NPs was determined (Bin Mobarak et al., 2022).

Testing of antioxidant action of biosynthesized CuO nanoparticles

By applying the DPPH technique, the antioxidant capacity of myco-prepared CuO-NPs was examined. By dissolving in high purity water (Milli-Q H_2O), different amounts of green myco-synthesized CuO-NPs (1000–1.95 µg/mL) were produced. Next, a test tube having one milliliter of DPPH (produced in methanol) and four hundred microliters of Tris-HCl buffer (pH 7.6, 50.0 mM) was filled with one milliliter of the produced solution. The tube was thoroughly combined and allowed to stand for thirty minutes at 36°C with 100 rpm agitation in a dark environment. The same parameters and levels were applied in another group of experiments with ascorbic acid (positive control). Additionally, the test was conducted with the identical incubation conditions for the negative control, consisting of DPPH and Tris-HCl buffer without CuO-NPs or ascorbic acid (Kaningini et al. 2023).

Detection of anti-inflammatory impact of prepared CuO nanoparticles

Prepared CuO-NPs' *in vitro* anti-inflammatory effectiveness has been assessed by the human red blood cells -membrane stabilization procedure. Specimens used in this experiment were dissolved in a hypotonic solution of distilled water. The graded dosages of the specimen (100-1000 µg/ml) in a hypotonic solution (5 ml) were put in duplicate pairs (per dose) within the centrifuge tubes. Additionally, duplicate pairs for every level of the centrifuge tubes were filled with isotonic solution (5.0 ml) containing graded levels of the specimen (100–1000 µg/ml). Five milliliters each of the distilled water and indomethacin at 200 µg/ml were found in the control tubes. Every tube was filled with 0.1 ml of erythrocyte suspension, which was well mixed. The solutions had been spun at 1300 rpm for three minutes after being left for one hour at the ambient temperature (36°C). The supernatant's hemoglobin concentration was estimated to be absorbed at 538 nm (Anosike et al., 2012).

Determination of cytotoxic effect of CuO nanoparticles

The cytotoxic effect on PANC1, Caco2 as well as MCF-7 which represent tumor cells was identified using the MTT method following the sample's dissolution in DMSO.

Applying standard levels yields a blue color whose values correspond to the number of live cells. With a computerized microplate analyzer (Tecan Life Science Infinite F50, USA), the absorbance was determined at 560 nm. After adding samples at several concentrations (from 1000 to 31.25 µg/mL) and waiting 24 hours for adhesion to merge, the cells were left at 35°C for additional 24 hours. After adding the fresh medium and letting it sit at 35°C for four hours, 100 µL of MTT solution (5.0 mg/mL) was put on. A CCD camera attached to a microscopy (OMAX, USA) for cell observation (Examinati et al. 2018).

Evaluation of cytotoxic effect towards Vero cells and antiviral potential t of prepared CuO nanoparticles

The cytotoxicity and maximal non-toxic level of CuO nanoparticles towards Vero cells were determined according to Fouda et al. (2022). Then, Vero cells were placed in 96-well micro-titer plates at a density of 10^4 cells/well with 200 µL growth media to examine the impact of CuO-NPs on the reduction of the Adenovirus-22, Adeno-40, and Coxsackie-B4 virus infection capacity. The cells were then left adherent for the entire night at 36°C in 5% CO_2. For one hour, a viral suspension was incubated at ambient temperature with non-lethal doses of CuO-NPs (1:1, v/v). Following incubation, one well containing Vero cells received 100 µL of the viral/sample solution, whereas the remaining three wells, which held only Vero cells as well as development media, were regarded as non-infected cells (control). The plates were shaken for six minutes at 160 rpm, and then they were incubated for twenty-four hours at 36°C with 6% CO_2 to enable the virus to multiply. Using the formazan crystal absorbance measurements employed in the MTT solution as specified for the cytotoxicity experiment, the cell viability of both affected and non-infected Vero cells was assessed. The variation in measurements across the optical densities of infectious and unaffected cellular viabilities was used to calculate the anti-Adenovirus-22, anti-Adenovirus-40 and anti-COX-B4 activities (Fouda et al., 2022).

Statistical evaluation

The collected data were quantitatively examined using (Graph Pad Prism V5, USA), and the mean values of three separate repetitions were used to convey the findings. To assess the variance across measures, the *t*-test or ANOVA was utilized, accompanied by the Tukey step at $p < 0.05$ to detect the dramatic change.

