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Original Article

Green-Synthesized *Tribulus terrestris*-Mediated CuO Nanoparticle-Loaded CS/PVA Hydrogels: A Multifunctional Platform for Cancer Therapy and Antidiabetic Applications

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ABSTRACT

Objectives: In this work, we report on the preparation of CuO nanoparticles using extract from *Tribulus terrestris* as a natural reducing and stabilizing agent, followed by the loading of the nanoparticles into the Chitosan/Poly (vinyl alcohol) (CS/PVA) hydrogel in order to design a multifunctional system for treating cancer and diabetes. **Materials and Methods:** Firstly, CuO nanoparticles were synthesized using *T. terrestris* extract as natural sources for the reduction and stabilization process. Then, the CuO nanoparticles were introduced into the hydrogel by simple cross-linking process. **Results:** The TEM results showed the homogeneous dispersion of CuO nanoparticles in the hydrogel. Moreover, FTIR studies proved the formation of chemical bond in both the polymeric matrix and plant extracts. Cytotoxic analysis of the loaded hydrogel indicated significant cell death in A549 cells with IC_{50} of 8 µg/mL and cell morphology changes as well as fluorescent labeling, suggesting apoptosis. *In vitro* drug release test showed a two-stage release profile including a rapid initial release phase and sustained release for up to 24 hr. Moreover, the formulation also showed potential anti-diabetic activities due to its inhibition of both α-amylase and α-glucosidase enzymes. **Conclusion:** The *T. terrestris*-mediated CuO nanoparticle-loaded CS/PVA hydrogel demonstrated promising anticancer and antidiabetic activities, indicating its potential as a multifunctional platform for cancer therapy and diabetes management.

Keywords: A549 Cells, Anti-Cancer Activity, CS/PVA Hydrogels, CuO Nanoparticle, *Tribulus Terrestris*.

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INTRODUCTION

The advent of nanotechnology into biomedical research has created a unique chance for the development of multifunctional materials which facilitate targeted therapeutic approaches. Here, Copper Oxide (CuO) Nanoparticles (NPs) have been described as a universal nanomaterial due to their distinct physicochemical characteristics and biological behavior (Khan *et al*., 2025). CuO NPs display strong anticancer activity by generating ROS and causing cell death through mitochondrial injury. Besides this, they also show antimicrobial and pro-angiogenic effects, aiding wound repair, and serve as antidiabetics through insulin-like actions, enzyme inhibition, and glucose absorption enhancement (Devaraji *et al*., 2024). The wide range of functionalities offered by Copper Oxide Nanoparticles (CuO NPs) make them a good choice for future applications in integrated therapy (Woźniak-Budych *et al*., 2023). The conventional chemical approach in synthesizing CuO NPs uses toxic chemicals, high temperatures and large amounts of energy. The use of these processes can result in the degradation of the environment and even biological organisms. As an attempt to counteract these shortcomings there has been a move to the green method. This involves the use of phytochemicals obtained from plants as reducing and stabilizing agents in the production of nanoparticles. Apart from reducing the need for toxic chemicals, the green process also endows the nanoparticles with biological activity (Naz *et al*., 2023; Ying *et al*., 2022). *Tribulus terrestris*, a traditional medicine plant, has an abundant phytochemical content such as flavonoids, saponins, alkaloids, and polyphenols and is well-known for its antioxidant, anti-inflammatory, antimicrobial, antihyperglycemic, and anticancer properties (AlSedairy *et al*., 2025). The bioactive compounds not only aid in reducing copper ions to CuO nanoparticles but also help in preventing the aggregated state of

