Polymer nanocomposite prepared using copper oxide nanoparticles derived from Sterculia foetida leaf extract with biological applications

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Abstract
The new hydrogel network was prepared by using glutaric acid, ethylene glycol and acrylic acid (GEA) through condensation polymerization without cross linker and it was fabricated by incorporation of green synthesized CuO nanoparticles (CuO NPs). The CuO NPs were synthesized by green route using Sterculia foetida leaf aqueous extract. Green synthesized CuO NPs were incorporated with GEA hydrogel resultant the GEA-CuO nanocomposite. The formation of green synthesized CuO NPs and GEA-CuO nanocomposite was confirmed by UV-Visible and FT-IR spectrum. The structure of GEA hydrogel was determined by 1H and 13C NMR technique. Morphology of synthesized GEA hydrogel and GEA-CuO nanocomposite was observed as bulged layer and uneven plates with cavities in SEM analysis, moreover the size of the material was evaluated by TEM analysis. Thermogravimetric analysis has revealed the GEA-CuO nanocomposite owing significantly higher thermal stability than raw GEA hydrogel. However, biological effect of synthesized GEA hydrogel and GEA-CuO nanocomposite was scrutinized by antibacterial activity against selected bacterial organisms and anticancer activity against lung cancer cell line A549.

1. Introduction

Hydrogels are hydrophilic polymer chain network that are interconnected with each other monomers by cross linkers. Hydrogels have rich water absorption capacity through hydrogen bonding and which depends on presence of hydrophilic moieties (carboxyl, hydroxyl, amido, and amino) within that polymer chains. However their high water content (70%–99%) provides hydrated porous which being similar to living tissues. Thus hydrogels have excellent biocompatibility, therefore commonly used in wide range of applications including tissue engineering, diagnostics, cellular immobilization, biological adhesions, wound dressings, and drug delivery etc, [1]. Hence the nanocomposite hydrogels are one of the fast emerging fields in the combination of nanotechnology and biomaterials for wide pharmaceutical applications. Glutaric acid is a dicarboxylic acid and it is a metabolic intermediate of fatty acid, tryptophan, and lysine metabolism [2]. However the glutaric acid is widely used in fiber formation, superconductors, semiconductors, and in biodegradable, thermally stable, or corrosion-resistant material [3]. Ethylene glycol is a di-functional monomer, its flexibility and biocompatibility improves the properties of hydrogels. Polymers containing carboxylic acids such as polyacrylic acid and polymaleic acid are the commonly used polymeric scale inhibitors [4]. Poly acrylates have excellent weather ability, water and alkaline resistance and they can act as anti-scaling agent on heat exchangers, cooling water [5–7]. The COOH group in acrylic acid can be utilized for immobilization of bio-molecules, high retention of carboxylic acid groups and wrinkle recovery angle of some textile surfaces [8–10]. Hence the polymer nano hydrogels has significant attention due to their applications in antibacterial, antifungal and in drug delivery [11].
Generally hydrogel nanocomposite have low mechanical strength, stiffness and thermal properties and it have better dielectric properties. Thus hydrogel nanocomposite has not exhibit long durability and more effective in drug carrier and other pharmacological treatments. However incorporation of metal nanoparticles with polymer composites will change and enhanced distinctive properties in application of diverse field. Because the metal nanoparticles have unique electronic and optical properties which are naturally depends on their size and shape [12]. Metal oxides nanoparticles like copper oxide, iron oxide and zinc oxide have shown more biological properties such as anti-inflammatory, antibacterial, anti-cancer, anti-diabetic and wound healing [13]. Among them, CuO nanoparticles have much interested by their potential applications in diverse fields such as heterogeneous catalysts, anti-microbial, anti-cancer, anti-oxidants, imaging agents and drug delivery agents [14]. Moreover CuO nanoparticles forms a complexes with electron-rich compounds (hydrogels) containing O, N and S elements which increases the swelling capacity in the polymer nanocomposites due to the more electrostatic repulsion [15]. Hence the current researchers have much focused on synthesis of metal polymer nanocomposite using CuO nanoparticles for the effective drug carrier applications.