Results

Synthesis of CuO nanoparticles

The objective of the present investigation was to isolate soil fungi and examine their potential for diminishing, topping, and stabilizing the metal oxide precursor $Cu(CH_3COO)_2$-H_2O to generate CuO-NPs. Upon combining the fungal biomass filtrate with the metal oxide precursory forms, Mekky 1502, one of the four fungal strains obtained from soil, was chosen due to the intensity of its greenish color. According to microscopic and culture analysis, this strain was recognized as *Aspergillus* sp., and its identity was further validated by the ITS gene sequence. With an identity rate of 99.26%, molecular evidence shows that the fungal strain Mekky 1502 was comparable to *Aspergillus niger*. As a result, *A. niger* Mekky 1502 (Figure 1) was identified as the chosen fungal strain, and the pattern that was produced was entered into GenBank with an accession code of **PQ218992.**

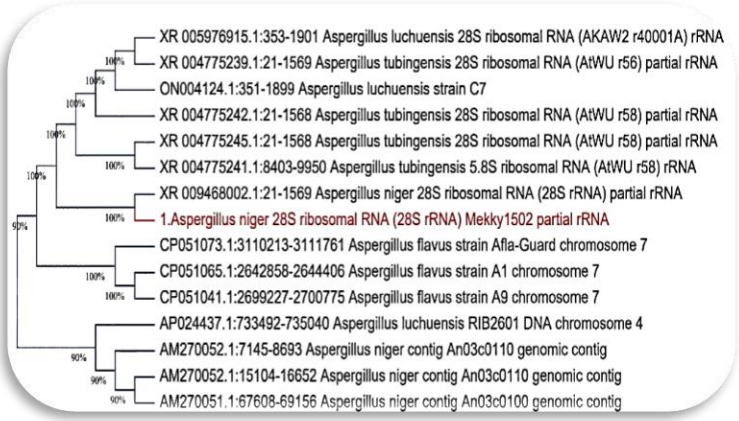

Fig 1. the phylogenetic tree of the chosen fungal strain FU.1 was determined by the neighbor-joining technique, MEGA-6.1 program built the tree using ITS analysis of sequence and sequence published in the NCBI.

Characterization by different instruments

UV visible spectrophotometer

To find the highest level of surface plasmon resonance, the greenish color's amplitude was determined with an ultraviolet spectrophotometer at several wavenumbers between 200 and 700 nm. Based to the UV-Vis chart, the largest peak was recorded at a wavenumber of 345 nm, indicating the CuO pattern as depicted in figure (2).

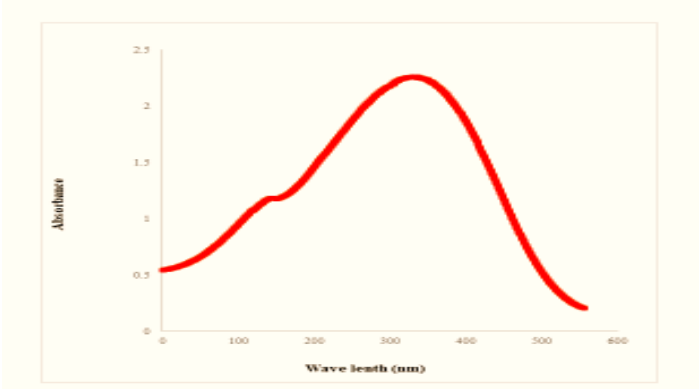

Fig 2. UV pattern of the myco-synthesized CuO nanoparticles.

FTIR analysis

By using FT-IR, it was possible to examine various functional groups in the nanoparticles that were generated, in addition to the new peaks associated with newly functioning groups in the synthesized NPs. Four peaks of absorption may be found in the biomass filtrate at wavenumbers of 3192, 2206, 1528, and 1006 cm^{-1}. The O-H bending of hydroxyl groups coincided with the N-H extending of aliphatic primary amines is responsible for the massive and wide peak observed at 3192 cm^{-1}. The peak at 2206 cm^{-1} may be caused by an unsaturated ester or carboxylic molecule CO expanding, or it may be connected to aromatic molecule overlap with (C–O). The prominent peak at a wavenumber of 1528 cm^{-1} indicates that polysaccharides are C–O expanding, or represent the protein amides I and II. The C-S, C= S (merged) relationship is related to the broadness peak at 1006 cm^{-1} (Fig. 3)

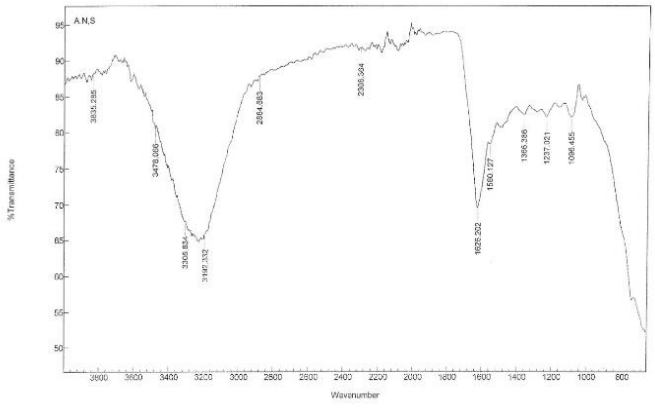

Fig 3. FITR with various peaks of the myco-synthesized CuO nanoparticles.