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CuO nanoparticles through their coating action. The presence of bioactivity in the phytochemical-coated nanoparticles can be used for enhancing the biological activity of the nanoparticles. For biomedical applications, the nanomaterials require a proper carrier system for stabilization purposes (Latha *et al*., 2026). Chitosan (CS)/Polyvinyl Alcohol (PVA)-based hydrogels represent a favorable system as they have the advantages of being biodegradable, hydrophilic, and adjustable in mechanical strength. As a derivative of chitin, chitosan is inherently antimicrobial and biocompatible, whereas polyvinyl alcohol provides additional benefits in terms of physical properties and swelling capacity (Hong *et al*., 2024). The incorporation of Copper Oxide Nanoparticles (CuO NPs) that are produced by using *T. terrestris* into CS/PVA hydrogels results in a hybrid material capable of local and targeted drug delivery (Stevanović *et al*., 2025). Indeed, there is great potential of these nanocomposite hydrogels in many aspects of biomedical engineering. For instance, in cancer therapies these hydrogels can use the property of CuO nanoparticles of generating ROS and initiating apoptosis in order to selectively eliminate cancer cells without damaging other cells (Cheng *et al*., 2025). In wound healing, these types of hydrogels are effective in stimulating the proliferation, migration, and angiogenesis processes, hence making it possible for tissue regeneration even in the challenging environment provided during the tests in aquatic organisms (Gounden *et al*., 2024). When it comes to antidiabetic medications and drug delivery systems, CS/PVA hydrogels-based nanocomposites will be helpful in inhibiting carbohydrate metabolism enzymes, facilitating glucose absorption, and providing a pH-responsive drug delivery system (Zhao *et al*., 2020; Ger *et al*., 2026). Therefore, with the help of CuO nanoparticles manufactured through the eco-friendly process discussed above this project aims at designing an environmentally friendly and biocompatible material for various biomedical purposes (Figure 1).

MATERIALS AND METHODS

Materials

The fresh collection of *Tribulus terrestris* plant was identified and included in the current study. Medium MW chitosan, having the degree of deacetylation above 75%, and polyvinyl alcohol, having the molecular weight in the range of 85,000-124,000 and hydrolysis value at 99%, were purchased from Sigma Aldrich (USA). Copper Sulphate Penta Hydrate, Sodium Hydroxide, Glacial Acetic Acid, Glutaraldehyde (25%), And Potassium Bromide Were Provided by Merck (India).

Preparation Methods

Preparation of Tribulus terrestris *Aqueous Extract*

The freshly harvested aerial parts of *T. terrestris* were first washed with distilled water, dried in shade and then ground to powder form. Plant powder (10 g) was added to 100 mL of distilled water and heated at 60-70°C for 30 min while continuously stirred. The mixture was allowed to cool to room temperature before being filtered using Whatman No. 1 filter paper.

Green Synthesis of CuO Nanoparticles

The aqueous solution of copper (II) sulfate pentahydrate (0.01 M) was heated to 60°C. In this solution, *T. terrestris* extract was slowly added drop-wise with constant stirring in 1:1 volume ratio. To keep the pH value at ~10, 1 M sodium hydroxide solution was used, which facilitates the synthesis of nanoparticles. As soon as black particles appeared, after 2 hr of stirring it is clear that nanoparticles of copper oxide are synthesized. The obtained precipitates were filtered washed multiple times with distilled water and ethanol and then dried at 60°C.

Preparation of Chitosan (CS) Solution

Chitosan (2% weight/volume) was dissolved in 1% (volume/volume) acetic acid solution by stirring continuously for 4 hr at ambient temperature, ensuring complete dissolution into a homogeneous solution. This solution was then filtered to remove any undissolved impurities.

Preparation of Polyvinyl alcohol (PVA) Solution

The PVA (10% w/v) was dissolved in distilled water by heating between 80-90°C while continuously stirring until full dissolution. Afterward, the solution was allowed to cool to room temperature prior to hydrogel synthesis.

Fabrication of CS/PVA Composite Hydrogel

The CS and PVA solution were combined in equal volumes under magnetic stirring conditions to form an even blend of polymers. This solution was then degassed to eliminate any entrapped air and was immediately used for loading nanoparticles.

Incorporation of CuO Nanoparticles into CS/PVA Hydrogel

The CuO nanoparticles prepared via a green method were dispersed in distilled water with the help of ultrasonic waves and then mixed with the CS/PVA blend at specific concentrations.