The green synthesis of metal nanoparticles using plant extracts is being an ecofriendly, efficient and facile benign method without using any capping or stabilizing agents [16]. The plant Sterculia foetida (S. foetida) belongs to Malvaceae family and has many medicinal properties such as anti-inflammatory activity [17], anti-obesity [18], anti-fertility [19] and anti-oxidant [20] properties. The present study aims to synthesize GEA-CuO nanocomposite by condensation polymerization method. The CuO nanoparticles were prepared using S. foetida leaf extract as reducing agent. The above synthesized CuO nanoparticles were introduced into polymer hydrogel to form GEA-CuO network. The biomedical efficiency of synthesized metal polymer nanocomposite was carried out by antibacterial activity against selected organisms, subsequently anticancer effect was studied against lung cancer cell line (A549).

2. Experimental

2.1. Chemicals used

Glutaric acid (GA) 99% purity, Ethylene Glycol (EG) (99% purity), Acrylic acid (AA) and Copper(II) nitrate trihydrate (Cu(NO$_3$_)$_2$·3H$_2$O) 99% purity were purchased from Merck (India).

2.2. Preparation of plant extract

The S. foetida plant leaves were collected from Bangalore, India. The leaves were cleaned using water to remove fine dust particles deposited on its surface and dried in the absence of sun light under room temperature. Then the leaves were powdered mechanically using mixer grinder, sieved and subjected to extraction through soxhlet apparatus by the use of de-ionized water for 68 h at the temperature of 90 °C–95 °C. The obtained aqueous solution is subjected to concentration by the use of rotary flash evaporator (Buchi, Flawil, Switzerland), then the sample was dried in hot air oven at 55 °C–60 °C, from the dried crude extract small amount is used for the nanoparticle synthesis.

2.3. Synthesis of CuO nanoparticles (CuO NPs)

Solution combustion method was adopted to synthesize CuO NPs using aqueous leaf extract of S. foetida as green reducing agent. In this process 0.2 g of dried crude aqueous leaf extract of S. foetida and 0.6 g of copper(II) nitrate trihydrate Cu(NO$_3$_)$_2$·3H$_2$O was dissolved in 10 ml of distilled water and constantly stirred for 10 min using magnetic stirrer to get homogeneous mixture. The muffle furnace was pre-heated and the temperature was maintained at 400 ± 10 °C, the above prepared homogeneous mixture was kept inside the furnace for 5–10 min. The synthesized CuO nanoparticles were calcinated for 2 h to attain purity. The material was removed from muffle furnace, cooled to room temperature and the obtained black colored CuO powder sample was stored in air tight container for further analysis [21–23].

2.4. Synthesis of GEA hydrogel and GEA-CuO nanocomposite

The round bottom flask was taken for the hydrogel preparation, Glutaric acid (0.025 mol) was dissolved in 10 ml of deionized water. The above prepared solution was taken in a round bottom flask, and then the flask was fixed with a condenser tube on the mechanical stirrer with nitrogen inlet. Ethylene glycol (0.025 mol) was added drop wise with the help of a dropping funnel in the round bottom flask. The mixture was stirred for 1 h at room temperature and then poured into a round bottom flask kept in silicone bath oil at 160 °C in a nitrogen atmosphere. The white color sticky gel was formed at the completion of the reaction and third monomer of acrylic acid (0.025 mol) was added dropwise and further stirred for 30 min and finally white color hydrogel (GEA) was obtained. Afterwards the obtained hydrogel was subjected to synthesis of nanocomposite, 0.2 g of green synthesized CuO NPs was added into GEA hydrogel with constant stirring at 160 °C for 4 h. Eventually the
lite blue colored gel material was obtained named as GEA-CuO nanocomposite and it was washed several times using deionized water the nanocomposite was dried at lukewarm condition for 48 h.