Morphological testing

The most likely method for identifying the morphological features of manufactured nanomaterials, such as dimensions, forms, is transmission electron microscopy. The sphere-like form and orderly arrangement of the fungal-mediated CuO-NPs synthesis are demonstrated in Figure 4 A. Furthermore, the mean diameter of the prepared CuO-NPs is 9.3 ± 1.1 nm, with diameters ranging from 4–18 nm (Figure 4).

XRD analysis

We evaluated the crystalline quality of fungal strain-based CuO-NPs using an X-ray diffraction pattern (Fig. 5) where eleven Bragg's diffraction peaks can be found at planes of (109), (-110), (111), (-201), (020), (201), (-112), (-301), (210), (301), and (-202). These correspond to 2θ values of 16.2°, 26.7°, 28.8°, 33.6°, 37.8°, 51.6°, 54.6°, 61.4°, 65.5°, 68.6°, and 71.3°, sequentially. According to the standard (JCPD, 80-1268), the acquired pattern verified that the fungal mediated-CuO-NPs have a face-centered cubic phase with a crystalline structure.

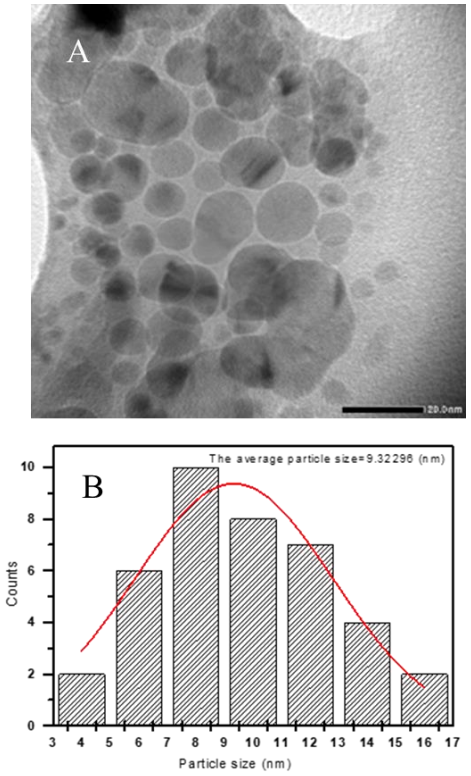

Fig 4. A: TEM pattern of the myco-synthesized CuO nanoparticles. B: Size distribution of the prepared CuO nanoparticles.

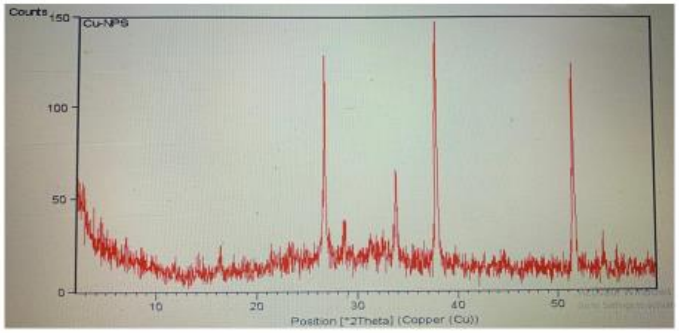

Fig 5. XRD with various peaks of the myco-synthesized CuO nanoparticles to illustrate crystal structure.

Antioxidant impact of biosynthesized CuO nanoparticles

The green synthesized CuO-NPs demonstrated reducing behavior at large concentrations (1000 µg/mL), with proportions of 83.2 ± 1.1%, in contrast to ascorbic acid (standard), which reported reducing ability of 98.2 ± 0.3% (Figure 6). At lower levels, these proportions declined. For example, at 250 µg/mL, the reduction rates for ascorbic acid and CuO-NPs were 92.4 ± 0.1% and 71±

3.1%, consequently. The produced CuO-NPs still exhibit antioxidant potential at the smallest level (1.95 µg/mL) at a rate of 28.1 ± 1.2% where IC_{50} for the standard was 1.89 ±0.2 µg/mL, While IC_{50} for the prepared nanoparticles was 27.93±2.4 µg/mL.