Hydrogel Crosslinking and Glutaraldehyde

The CS/PVA mixture containing *T. terrestris*-mediated CuO nanoparticles synthesized using a green method was treated with 0.5% (v/v) glutaraldehyde solution while stirring with a magnetic stirrer in order to achieve the stabilization of the polymeric network. In order to enable effective cross-linking and formation of a stable hydrogel structure, the reaction mixture was allowed to stand at room temperature for a sufficient period of time. To eliminate any remaining unreacted glutaraldehyde and lessen surface acidity, the resulting CuO nanoparticle-loaded CS/PVA hydrogel was repeatedly cleaned with distilled water. Prior to additional physicochemical characterization and biological assessments pertaining to anticancer and antidiabetic applications, the cleaned hydrogel was equilibrated to near-neutral pH, cut into the necessary dimensions, and exposed to UV irradiation for surface sterilization.

Physicochemical Characterization

Transmission Electron Microscopy

TEM analysis was performed to examine the morphology and dispersion of *T. terrestris* mediated CuO nanoparticles within the CS/PVA hydrogel. Ultra-thin sections (~70-100 nm) of the hydrogel were prepared using an ultramicrotome and mounted on carbon-coated copper grids. For isolated nanoparticles, a drop of the nanoparticle suspension was placed on grids and air-dried. Imaging was carried out at an accelerating voltage of 100-200 kV, and energy dispersive X-ray Spectroscopy (EDS) was used to confirm the elemental composition of copper and oxygen (Jayaprakash *et al.*, 2023).

FTIR Characterization

Fourier Transform Infrared (FTIR) spectroscopy was employed to examine the functional groups present on the copper oxide nanoparticles using the KBr pellet technique. Spectral measurements were carried out using a Nicolet 5700 FTIR spectrometer (Thermo Scientific, USA) over the range of 500-4000 cm^{-1}. This analysis was performed to identify characteristic absorption bands corresponding to CuO nanoparticles and to elucidate the involvement of plant-derived biomolecular components in nanoparticle reduction, capping, and stabilization (Pasieczna-Patkowska *et al.*, 2025).

Anti-cancer Activity

Cell Culture Maintenance

The human cancer cells line (A-549) was cultivated in Dulbecco's Modified Eagle Medium (DMEM) containing 10% Fetal Bovine Serum (FBS) and 1% penicillin-streptomycin. They were incubated under humid conditions at 37ºC in a 5% CO_2 environment. The cells were sub-cultured when they achieved 70-80% confluency by trypsin-EDTA treatment and subsequently seeded onto their respective culture plates. All procedures were done under sterile conditions (Arjunan *et al.*, 2021).

Critique of Morphology

The cytotoxic behavior of Cs/PVA-TT hydrogels can be confirmed by morphological analysis. While control cell morphology appears homogenous and normal, the morphological hallmarks of apoptosis in A-549 cells are shown in a contracted cell body, membrane blebs, and detachment from the substratum. To aid statistical confirmation of results, the morphological analysis could further benefit from quantitative measurements via image analysis software, flow cytometry, and other tests, although the results obviously point toward apoptosis. In addition, the use of time-lapse imaging and/or comparison between multiple cell lines would add robustness to the morphological assessment (Suhail *et al.*, 2022).

Cytotoxicity Assay

The MTT assay was conducted to assess the cytotoxicity of the Cs/PVA-*Tribulus terrestris* hydrogel on the A549 lung adenocarcinoma cell line cell line. The results demonstrated dose-dependent cytotoxicity against the cancerous cells, with an IC_{50} value of 8 μg/mL. There was evidence of selective cytotoxicity due to the hydrogel's minimal cytotoxic effects on normal cells. The findings indicate the therapeutic utility of phytochemicals from *Tribulus terrestris* delivered via a hydrogel system (Akbaba *et al.*, 2021).

Cell Death Analysis Using Fluorescent Microscopy

Using a fluorescence microscope and dual labeling with Acridine Orange (AO) and Ethidium Bromide (EtBr) cell death was assessed. Cells in control samples fluoresced green suggesting vitality but cells treated with Cs/PVA-TT at IC_{50} (8 μg/mL) fluoresced orange to red, indicating early and late apoptosis because of reduced membrane integrity. Apoptotic morphology was further validated by the presence of broken chromatin and condensed nuclei. Although qualitative, this approach successfully visualized apoptotic events, nonetheless, more accurate apoptotic index measures would be obtained through quantitative analysis employing flow cytometry tests (Gökalp *et al.*, 2011).