**Mechanism**

![Chemical reaction diagram]

2.5. Characterization

The UV-Visible spectrum of above synthesized CuO NPs, GEA hydrogel, GEA-CuO nanocomposites were recorded by UV-2301 (Techcom) spectrometer. FT-IR spectrum was recorded for the organic and inorganic constituents in wavelength ranging from 400–4000 cm⁻¹. EDAX analysis was carried out by x-ray diffractometer instrument. The SEM and TEM analysis were carried out using (TESCAN-VEGA3 LMU) and (Jeol/JEM2100) instruments. TGA analysis was done by Perkin Elmer STA 6000 instrument. The NMR studies were carried out by Bruker instrument (Avance III HD Nanobay 400 MHz FT-NMR) using DMSO-d₆ solvent.

2.6. Antibacterial activity

The antibacterial activity of synthesized GEA hydrogel and GEA-CuO nanocomposite was carried out by disc diffusion method against *Staphylococcus aureus* (S. aureus) and *Escherichia coli* (E. coli) organism. Mueller Hinton broth Agar (MHBA) plates were injected with test organisms. The plates were uniformly spread out. Then wells were prepared in the plates with a cork borer. Each well was loaded with 500, 1000, 1500 and 2000 μg ml⁻¹ of corresponding concentration of sample and 2 mg of *Ciprofloxacin* dissolved in 1 ml of 10% DMSO was used as a positive control. The plates were brooded for 24 h at 37°C. The development of inhibition zone around the well was measured in diameter and recorded [24–26].

2.7. Anticancer activity

The anticancer activity of synthesized GEA hydrogel and GEA-CuO nanocomposite was explored by MTT cytotoxicity assay on A549 lung cancer cell line. The lung cancer cells A549 sample code B1800044 procured from the south India textile research association, Coimbatore, Tamil Nadu, India. The fresh medium was replaced by the culture medium from the A549 cells. Test samples in triplicates were added on the cells. The incubation is carried out at the temperature of 37±1°C for 18 h, then (1 mg ml⁻¹) of MTT solution were added in all the wells and incubated for 4 h then 0.5 ml of DMSO was added. The absorption test aliquots were measured at 570 nm in spectrophotometer. The following formula is used to calculate the percentage of cytotoxicity,

\[
\text{Cytotoxicity} = \left(\frac{\text{Control} - \text{Treated}}{\text{control}}\right) \times 100
\]

3. Results and discussion

3.1. UV-Visible analysis

UV-Visible analysis was carried out for *S. foetida* leaf aqueous extract, green synthesized CuO NPs, GEA hydrogel and GEA-CuO nanocomposite. Figure 1(a) shows the UV-Visible spectrum of aqueous extract of *S. foetida* leaf exhibits two bands at wavelength of 312 nm corresponding to π → π⁺ transition and 370 nm corresponding to n → π⁻ and π → π⁺ transitions. However this absorption bands are exhibited by
characteristic of phenolic and flavonoid compounds, hence the result of UV-Visible analysis disclosed the plant extract contains these types of phytoconstituents [27].

The formation of green synthesized CuO NPs and incorporation of CuO NPs with prepared hydrogel were examined by UV-Visible analysis. The green synthesized CuO NPs using S. foetida leaf extract was exhibited a band at ($\lambda_{\text{max}}$) 360 nm (figure 1(b)), which confirms the formation of nano sized CuO metal particles. Metal nanoparticles are exposed the absorbance in UV spectrum, it is occurred by the surface plasma resonance (SPR) of nano sized particles. However the absorbance spectra of green synthesized CuO NPs is well correlated with earlier report [28, 29]. The absorbance spectrum of GEA-CuO nanocomposite has revealed the small band at 345 nm and the prepared hydrogel did not shows any absorbance in the UV spectrum analysis (figure 1(b)), which shows the incorporation of green synthesized CuO NPs is done with the prepared hydrogel. Moreover the similar observation was recorded in the synthesis of chitosan/copper oxide nanocomposite (330 nm) [30], which is being supportive evidence of present study.

The absorbance spectrum of present study ensured the formation of green synthesized CuO NPs and metal nanoparticles incorporated GEA-CuO NCs. The metal oxide incorporated polymer nanocomposite is much attracted by current researchers to build the vehicle of nanoscale-based smart drug delivery system in chemotherapeutic treatments due to its high drug loading capacity, controlled drug releasing ability and target delivery efficiency [31].

