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BIOGENICALLY SYNTHESIZED CUO NANOPARTICLES FROM SOLANUM MELONGENA FOR ANTICANCEROUS POTENTIAL AGAINST HCT-116 CELL LINES

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ABSTRACT

Biogenically synthesized copper oxide (CuO) nanoparticles for anticarcinogenic drug theraphy is documented in this research work by utilizing the skin (peel) extract of Solanum melongena (SM) . The structural (XRD) profile exhibited a monoclinic phase of superior nanocrystallinity with an average crystallite size of 31.54nm and BET surface area of 11.64m^2/g. The existence of Cu^{+2} and O^{-2} was confirmed from XPS and EDS spectral survey. UV-DRS and fluorescence studies were done to inquire the optical behaviour of CuO nanoparticles, where a band gap of 2.45eV and extreme peak of emission near visible region (480eV) was distinguished. A surface morphology of spherical pores were confirmed with SEM analysis and the TEM micrograph displayed the nanospheres of size upto 50 nm. The cytotoxic investigation of Solanum melongena mediated CuO-Nps has shown excellent cytotoxic efficiency against HCT 116 cells and suggested for restoration of chemotherapaeutic drugs and flourescent anticarcinogenic diagnosis applications to root out the complexity of colorectal cancer.

Keywords: copper oxide; solanum melongena; optical; nanosphere; anticancerous

1. INTRODUCTION

According to the specified reports of WHO, Carcinoma(cancer) is the lethal trait in many countries which increases the mortality rate and also it is the toxic health threat to our mankind, which is focused to report diagnosed cases of 25 million in a year. Generally, the convenient treatments like alkylating drugs, antimetabolite drugs and analgesics have lots of side effects because of the shortcomings to discriminate oncogenes(cancer cells) from healthy human cells, further results in lethal closure. Consequently, it is very vital to focus on exploring advanced and effective replaceable therapeautic agents and drugs, especially if it is derived from natural assets means, the toxicity level from chemical therepeautics is so much avoided[1-2]. This research work focuses on the biogenic green synthesis of anticancerous nanostructured CuO from the naturally anticancerous source, *Solanum melongena* (SM). The skin (peel) of *Solanum melongena* is rich in anthocyanins. Further, anthocyanins (a derivative of flavonoid) have anti oxidative, anti mutagenic and anticancerous activities. To date, CuO-NPs have been synthesized from considerable extracts of plants extracts such as *Cardiospremum halicacabum, Punica granatum, Catha edulis, Aervajavanica* and *Caesalpinia bonducella* seed and their pest control, antimicrobial, cytotoxic/anti inflammatory and electrochemical properties were investigated [3-7]. To the best of our knowledge, rare reports are available on the biogenic synthesis of CuO-NPs using *SM* skin extract with enriched anticancerous effect. It paves a new way for cancer drug from anticancerous *Solanum melongena*. The present study explores the biogenic synthesis of CuO-Nps, their characterization and investigation of invitro cytotoxic potency of CuO-NPs.

2. EXPERIMENTAL FEATURE

Copper acetate monohydrate [$Cu_{(2)}CH_3COO.H_2O$], ethanol and sodium hydroxide (NaOH) of AR grade were purchased from Sigma-Aldrich. *Solanum melongena* fruits were gathered from home farm, Thoothoor, India. The extract preparation is done according to documented literature [8] with slight modifications. The biogenic synthesis of CuO-NPs begins with the preparation of 1mM $Cu_{(2)}CH_3COO.H_2O$ solution which was kept in stirrer for 7h. pH of the solution was modified to 8 by adding IM NaOH solution dropwise. Then 30ml of *SM* extract

was added to the above solution with perpetual stirring condition of 400rpm. Subsequently after the prescribed time, a change of dark green reaction mixture to blackish precipitate is noted. Succeedingly, the solution was again kept in the stirrer for 3 hours after one hour of incubation which affirmed the formation of CuO-NPs. The resultant precipitate was separated by centrifugation (6000rpm/20min) by washing thrice with distilled water/ethanol. The precipitate was then calcined (250°C) for overnight and preserved in airtight container for further characterization purposes [9]. MTT (calorimetric) assay is performed to check the cytotoxic potency against HCT 116 cell lines.