Drug Release and Antidiabetic Activity

In vitro ***Drug release Assay***

The Cs/PVA *T. terrestris* hydrogel's *in vitro* drug release profile was assessed over the course of 24 hr at 37ºC in phosphate-buffered saline (PBS, pH 7.4). After a brief burst release during the first 5 hr, the hydrogel showed a steady and regulated release pattern, culminating in a total release of roughly 98-100% by the end of the day. This hydrogel's capacity to sustain therapeutic levels over time, improve bioavailability and lower dose frequency is demonstrated by its biphasic release pattern. The cross-linked polymer matrix, which controls the diffusion of *T. terrestris* bioactives, is responsible for the regulated release (Chopra *et al.*, 2022).

Anti-Diabetic Activity

The assay for α-amylase and α-glucosidaseinhibition

In vitro α-amylase and α-glucosidase inhibition tests were used to evaluate the Cs/PVA-*T. terrestris* hydrogel's anti-diabetic potential. With 67.1% inhibition of α-amylase at 50 μg/mL and similar performance in α-glucosidase inhibition, the hydrogel showed dose-dependent inhibitory efficacy, suggesting efficient suppression of glucose release and carbohydrate breakdown. The hydrogel continuous release and plant-derived

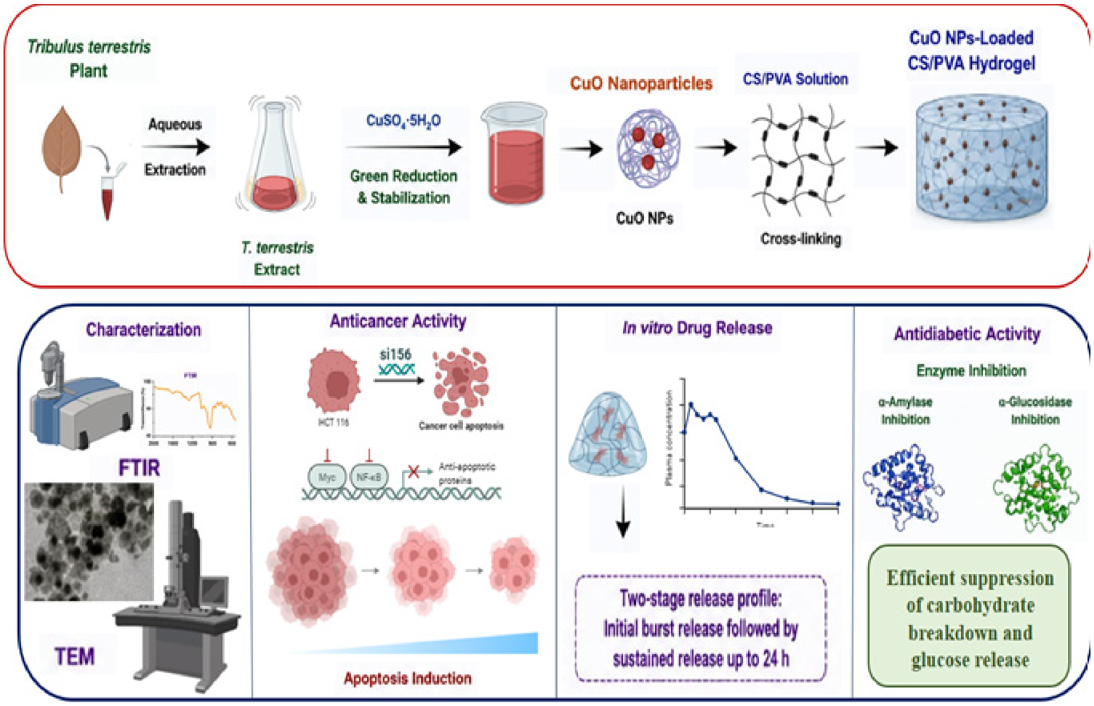


Figure 1: The green synthesis of Tribulus terrestris-mediated CuO nanoparticle-loaded CS/PVA hydrogels and their characterization, controlled drug release, anticancer efficacy against A549 cells, and antidiabetic enzyme inhibitory activities.

bio actives including calanolides and flavonoids indicate a natural and biocompatible alternative for treating postprandial hyperglycemia in diabetic therapy, even if metformin (positive control) demonstrated stronger inhibition (~79.3%) (Magwaza *et al.*, 2023).