3.2. FTIR analysis

FT-IR analysis of S. foetida leaf aqueous extract has shown the presence of possible phytoconstituents. Figure 2(a) shows FT-IR of plant extract, the broad peak at 3354 cm$^{-1}$ is corresponding to O–H stretching vibration for
water, phenols, amide (–NH) groups of phytoconstituents. The peaks at 1654 and 1610 cm$^{-1}$ revealed the stretching of C=O and C=C. Moreover the sharp peaks at 1019 and 880–800 cm$^{-1}$ represented the C–O stretching of carboxylic group and =C–H alkene bending vibration. However the IR peaks at higher wavenumber regions indicates the phytoconstituents such as phenolic, flavonoid, alkaloids etc which served as a green reducing and stabilizing agent in the metal nanoparticle synthesis [32].

The formation of green synthesized CuO NPs was examined by FT-IR analysis and shows the contribution of phytoconstituents from S. foetida leaf aqueous extract to synthesis of metal oxide nanoparticles. Figure 2(b) shows the FT-IR spectrum of green synthesized CuO NPs, the sharp peak at 500 cm$^{-1}$ is corresponding to stretching vibration of metal oxygen bond which confirms the formation of CuO NPs [33].

Moreover the characterization of prepared hydrogel polymer (GEA) was carried out by FT-IR analysis (figure 2(b)), which exhibited their corresponding functional groups peaks at 3511–3221 (O–H stretching), 2956 cm$^{-1}$ (aliphatic –CH stretching), 1680 (C=O stretching of ester), 1260 cm$^{-1}$ (C–C stretching).

Subsequently the green synthesized CuO NPs incorporated polymer nanocomposite was explored by FT-IR analysis (figure 2(b)), the peaks at 490–523 cm$^{-1}$ shows the presence of metal oxide nanoparticles and this result also ensured the formation of GEA-CuO nanocomposite [34].

3.3. XRD analysis
In the present study, XRD analysis of green synthesized CuO NPs using S. foetida leaf aqueous extract was carried for exploring the crystalline nature. Figure 3 shows typical XRD pattern of green synthesized CuO NPs and 11 diffraction peaks are detected at 2θ of 32.61°, 35.64°, 38.97°, 48.93°, 53.55°, 58.35°, 61.08°, 66.27°, 68.19° 72.48° and 75.27° were matched with the corresponding planes like (110), (002), (111), (−202), (020), (202), (−113), (310), (220), (311) and (−222) respectively. The observed diffraction peaks of green synthesized CuO NPs are well matched with JCPDS (Joint Committee on Powder Diffraction Standards) Card 48-1548, which exhibits the CuO NPs are crystalline nature with monoclinical structure and its lattice constant a = 4.688, b = 3.422, c = 5.131, β = 99.506. However the average crystalline size of green synthesized CuO NPs was calculated by using Debye–Scherrer’s equation.

\[ D = \frac{(K\lambda)}{\beta \cos \theta} \]

where D is the particle size (nm), K is constant (0.94), λ is wavelength of x-ray radiation (1.54 × 10$^{-10}$), β is full width half maximum (FWHM), θ is Bragg angle (Degree). Thus average crystalline size of green synthesized CuO NPs was calculated as 32 nm. Moreover the same diffraction peaks and crystalline properties were found in recent report on characterization of green synthesized CuO NPs using Tinospora crispa leaves aqueous extract [29]. The XRD study is not applicable for characterization of prepared GEA hydrogel and GEA-CuO nanocomposite because powder like material only preferable for analysis.
3.4. NMR analysis

The synthesis of GEA hydrogel and its structure were confirmed by the data of $^1$H NMR and $^{13}$C NMR analysis. Figure 4 shows the $^1$H NMR spectrum of synthesized GEA hydrogel, the peaks at $\delta_H$ (ppm) value 1.68 (3H, t) indicates the $-\text{CH}_3$ proton of acrylate terminal of hydrogel, 2.26–2.50 (6H, m) related to the $-\text{CH}_2$ protons of glutaric acid methylene groups [35], 2.51 (1H, m) observed for one proton in $-\text{CH}_2$ group of acrylic acid moiety, 3.36 (2H, m) noticed the methylene protons of ethylene glycol [36], 3.48 (1H, m) supported the remaining one methylene proton of acrylic acid moiety, 4.22 (2H, m) denoted $-\text{CH}_2$ protons of ethylene glycol moiety and 12.13 (1H, br s) noticed $-\text{OH}$ proton of glutaric acid terminal [37]. However the data of $^1$H NMR strongly supported the incorporation of hydrogel monomer units.