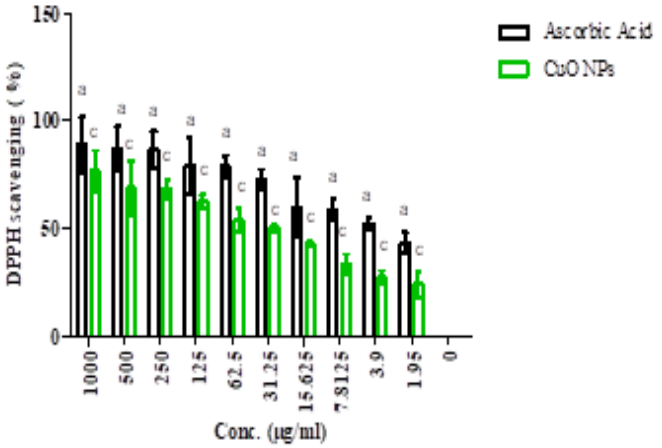

Fig 6. Elucidation of antioxidant potential of prepared CuO-NPs compared to standards. CuO-NPs produced by fungus had effective antioxidant compared to ascorbic acid (Standard). At the comparable level, distinct letters represent values that are significant ($p \leq 0.05$).

Anti-inflammatory action of prepared CuO nanoparticles

It could be noticed that upon using a descending level of CuO-NPs the hemolysis inhibition percentage decreased compared to indomethacin as standard where at a level of 1000 µg/mL the inhibition percentage were 98.6 %, 99.7 for the sample and standard consecutively. While, at a level of 600 µg/mL the inhibition percentage were 79.7, 97.7 % for the CuO-NPs and indomethacin respectively. Lastly, at a level of 100 µg/mL the inhibition percentages were 48.9, 94.4 % for the CuO-NPs and indomethacin, consecutively as shown in figure 7.

Anti-proliferative role of myco-synthesized CuO nanoparticles

For cancer cell lines, the highest concentrations of the produced CuO-NPs at 1000 and 500 µg/mL resulted in the lowest cell viability. The results indicate that, following 48 hours of incubation, the cell viability percentages for PANC1, Caco2, and MCF-7 cell lines were (4.9 ± 0.3, 5.2 ± 0.6; 5.3 ± 0.3, 6.0 ± 0.5% and 2.7± 0.4%, 3.2± 0.6%) and (11.4 ± 0.9, 7.0 ± 0.4, 6.5 ± 0.5, and 11.3 ± 0.4%) for PANC1, Caco2, and MCF-7 cell lines, respectively. When the concentration of CuO-NPs decreases, cell viability increases, Where IC_{50} for the prepared CuO-NPs towards PANC1, Caco2, and MCF-7 where 151.45 ± 0.89, 91.45 ± 1.89 and 82.02 ± 1.04 µg/mL as shown in figures 8 and 9.

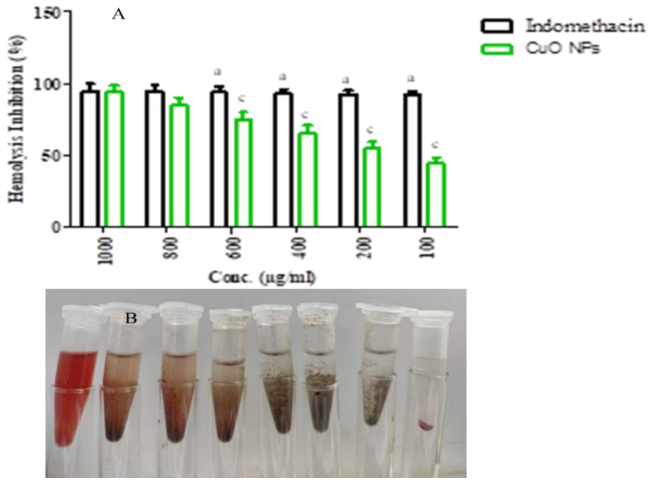

Fig 7. (A) Determination of anti-inflammatory capacity of produced CuO-NPs relative to standard. CuO-NPs prepared by fungus had effective anti-inflammatory impact than ascorbic acid relative to indomethacin (Standard). At the comparable level, distinct letters represent values that are significant ($p \leq 0.05$); (B) Different levels of specimen with various levels of hemolysis inhibition.