Statistical Analysis

All experiments were performed in triplicate (n=3), and data are presented as mean ± SD. Statistical analysis was carried out using GraphPad Prism. Differences among groups were analyzed by one-way ANOVA followed by Tukey's *post hoc* test, with p<0.05 considered statistically significant.

RESULTS

Characterization

TEM

The TEM image reveals a single, irregularly shaped CuO nanoparticle embedded within the CS/PVA hydrogel matrix, synthesized using *Tribulus terrestris* extract. In Figure 2A, the particle exhibits a quasi-spherical to slightly elongated morphology with a smooth outline and non-uniform contrast, indicating variations in internal density and possible crystalline domains. The average size appears to be below 50 nm, as confirmed by the scale bar, suggesting successful nanoscale synthesis suitable for biomedical applications.

FTIR Analysis

There are specific absorption bands in the FTIR spectrum, which show that there are more than one functional group present in the sample. There is a large absorption area between 1600-1500 cm^{-1}, which might indicate amide or aromatic C=C stretching vibration. Peaks at the range of 1200-1000 cm^{-1} represent the C-O-C and C-O stretching vibration, respectively. It may indicate polysaccharide or polymeric compounds (Figure 2B).

Cytotoxicity Assay

It may be noted from the results obtained from the cytotoxicity assay that there is a clear dose response relationship between reductions in cell viability due to increase in concentration of nanoparticles. As evident from (Figure 3A), at a concentration level of 0 µg/mL, cell viability is 93%. At 5 and 10 µg/mL, cell viability decreases to 63% and 59%, respectively. Cell viability decreases even more to 46%, 25%, and 20% at 25, 50, and 100 µg/mL, respectively (Table 1).

Cell Morphology Analysis

The cellular images illustrate the alterations in cell morphology both before and after the nanoparticle treatment. The untreated cells (Figure 3B) show healthy morphology, with cells elongated and spread out on the surface, representing high confluency. On the other hand, the treated cells (Figure 3C) reveal a decrease in the number of cells on the surface, with cells rounded up and shriveled, indicating cell membrane disruption and death.

Cell Death Analysis Using Fluorescent Microscopy

The differences seen between (Figures 3D and 3E) are mainly because of viability of cells in both cases. In case (d), it is observed that there is presence of green colored cells which show high viability of cells and no cytotoxic effect on cells. On the other hand, in case (e), there are considerable numbers of red/orange stained cells which show less viability of the cells due to their death.

In vitro Drug Release Assay

The Cs/PVA-TT system follows a two-stage drug delivery process that is marked by an initial rapid release phase within the first 5 hr, and then there is a second slower phase until 24 hr (Figure 4A). This suggests the potential of the Cs/PVA matrix for effective drug delivery, providing both immediate therapeutic effect and prolonged drug availability.

Anti-diabetic Activity

α-Amylase and α-Glucosidase Inhibition Assay

The Cs/PVA-TT formulation exhibits significant antidiabetic potential by inhibiting alpha-amylase activity in a concentration-dependent manner. Although the inhibition is lower compared to standard metformin, a steady increase from 25% to 42% was observed with rising concentrations (25-75 μg/mL), indicating its promising role in managing postprandial hyperglycemia (Figure 4B).