Figure 5 illustrates the chemical shifts of carbons in synthesized GEA hydrogel by $^{13}$C NMR technique, the peaks at $\delta_c$ (ppm) value: 20.16 ($-\text{CH}_3$ carbon terminal), 32.96 ($-\text{CH}_2$ carbon of glutaric acid), 33.16 ($-\text{CH}_2$ carbon of acrylate acid), 39.28 and 39.69 ($-\text{CH}_2$ carbons of glutaric acid), 62.27 and 62.31 ($-\text{CH}_2$ carbons of ethylene glycol) [38], 170.78 ($-\text{C}=\text{O}$ carbon of glutaric acid), 172.44 ($-\text{C}=\text{O}$ carbon of acrylic acid moiety) and 174.48 ($-\text{C}=\text{O}$ carbon of glutaric acid) [39]. The NMR analysis confirmed the desired structure of synthesized GEA hydrogel, besides the results are well correlated with UV-Visible and FT-IR spectral analysis.

![Figure 4. $^1$H NMR spectrum of synthesized GEA hydrogel.](image)

![Figure 5. $^{13}$C NMR spectrum of GEA hydrogel.](image)
3.5. Thermogravimetric analysis (TGA)
Thermal stability of synthesized GEA hydrogel and GEA-CuO nanocomposite was determined by TGA analysis and shown in figure 6. GEA hydrogel exhibits 10% of weight loss at 82 °C as initial degradation is due to removal of water moiety. Subsequently 75% of weight loss was observed at 244 °C as second stage degradation is due to decompose of monomers of hydrogel (figure 6(a)). However the complete degradation of GEA hydrogel was exhibited at 394 °C, hence this present study portrayed the newly synthesized GEA hydrogel will be an efficient polymer network under difficult thermal state. Figure 6(b) illustrates the weight loss tendency of GEA-CuO nanocomposite, which exhibit the initial degradation at 95 °C about 2% of weight loss. Afterwards second stage degradation of GEA-CuO nanocomposite was noted at 253 °C as 71% of weight loss. However in higher temperature at 421 °C, the GEA-CuO nanocomposite is exhibited 98% of weight loss and remaining residue is exposed the CuO NPs. The resultant of TGA analysis disclosed the incorporation of green synthesized CuO NPs with prepared hydrogel is increased their thermal stability as well as durability in higher temperature [40].

There are several research groups have been studied about thermal stability of polymers and the report stating that the thermal stability of polymer raised by converted into metal nanocomposites [41]. The dispersed nanomaterials such as CuO NPs might ruin the flux of degradation products and thereby delay the onset of degradation. The improved thermal stability and higher thermal conductivity of polymer nanocomposites accelerate the heat dissipation within the composite [42].

3.6. SEM analysis
The SEM analysis was carried out to explore the morphology of green synthesized CuO NPs, GEA hydrogel and GEA-CuO nanocomposite (figure 7). SEM image of green synthesized CuO NPs revealed the particles are spherical in shape and seems like clusters form (figure 7(a)), besides number of pores between the clusters were found due to removal of phytoconstituents by calcination process in the green synthesis method of metal nanoparticles [43]. Figure 7(b) shows the morphology of synthesized GEA hydrogel, it has uneven bulged surface in layer form. Subsequently the incorporation of green synthesized CuO NPs with GEA hydrogel offered the more number of uneven plates with cavities were observed in GEA-CuO nanocomposite (figure 7(c)).