3. CHARACTERIZATION TECHNIQUES

The Structural analysis (X ray diffraction studies) of the synthesized CuO nanoparticles were investigated by monochromatized X'Pert PRO powder X-ray diffractometer with CuKα radiation (λ= 1.54060Å) which depicts the structure, crystal purity and crystallite size of nanoparticles. The optical analysis (UV DRS spectrum) of synthesized CuO nanoparticles were recorded with Perkin's Elmer 2450 spectrophotometer. The Fourier transform infrared (FTIR) spectrum was recorded by using SHIMADZU 8400 FTIR Spectrophotometer. The results of FTIR can be utilized to find the functional groups present in prepared nanoparticles as well as extracts which results in changes in structure of molecules. The specific surface area determination is done by BET method for nitrogen adsorption which was conducted using Micrometrics ASAP 200. The elemental chemical surface analysis was done by using X ray photoelectron spectroscopy (XPS) by spectrophotometer of Thermo scientific K-alpha device. Morphological analysis of the prepared biogenic CuO nanoparticles and EDS spectrum which reveals the elemental contents were recorded by using scanning emission electroscope Quanta FEG-250. The Transmission electron microscopy is done by using JEOL/JEM 1101 instrument. The fluorescence spectra was recorded at room temperature under emission spectra and the results were noted in between 300-800nm. For cytotoxicity studies, the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay was utilized to assess the effects biogenically synthesized CuO NPs on HCT-116 cells.

4 RESULT AND DISCUSSION

The XRD pattern (Figure.1a) stated the presence of a fine intense band of Bragg's peak of CuO-NPs nanoparticles. It is because of the stabilization of the nanoparticle by a mixture of reducing agent of the skin extract of SM. The XRD pattern accords the formation of CuO nanoparticles with diffraction peaks at 2θ=38.2°,46.13°,48.31°, 51.20°, 54.02°, 58.15°, 61.89°, 64.83°, 65.24°, 67.21°, 72.57°, 75.05°,75.50° and 78.13° as shown in the Figure.1a. All the diffraction peaks corresponded to the monoclinic structure of CuO-NPs that finely fits with the JCPDS no:89-5899. Impure peaks were elsewhere because of the anisotropic growth and a favored nanocrystallite coordination further based on the relatively high intensity peak of 2θ value, 38.2°[10]. The crystallite size of the CuO-NPs obtained from full-width half maximum of the diffraction peak was 31.54 nm. The higher value of dislocation density ($21.5\times10^{14}m^{-2}$) illustrates its higher hardness value. The estimated 'a' and 'V' values (4.067Å and 67.271$Å^3$) have slight deviation with the standard values. This might be due to the bio-active constituents, probably flavonoids (anthocyanins) of SM skin extract [11]. The surface area and strain is determined to be 3.014 m^2g^{-1} and $0.0628x10^{-3}$ respectively. It is also noticed that the number of unit cell (N=0.77) and cell volume were inversely proportional to the dislocation density which is well agrees with the inferences of earlier literature [12]. This makes an evidence of capable binding hindrance of biomolecules with Cu metal ions to get scaled down into CuO-NPs. The XPS spectrum discloses peaks analogous to Cu_{2p} (79.4%) and O1s (20.6%). The peak at 934.57 eV (Figure.1b) and 531.76 eV (Figure.1c) is attributed to CuO, interrelated to the Cu 2p and O 1s state, respectively. The BET surface area ((Figure.1d) was determined as 11.64m^2/g. The elegant existence of Cu and O was doubly accorded with EDS spectrum (Figure.2a) too.