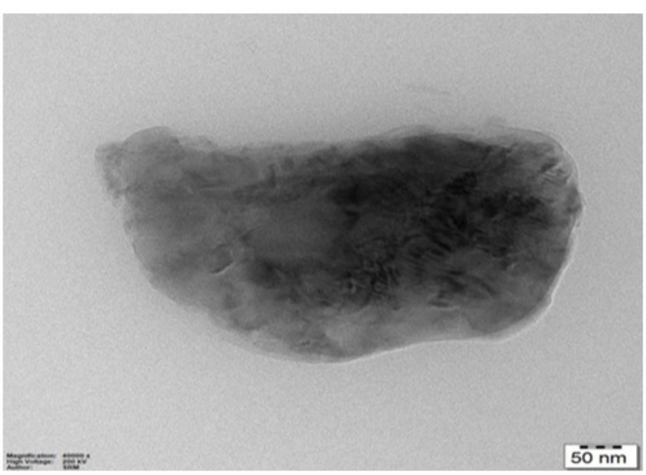
DISCUSSION

The present study demonstrates the successful development of a multifunctional CS/PVA hydrogel incorporated with green synthesized CuO nanoparticles using *T. terrestris* extract highlighting its potential for integrated anticancer and antidiabetic applications (Ahamadian *et al*., 2019). Plant mediated synthesis can be considered a more eco-friendly approach compared to chemical synthesis, with reduced ecological footprint and improved bioactivity via the process of phytochemical capping (Edo *et al*., 2025).

From the TEM results, it is evident that the CuO nanoparticles are homogeneously distributed with particle size less than 50 nm within the CS/PVA hydrogel scaffold. Nanosize is essential in biomedical applications due to its increased ability to penetrate cells and interact with intracellular targets (Kamyab and Raja *et al*., 2024). This morphological observation is typical for the green-synthesized metal oxides nanoparticles, which may be due to the combination of different flavonoids, saponins, and polyphenolic components that can serve as reducing agents and stabilizers in *T. terrestris* (Yazhini *et al*., 2025). The identified FTIR peaks suggest that there are important functional groups related to polymeric and bioactive components. The major

Table 1: ***Tribulus terrestris*-mediated CuO nanoparticle-loaded CS/PVA hydrogel. IC_{50} against A549 cells (Mean ± SD): 8±0.5 μg/mL.**

Concentration (μg/mL)	Mean Viability (%)	SD	Mean Inhibition (%)	SD	95% CI
0	93.0	1.20	7.0	1.20	2.98
5	63.0	1.10	37.0	1.10	2.73
10	59.0	1.05	41.0	1.05	2.61
25	46.0	0.96	54.0	0.96	2.38
50	25.0	0.82	75.0	0.82	2.03
100	20.0	0.71	80.0	0.71	1.76



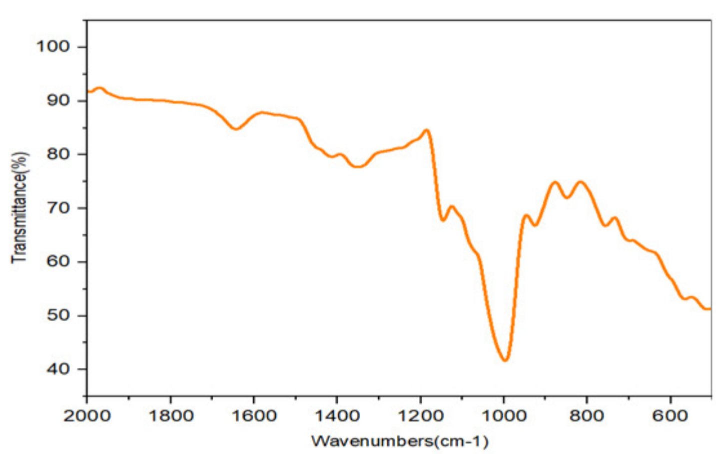


Figure 2: Characterization of CuO nanoparticle-loaded CS/PVA hydrogel: (A) TEM image showing nanoparticle morphology and distribution; (B) FTIR spectrum confirming functional groups and polymer-nanoparticle interactions.