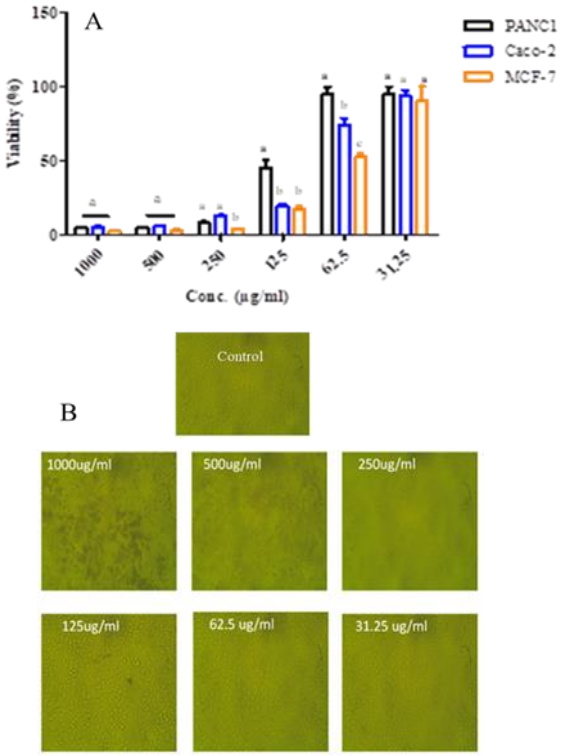

Fig 8. (A) Statistical comparing of CuO-NPs versus PANC1, Caco-2 and MCF-7 tumor cells; data are drawn as means ± SD. (B) PANC1 (C: untreated cells), morphological variations upon using various levels of CuO-NPS.

Cytotoxicity and Anti-viral activity of myco-synthesized CuO nanoparticles

Prior to evaluating the produced CuO-NPs' antiviral efficacy, the sample's toxicity on Vero normal cells was investigated. The sample should have a strong antiviral impact on the viruses without causing noticeably harmful effects on the host cells. CuO-NPs were found to exhibit obvious cytotoxic on treated Vero cells within the 1000−125 µg/mL range, and their cytotoxic level that inhibit 50 % of cells (CC_{50}) was measured with a value = 196.91 ± 4.02 µg/mL revealed minimal cytotoxicity of CuO-NPS (Figure 8). While, the maximum non-toxic level of CuO-NPs was at 62.5 µg/mL was applied to test their antiviral action. The antiviral impact (%) of CuO-NPs towards Adeno-22, Adeno-40 and Co-B4 viruses increased upon applying ascending levels of CuO- NPs as expressed in figures 10 and 11.

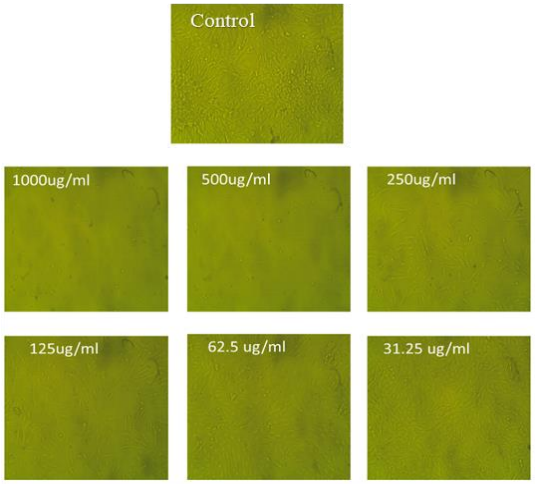

Fig 9. (A) Caco-2 (C: untreated cells), morphological variations upon using various levels of CuO-NPS. (B) MCF-7 (C: untreated cells), morphological variations upon using various levels of CuO-NPS.

Discussion

Metal and metal oxide nanoparticles have many uses in many fields (Soliman et al. 2021; Zahra et al. 2022). Thus, enormous efforts are made to quickly, cheaply, environmentally, and biocompatible synthesize these compounds. Because fungal strains produce large amounts of molecules that are employed to reduce metal and metal oxides to nanostructure, resulting in long-term capping and stabilization of products, Green-producing NPs employing these strains is recommended (Bahrulolum et al. 2021; Eid et al. 2021). Furthermore, the issues that arose from the use of chemical and physical procedures are resolved by the production of NPs utilizing these methods (Salem & Fouda, 2021).

Since fungi are uncomplicated to handle, accumulate elements resistant, secrete a high amount of extrinsic compounds, are simple for growing up, and provide a substantial biomass output, they are chosen over other microorganisms (Šebesta et al. 2022).