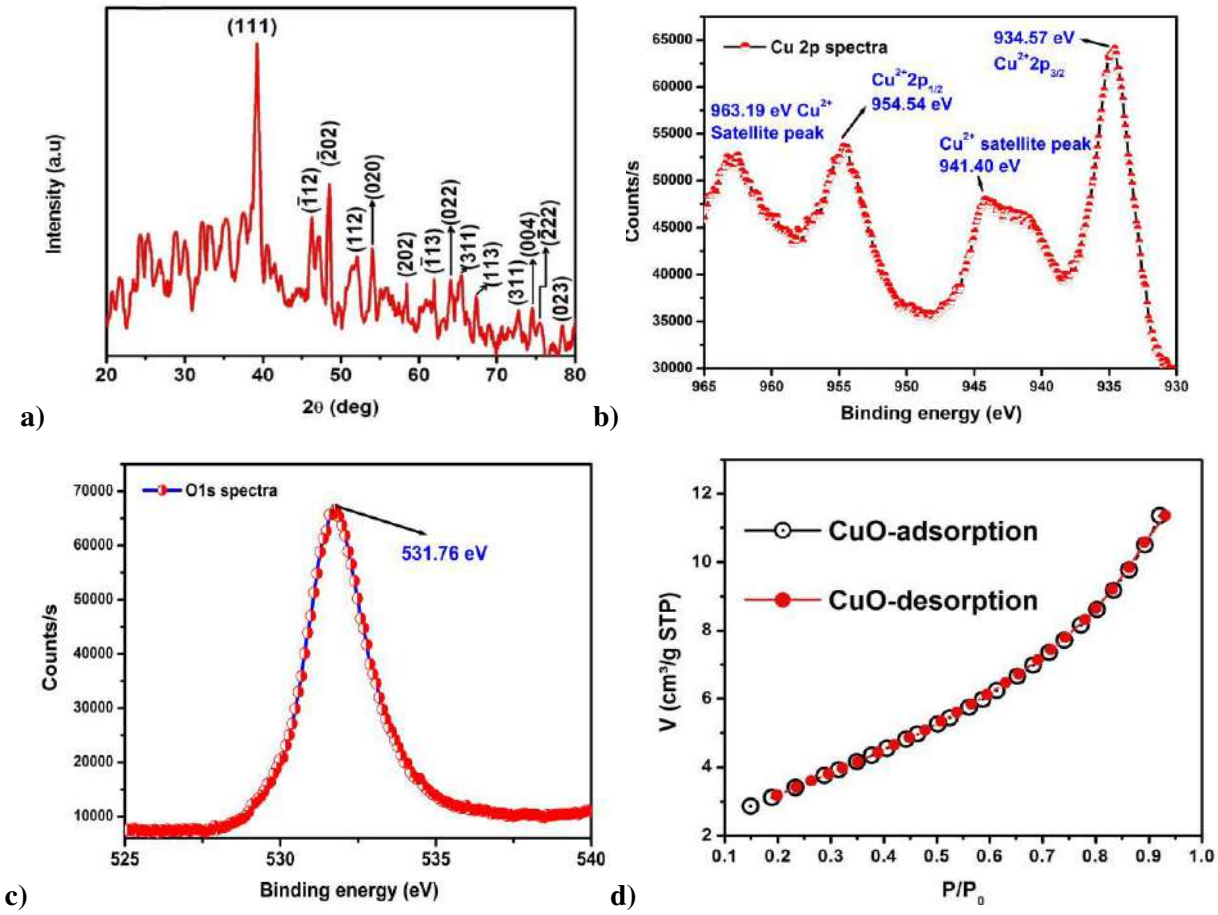


Figure.1. a) Structural profile **b)**Cu2p **c)**O1s core and **d)**BET spectrum of CuO-NPs.

The major characteristic peaks of SM skin extract and CuO-NPs were observed in the spectrum (Figure.2b). Intense and broad peak at 3462cm^{-1} corresponds to hydrogen bonded OH groups of alcohols and phenols. The FTIR spectrum of CuO-NPs showed a fine peak at 562cm$^{-1.}$ The strong peak observed at 1622cm^{-1} and 1457cm^{-1} (C=C stretching of C=O amide conjugated C=O and C=C stretching of C-OH) is correlated to proteins. This depicts that the skin of *SM* extract can strongly bind to CuO-NPs via hydroxyl and carbonyl groups (proteins) of the extract firmly proved by phytoconstituent screening, hence acts as stabilizing reducing, capping and dissipating agent for development of biogenically synthesized CuO NPs [13].