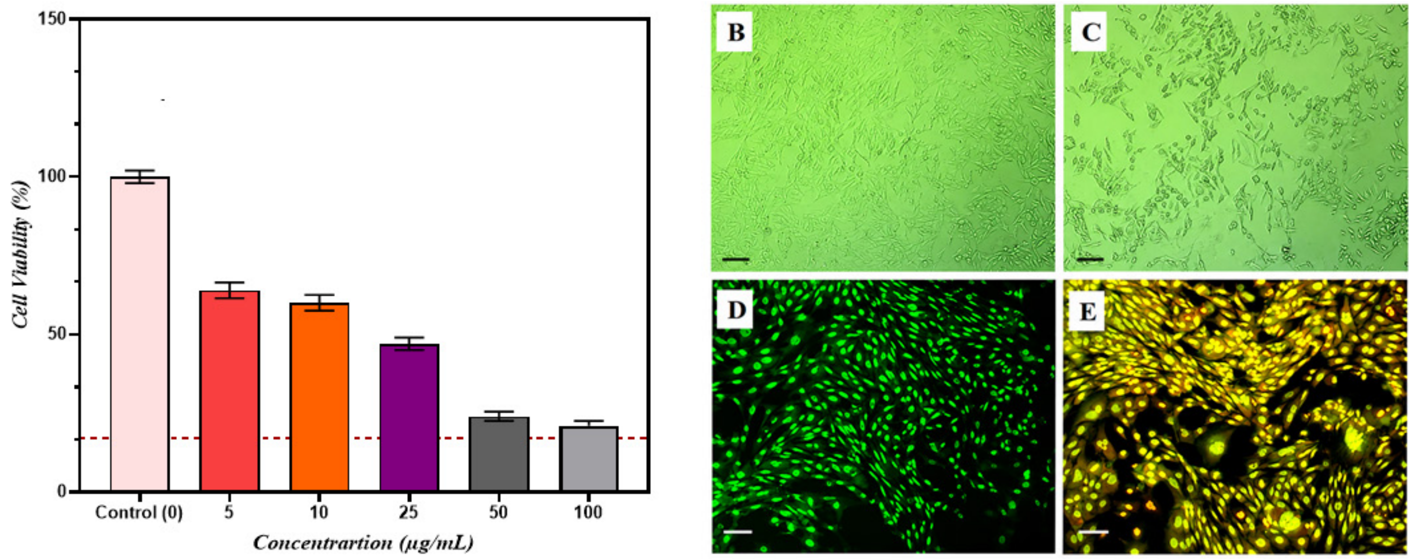


Figure 3: Anticancer activity of CuO nanoparticle-loaded CS/PVA hydrogel against A549 cells: (A) MTT cytotoxicity assay; (B) untreated cells; (C) treated cells; (D) AO/EtBr staining of control cells; (E) AO/EtBr staining of treated cells showing apoptosis.