SEM analysis of GEA-CuO nanocomposite is being an evident for this material may serve as efficient drug carrier due to having large number of cavities and metal interactions for drug loading and releasing. Formerly, CuO NPs incorporated hydrogel nanocomposite (polyvinyl alcohol/CuO) demonstrated as efficient carrier for ibuprofen drug and it inspired the evaluation of present study [15].

3.7. EDAX analysis
The presence of elements composition in the test samples were studied by EDAX spectroscopy. Figure 8 shows the EDAX spectrum of GEA hydrogel and GEA-CuO nanocomposite. EDAX spectrum has revealed the atomic percent of C 57.62% and O 42.38% in GEA hydrogel (figure 8(a)) which declared the hydrogel having no impurity elements. However, EDAX spectrum of GEA-CuO nanocomposite exhibited the elements such as C, O and Cu with atomic percent of 54.97%, 42.43% and 2.6% (figure 8(b)). However the result of EDAX spectrum for GEA-CuO nanocomposite confirms the incorporation of green synthesized CuO NPs and ensured the metal nanocomposite is constructed without any other elements.
3.8. TEM analysis
The exact size and morphology of green synthesized CuO NPs and GEA-CuO nanocomposite were examined by TEM analysis. The morphology of green synthesized CuO NPs is like agglomerated spherical in shape majorly and few particles are like diamond in shape, it was observed in the magnification of 50 nm (figure 9(a)) and TEM analysis portrayed the exact size of green synthesized CuO NPs is in the range of 10–50 nm. Subsequently GEA-CuO nanocomposite was scrutinized in the magnification of 50 and 10 nm with well dispersion condition. TEM images (figures 9(a) and (c)) shows GEA-CuO nanocomposites are spherical in shape with the size range of 5–10 nm. Moreover the cloudy surface on nanocomposites shows the presence of hydrogel accumulation. The similar morphology was observed for green synthesized CuO NPs using Tamarix gallica leaf extract [44]. Generally copper metal is highly focused for its catalyst property, besides in nano size level they can easily enter into micrometer sized human cellular which exhibits wound healing, antibacterial properties and used for targeted drug delivery application as in the form of polymer metal nanocomposites [17].

The TEM analysis also used to visualize the intercalation and orientation of nanoparticles in to polymer matrix [45]. Starch/CuO bio-nanocomposite with the incorporation of 35–40 nm sized CuO NPs demonstrated as potent drug carriers with inherent antibacterial activity. Hence green synthesized CuO NPs incorporated GEA-CuO nanocomposites can be employed in the biomedical applications [46].
3.9. Antibacterial activity

The antibacterial capability of synthesized polymer hydrogel (GEA) and nano composite of green synthesized CuO nanoparticles introduced polymer hydrogel (GEA-CuO) was assessed against *S. aureus* (gram positive bacteria) and *E. coli* (gram positive bacteria) (figure 10(a)). The GEA-CuO nanocomposite have exhibited significant antibacterial ability against two tested bacterial organisms, especially the higher zone of inhibition.
was observed for S. aureus (20 ± 0.52 mm) at 2000 μg ml⁻¹ concentration and 17 ± 0.52 mm zone of
inhibition observed for E. coli (figure 10(b)). However the GEA hydrogel has revealed very poor antibacterial
activity and shows zone of inhibition nil, which declared the raw synthesized hydrogel is not effective in bacterial
resistance. Notably the metal nanocomposite GEA-CuO exhibits potent antibacterial activity which might be
due to the presence of green synthesized CuO NPs, it enhance some electrostatic interactions with bacterial cell
wall and metal composite has tiny pores that allows the foreign agents to come in contact with the bacteria and
induced the cell decay [47, 48].

The green synthesized CuO NPs incorporated GEA-CuO nanocomposite exhibited significantly higher
antibacterial activity than earlier reported metal incorporated polymer nanocomposites of Ag, ZnO and CuO
with low-density polyethylene (LDPE) [49] and CuO NPs impregnated chitosan nanofibres [50].