In Figure.2c(inset), the *Solanum melongena* mediated CuO-NPs discloses a broad light absorption spectrum with the intense absorption peak pointed at 478 nm which is ascribed to the ideal intrinsic bandgap energy of CuO-NPs. It is due to the transition of valence band electrons to the conduction band energy level. On comparing with earlier documentation [14], a shift noted at the optical absorption peak is in view of the nanometric extent reaction of biogenically synthesized CuO nanoparticles. The optical absorption potential of the nanoparticles will directly interferes its bandgap energy. The calculation of bandgap energy of biogenically synthesized CuO-NPs was determined out by using Kubelka Munke (KM) plot as shown in Figure.2c. The bandgap energy of CuO-NPs was found to be 2.45eV. The *Solanum melongena* mediated nanoparticles are p type semiconductor of narrow energy gap and consisting of monoclinic space group C2/c. This accords well with previous literature [15] and as well as the XRD results. The attenuated (narrower) band gap energy of as synthesized CuO-NPs clearly exhibits that it is very much efficient in visible light irradiation for photocatalytic applications and hence the biogenically synthesized CuO-NPs can be used in sensors and opto-electronical gadgets.

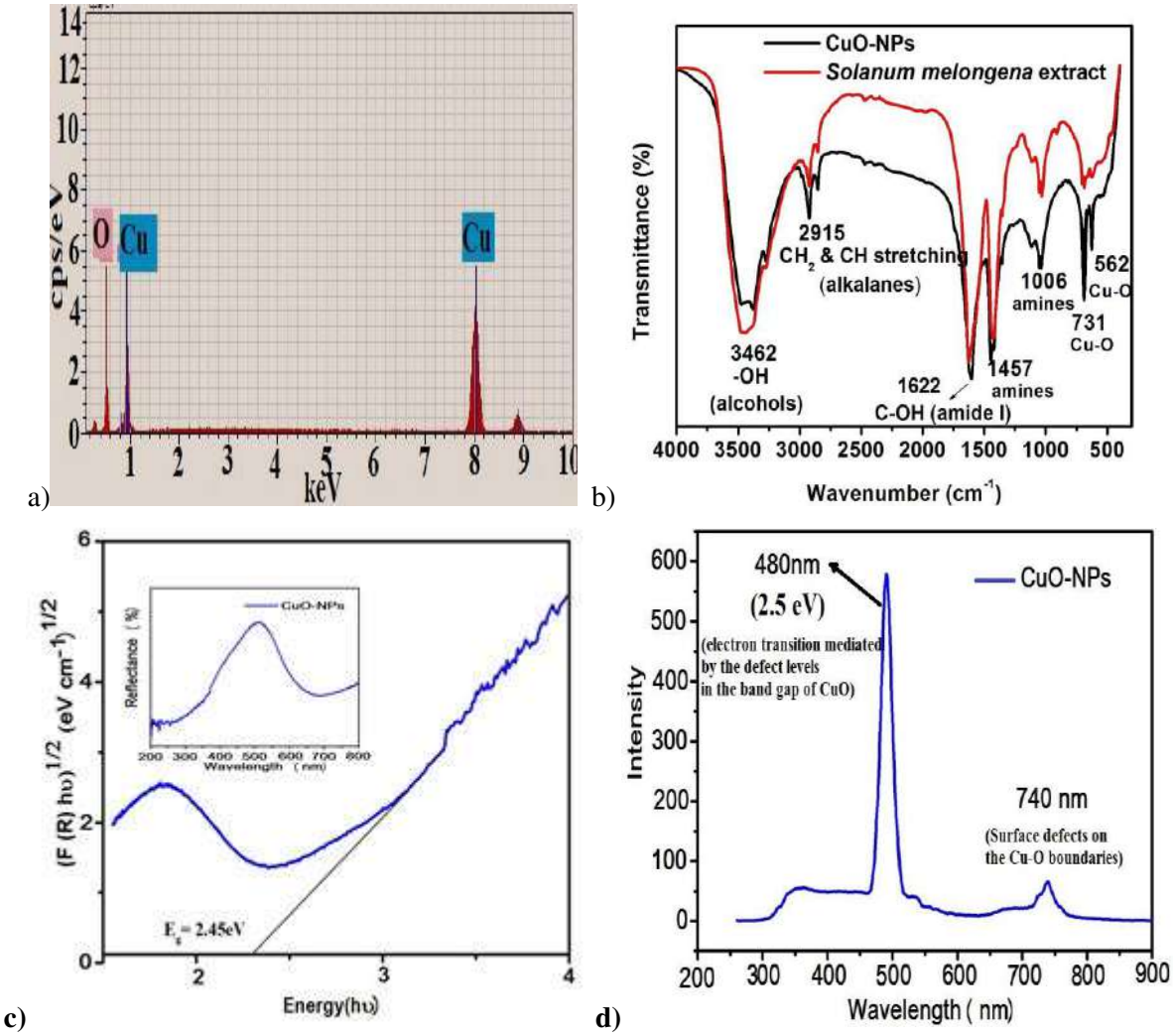
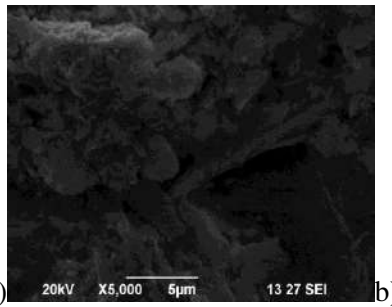
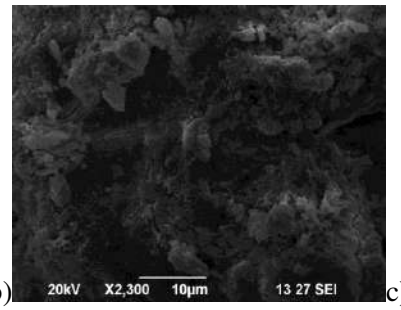