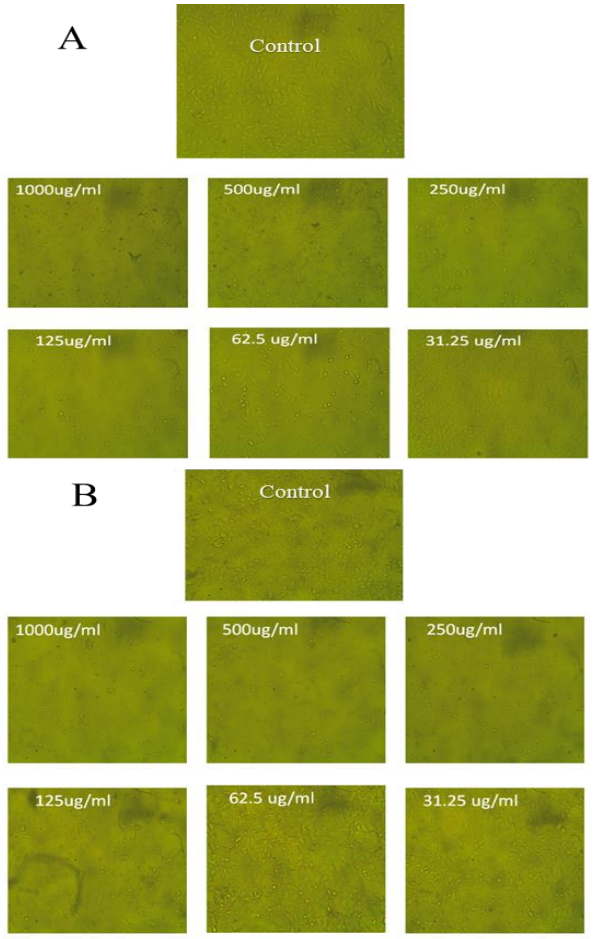

Fig 10. Determination of impact of CuO-NPs at various levels towards Vero cells where CC_{50}= 196.91 ± 4.02 µg/mL (C: control, levels of 1000 -31.25 µg/mL were used towards cells).

The present investigation examined the effectiveness of isolated fungus in decreasing, capping, and stabilizing the metallic oxide substrate to create CuO-NPs. After combining fungal biomass filtrate with the oxide of metal precursor form, FU.1 was chosen from among the many fungal strains that had been isolated, depending on the magnitude of its greenish color. According to investigations using microscopy, culture, and genetic analysis, this species was determined to be *Aspergillus niger*.

CuO-NPs, which have demonstrated promising function in treatment of tumors, were reportedly synthesized by using *Aspergillus terreus*'s active compounds (Mousa et al. 2020). In a related investigation,

a fungal species of *Aspergillus terreus* was obtained from the *Aegle marmelosa* botanical medicine and identified using both conventional techniques and ITS sequencing. CuO-NPs with diverse biological uses, such as antimicrobial, antifungal, anti-inflammatory (Salem & Mekky, 2024) and in vitro lethal effectiveness, were produced using this fungal species (Mani et al. 2021).

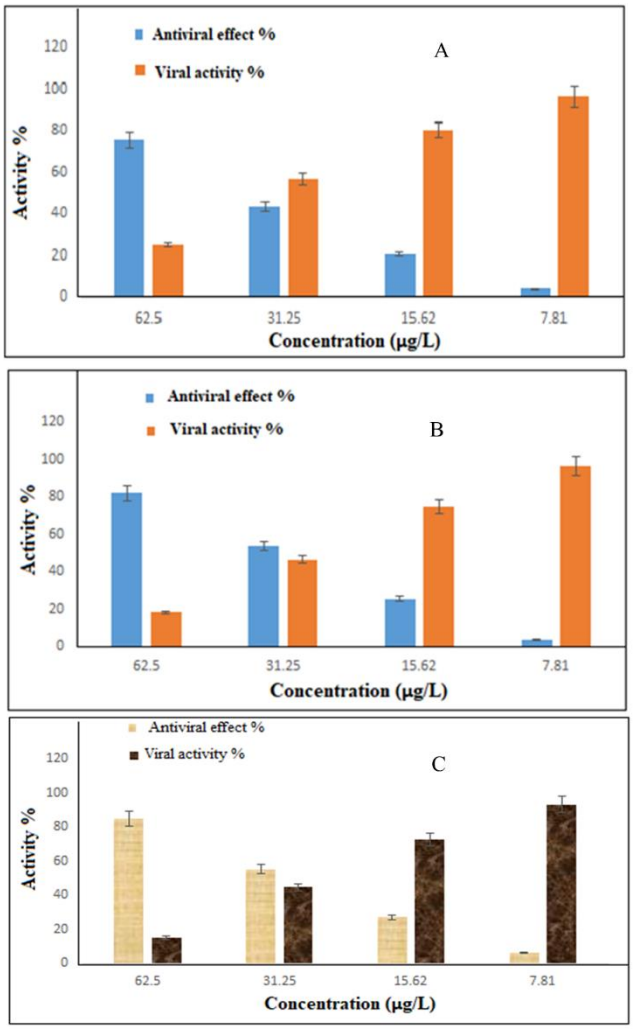

Fig 11. (A) Antiviral impact (%) versus Adeno-22 virus upon using various concentrations of CuO-NPs. (B) Antiviral activity (%) versus Adeno-40 virus upon using various concentrations of CuO-NPs. (C) Antiviral activity (%) versus Cox-B4 virus upon using various concentrations of CuO-NPs (Results are drawn as means ± SD).