Antibacterial mechanism of metal nanocomposites not precisely found yet but which occurred in two ways
such as biophysical and biochemical mechanism. Through biophysical mechanism, while the contact of nano
materials with bacterial surface which generates the physical stress on membranes. However the increasing
touch of bacterial membrane with nanostructure resultant the higher amount of physical stress on it and lead
to membrane rupture. Moreover in biochemical mechanism, the metal ions of nano materials make some
interactions with bacterial cell membranes which infuse the positive charge nanoparticles (Cu⁺) inside.
Subsequently the metal nanoparticles interacts with electro negative atoms of peptidoglycan layer of bacteria and
the metal (Cu⁺) ions generates the reactive oxygen species (ROS) such as hydroxyl radical (OH⁻) by the
oxidative stress. Therefore the regular transportsations of bacterial protoplasm and protein damaged which leads
to membrane rapture and cell death [32, 51].

3.10. Anticancer activity
The anticancer effect of synthesized GEA hydrogel and GEA-CuO nanocomposite was explored by MTT assay
method against lung cancer cell line (A549). The cytotoxicity of test samples on cancer cell line was
demonstrated at various concentrations like 5, 25, 50, 75, 100 μg ml⁻¹. GEA hydrogel and GEA-CuO
nanocomposite have exhibited significant cytotoxicity against lung cancer cell line which observed by increases
the death cell in morphological analysis (figures 11(a) and (b)). While the concentration of samples increases the
cell viability decreases and cytotoxicity increases (figure 11(c)). The higher cytotoxicity was exhibited by GEA-
CuO nanocomposite (50.8%) than GEA hydrogel (41%) at the concentration of 100 μg ml⁻¹. Based on the
results and ISO 10993-5:2009, the synthesized GEA-CuO nanocomposite considered as mild effective for lung
cancer treatment. The anticancer effect of GEA-CuO nanocomposite was accelerated by metal nanoparticles,
which might be due to agglomeration and shape of nanoparticles [52]. However the result of present study
exposed the polymer metal nanocomposite will act as efficient drug carrier in biomedical applications. The
GEA-CuO nanocomposite exposed the significant cytotoxicity effect on Lung cancer cell line (A549) at
100 μg ml⁻¹ concentration when compared with chitosan-CuO (CS-CuO) nanocomposite [30].

The anticancer efficiency of metal polymer nanocomposite is depends on metal nanoparticles, chemical
composition, shape and size. Anticancer mechanism of metal polymer nanocomposite is predicted as generation
of ROS due to oxidative stress on cancer cell. However, it might induced by electro chemical interaction of
specific negative charged surface groups (i.e. lipids) of cancer cell with positive charge of metal nanoparticles.

In case, size of metal nanoparticles is very small in nanocomposites, this increases the surface to volume ratio
and enriched the electro chemical properties and caused to more cytotoxicity on tested cancer cell. Besides, the
semiconductor metal Cu⁺ present in nanocomposite can trigger the oxidative stress extensively through Fenton
type reaction. It reacts with H₂O₂ on cancer cell environment produces OH⁻ and O₂⁻ radicals consequently
cytotoxicity increased [53]. Hence the CuO nanoparticles incorporated hydrogel nanocomposites will be
effective drug carrier in cancer chemotherapy.

4. Conclusion
The metal polymer nanocomposite was successfully prepared by incorporation of green synthesized CuO NPs
with synthesized GEA hydrogel. Synthesis of GEA hydrogel and GEA-CuO polymer composite were confirmed
by UV, FT-IR analysis. Besides the newly synthesized GEA hydrogel was separately characterized using NMR
and TGA analysis. SEM and TEM morphological analysis expressed the green synthesized CuO NPs and GEA-
CuO nanocomposite were agglomerated spherical in shape with size range 10–50 nm and 5–10 nm respectively.
However biological application of GEA hydrogel and GEA-CuO nanocomposite was studied by in vitro
antibacterial and anticancer activity. Green synthesized CuO NPs incorporated GEA-CuO nanocomposite
exhibited potent bacterial resistance against S. aureus and E. coli organisms than GEA hydrogel. Subsequently the
significant cytotoxicity effect on lung cancer cell A549 was exhibited by GEA-CuO nanocomposite. Hence the present study suggested GEA-CuO nanocomposite will be effective in biomedical applications.

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Conflict of interest

Authors declare that they have no conflict of interest.

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