Figure.2.a)EDX, **b)**FTIR, **c)**Kubelka-Munke-plot (inset:absorption), and **d)**florescent spectrum of CuO-NPs.

The SEM micrographs were shown in Figure.3(a-b) revealed that all the particles were interconnected randomly with one another to form a network system with spherical shape and pore which were individual cubic structured. The presence of porous clusters when magnified was clearly visible corresponding to a high specific surface area of CuO-NPs, which were distinctly spherical structured. TEM micrograph(Figure.3c) showed a mixture of porous and spherical shaped particles which agrees with the SEM results. It affirms some spherical morphology with mean particle sizes of 50 nm which well matches with the XRD results. The particles seen as spherical clusters are owing to the chemical composition and because of the coincidental biomolecules present in the *Solanum melongena* extract [16].

a) 

b) 

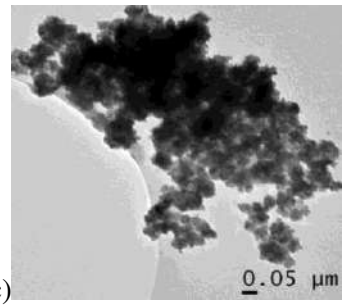
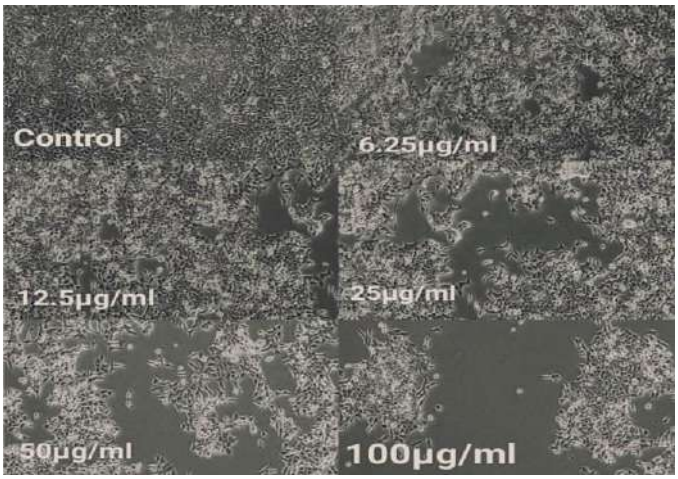
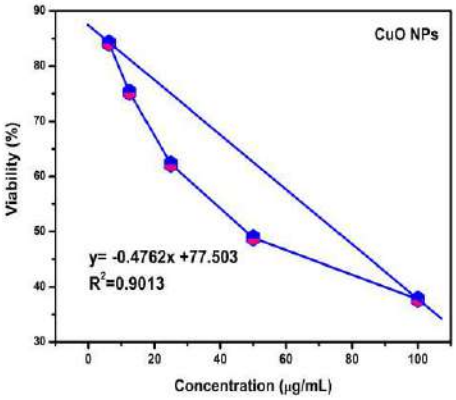
c) 