In the present research, the substances released by *A. niger* FU.1, including proteins, enzymes, and amino acids, were utilized as reducing agents in order to generate nanoscale shape in metal precursors, and then the resulted ultimate outcome was capped and stabilized. The color of the microbial filtrate changed from colorless to greenish after combining with metal, serving as the first clue for the precreation of CuO-NPs. By comparison, at the conclusion of the test, there is no shift in the color of the positive control. As stated earlier, the deep color denotes the total decrease of every Cu^{2+} ions to CuO. The strength of the greenish color is linked to the proportions that reduce metallic ions through fungal products (Nassar et al. 2023).

In this work, the greatest level of absorption was seen at 345 nm and was ascribed to the CuO-NPs' SPR absorbing spectrum. The production of CuO-NPs has been verified by the CuO-NPs SPR absorbing peak at 340 nm. It is evident that the development did not take place at an extensive wavelength because its absorption level declined as the wavelength grew. According to research, CuO-NPs produced around 200 and 350 nm (Aroob et al. 2023). This is in conformity with the forming of CuO-NPs (Akintelu et al. 2020).

In this work FT-IR pattern reveal the form of CuO-NPs The various functional structures found in protein molecules, carboxylic acids, complex sugars, and peptides in the filtrate of fungal biomass indicate their role in reducing the metal precursors to generate CuO-NPs and then covering them to boost their function. FTIR reflect the existence of peaks at 3192, 2206, 1528, and 1006 cm^{-1} in accordance with other investigators who reported similar results (Hamza et al., 2021; Hamza et al., 2022; Mekky et al., 2021, Fouda et al., 2022).

CuO-NPs produced by the soil-isolated fungal strain *A. niger* had a diameter of with diameters below 100 nm (Mani et al. 2021). Furthermore, utilizing the cell-free supernatant of the fungal strain *Trichoderma asperellum*, which has a median dimension of 22 nm, circular CuO-NPs were created (Gaba et al. 2022, Radwan et al. 2024). Nanomaterials' action is influenced by both their form and size, earlier published. For example, CuO-NPs synthesized by plants through an aqueous extract of *Aloe vera* and shaped like rods and platelets demonstrated dramatic antibacterial capabilities towards both various bacterial species when compared to circular forms (Tavakoli et al. 2019, Saied et al. 2023). The active regions on the outermost layer of the NPs and their varying surface energies based on their structural forms may be responsible for this function (Gao & Liu, 2015). Additionally, due to tiny dimensions' superior ability to release hazardous ions like Cu^{2+} more quickly than big sizes, their toxic effect was greater than that of large dimensions (Wongrakpanich et al. 2016).

The acquired XRD data for the environmentally friendly processes of CuO-NPs are consistent with the prior research (Rehman et al. 2011). The process of fungal molecules to produce CuO-NPs is indicated by the peak patterns of diffraction at 2θ values ranging from 35° – 39° (Naz et al. 2020). Furthermore, the production of the FCC crystal form of CuO-NPs with diameters less than 100 nm

was validated by the existence of distinct and strong XRD peaks. Based on XRD examination, it can be deduced that the formed CuO-NPs were homogeneous because of the distinct structure that was seen and the lack of extra diffraction peak values (Ahamed et al. 2014). Conversely, an XRD investigation revealed that the dimension of the crystallite of CuO-NPs produced from waste fishes was 86 nm (Bin Mobarak et al. 2022). A crucial step is to look into the antioxidant capacity of NPs that will be added to biological systems. The interplay among oxygen molecules and macromolecules generates free radicals in various biological processes (Dobrucka, 2018). These radicals, which have a number of unpaired electrons, are extremely unsteady and harm cells by taking electrons through them to make them steady. Multiple mechanisms, including the breakdown of peroxides, chain initiating blocking, molecular oxygen appropriation prevention, rescuing of reactive oxygen species, and reducing capacity, have been linked to the antioxidant properties of both manufactured and natural substances (Rehana et al. 2017, Mohammed et al. 2024).