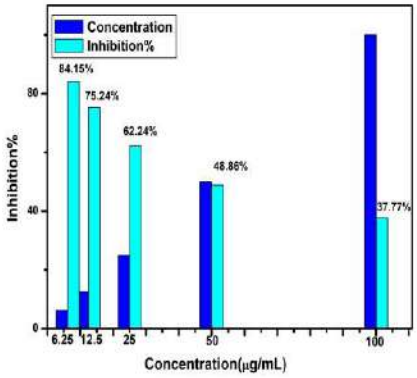
Figure.3. (a-b) SEM and (c) TEM images of biogenic CuO-NPs.

The images clearly exhibits that the biogenically synthesized CuO-NPs had incinerated cell lines of human colon cancer cell line (HCT-116) as shown (Figure.4a). This study reveals that the *Solanum melongena* mediated CuO-NPs were potent of hitting the cell membrane and colloborates with various biomolecules such as protein groups, alkaloids and flavonoids, especially anthocyanins. The previous literature reports [17] also correlates with this tendency of biomolecules of *Solanum melongena* have potency to fight against cancer cells. The images exhibited a slight variation occurred at the beginning of the assay with concentrations of CuO-NPs (6.25 and 12.5 µg/mL) but when the concentration level was increased to 25 µg/mL and 50 µg/mL , the sustainable efficiency of cells were very much reduced. At the peak concentration level(100 µg/mL), the cell morphology depicts mostly damaged cells. The cell survival potential was analyzed and the capability of destroying cells is obtained with the IC_{50} value of 59.530 µg/mL (Figure.4b,c)[18]. Dose based reduction in cell viability was noted in HCT-116 cells done with various concentrations of the sample. The highest potency was brought out by the sample at the concentration 100µg/ml. The CuO-NPs were investigated for fluorescence property and it is found to be excited at 240 nm (Figure.2d) , is of high intensity due to presence of phytoconstituents in *Solanum melongena*. Hence they were implemented in optical bio imaging and medical diagnosis. The findings suggests that increasing the concentration of phytoconstituents to reduce toxicity will attain maximum cell death as well as potential anti carcinogenic effect and hence they were utilized in chemotherapic drugs and other biomedical applications.

(a) 



(b)



(c)

Figure.4 (a,b,c) Cytotoxicity with cell survival various concentrations of SM mediated CuO-NPs

4 .CONCLUSION

The biogenically synthesized CuO-NPs using *Solanum melongena* skin extract depends up on various physicochemical properties like particle size, cell type, binding properties, aggregation potential, surface charge and type of capping agent used for the synthesis of CuO-NPs. The existence of flavanoids in *Solanum melongena* skin extract mainly played a vital role in reduction which was confirmed by FTIR analysis. The monoclinic phase structure crystal system was proved by XRD profile analysis and the nanoparticle behaviour was further highlighted by TEM results with the particle size of 50 nm. The porous spherical nanoparticles were revealed by SEM/TEM imaging. Optical analysis was done by UV-DRS analysis and the obtained bandgap of 2.45eV listed under semiconductor nanoparticles. The MTT cytotoxic assay endorsed the cell survival capacity of biogenically synthesized CuO and revealed the efficacy of anticancerous CuO-NPs, the extract as well as metal ions possess well defined anti-carcinogenic potency. Hence this cost effective, less toxic *Solanum melongena* mediated CuO-NPs were known to be effective drugs and chemotherapeutics with rare side effects.

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