The present work revealed a direct correlation among the levels of CuO-NPs and their protective capability. This result is consistent with the body of research on green synthesized CuO-NPs' antioxidant capacities that has been published (Rehana et al. 2017). In comparison to ascorbic acid (94.1 µg/mL) at the equivalent level, the CuO-NPs made from the purified extract of *Suaeda maritima* demonstrated antioxidant capacity, as measured by DPPH reducing techniques, with a value of 82.8% at a level of 39 µg/mL (Peddi et al. 2021).

Different concentrations (1000 µg/mL) showed diminishing rates of inhibition depending on the experimental work, suggesting increased anti-inflammatory action. According to the results of the present investigation, CuO-NPs significantly reduced inflammation. In same line with results of the experiment which conducted concurrently to investigate the anti-inflammatory properties of silver nanoparticles derived from *Cotyledon orbiculata* extract (Caroline et al. 2021). In contrast, *Avicennia marina* showed anti-inflammatory effect with a crude extraction rate of 68.92% and a synthetic AgNP content of 72.1% (Sultana et al. 2023).

The spread of cancer is one of the major hazards to world wellness that remain to be tackled. Recently, a number of active compounds, notably NPs, have shown significant efficacy towards a range of cancer cells, opening the possibility of using them in cancer treatment or as vehicles or means of administration for anti-cancer drugs (Ibrahim et al., 2022). In view of their biological acceptability, permeation, ease of scaling up, sustainability, and safety for normal cells at particular levels, nanomaterial-based therapies for cancer, particularly those made using environmentally friendly methods, are being researched extensively (Lashin et al. 2021). The MTT test technique is precise, delicate, colorimetric, and capable of assessing biochemical cell processes by the computation of live cells subsequent to the administration of active compounds (Ghasemi et al. 2021). Thus, using the MTT analysis approach, the efficacy of fungal-mediated CuO-NPs versus three cancer cell lines, PANC1, Caco-2, and MCF7, was examined in the present work. The results acquired are consistent with those published by (Amin et al. 2021) who found that the level of green synthesised CuO-NPs administration affected the lifespan of Neuroblastoma cell lines, increasing at low levels and decreasing at elevated levels. Additionally, CuO-NPs produced by endophytic fungi show dependent on concentration antitumor action towards colon cancer cell lines HT-29 (Mani et al. 2021). Besides, according to a number of published research findings, green synthesized CuO-NPs had a greater cytotoxic activity versus cancer cells than NPs made via both chemical and physical processes (Ali & Tareq, 2019; Andra et al. 2019).

The issue of viral illness and the numerous, sometimes fatal illnesses brought on by distinct viruses affects people all over the globe. Infectious breakouts in human cultures are being caused by newly developing and reappearance viral diseases. Drugs cannot totally prevent all viral illnesses, despite the fact that antiviral medication has occasionally made enormous advancements in this area. Moreover, there currently are very few antiviral options available for treating viral infections, and drug-resistant viruses are regularly discovered (Gaikwad et al. 2013, Mekky et al. 2024). In this study the produced nanoparticles showed a promising antiviral impact towards Adeno-22, Adeno-40 and Cox-B4. (Haggag et al. 2019) looked into how biosynthesized AgNPs might protect towards HSV-1 infection. According to the findings, AgNPs were antiviral against HSV-1 infection. Using the Feline calicivirus as a stand-in for the human norovirus, (Shionoiri et al. 2012) examined the antiviral potential of CuO-NPs on Crandell-Rees feline kidney (CRFK) cells in a different investigation. According to their research, CuO-NPs considerably decreased FCV's infectivity against CRFK cells. CuO-NPs may cause ROS to be produced, which in turn may cause viral capsid proteins to oxidase. Besides, Cu-ONPs' ability to inhibit HSV-1 was studied and reported that it reduced HSV-1 infectivity in a way that was dose-dependent. To explain CuO-NPs' anti-HSV-1 characteristic, they proposed a number of methods, such as interactions with viral proteins (Ahmadi et al. 2023).

Conclusion

In this work, CuO-NPs were effective synthesized by the fungal biomass filtrate, which was determined as

Aspergillus niger Mekky1508 by both conventional methods and genetic confirmation. At a wavenumber of 345 nm, the synthesized CuO-NPs had maximal SPR, indicating the SPR of CuO. Furthermore, TEM and XRD were used to identify round forms and crystalline frameworks of the prepared nanoparticles. The reducing capacity of CuO-NPs was tested using DPPH technique which display the scavenging ability especailly at low levels. Remarkable anti-inflammatory, anticancer towards PANC1, Caco-2, and MCF-7, and antiviral effects versus Adeno-22, Adeno-40 and Cox-B4 were demonstrated by the produced CuO-NPs.

Conflict of interest

The authors declare that they have no conflict of interest.

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