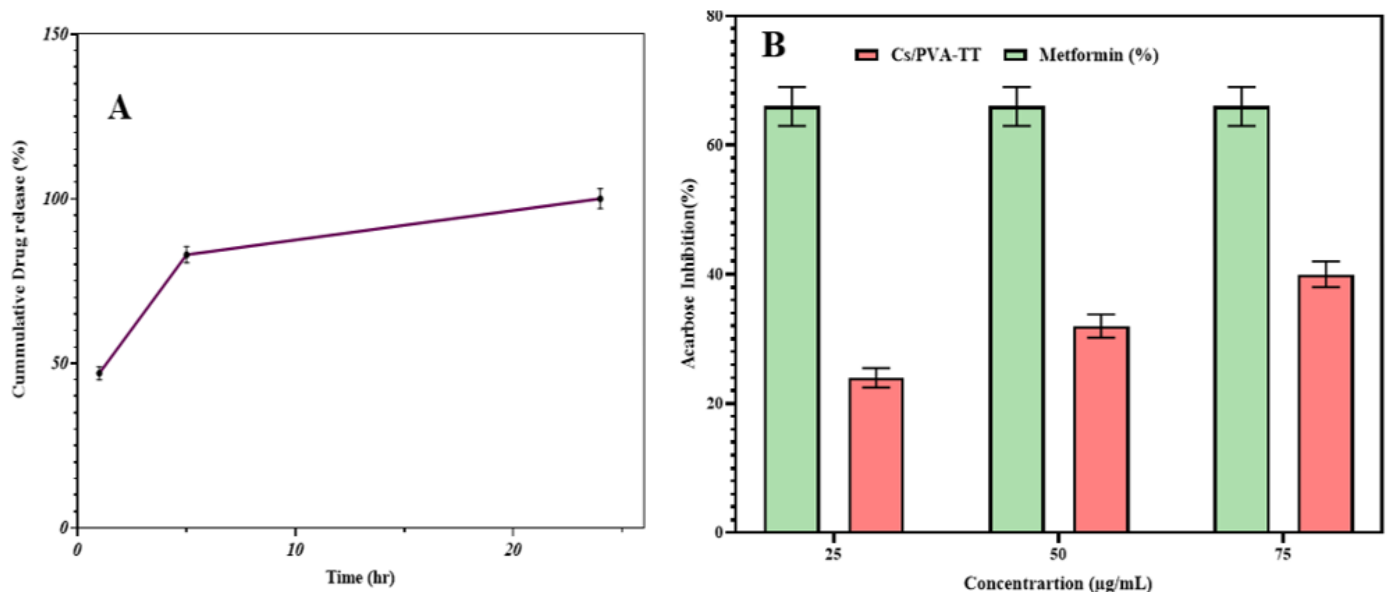


Figure 4: Functional evaluation of the hydrogel: (A) *In vitro* drug release profile showing biphasic release behavior; (B) Antidiabetic activity assessed by α-amylase inhibition assay.

peak regions in the fingerprint range (1200-800 cm^{-1}) relate to polysaccharide vibrational peaks, which might be responsible for the stability and functionality of this material, making it suitable for biomedical applications (Hanif *et al.*, 2023). From the cytotoxic analysis, there was a marked concentration-dependent decrease in the viability of A549 lung cancer cells with an IC_{50} value of 8 µg/mL. This suggests that the nanocomposite hydrogel exhibits strong antineoplastic potential with an IC_{50} of 8 µg/mL (Hwang *et al.*, 2022). The observed increased cytotoxicity may be due to the synergism between the CuO NPs and the phytoconstituents of *T. terrestris*. In this case, CuO NPs produce oxidative stress by generating excessive ROS. These effects ultimately cause mitochondrial malfunction, DNA damage, and apoptosis (Vel *et al.*, 2024). Further evidence which confirms the previous cytotoxic data was gathered using morphological techniques, whereby it is noted that cells exposed to the compound exhibited typical signs of apoptotic cell death including cell shrinkage, rounding, formation of membrane blebs, and detachment (Baeza-Morales *et al.*, 2025). These structural changes indicate alteration in cytoskeletal organization and cellular membrane, which occur during apoptosis but not necrosis. In particular, the application of AO/EB dye technique indicated the presence of apoptosis by change in fluorescence color from green to orange (Huang and Brazel *et al.*, 2001). From the study of drug release, it was evident that there are two stages in the process of drug release, with the first stage representing the burst release stage and the second stage representing the sustained release stage for 24 hr. In the burst release stage, this can be credited to the fact that there are active biological substances attached on the surface as well as the nanoparticles entrapment technique, while in the sustained release stage, it is affected by the diffusion of drug molecules within the crosslinked CS/PVA (Ratananikom *et al.*, 2024).

The CS/PVA-*T. terrestris* hydrogel proved to possess considerable antidiabetic efficacy due to dose-related inhibition of α-amylase and α-glucosidase enzymes. While the inhibitory potential was somewhat weaker compared to metformin, the gradual and continuous inhibition implies successful regulation of postprandial glucose (Dewanjee *et al.*, 2020). The demonstrated action can be attributed to flavonoids and saponins derived from plants, capable of inhibiting carbohydrate breakdown and glucose uptake. Moreover, the use of hydrogels as a drug delivery system could improve efficacy of enzymatic inhibition (Lin *et al.*, 2025). In summary, the incorporation of green-synthesized copper oxide nanoparticles within the biodegradable CS/PVA hydrogel scaffold material provides an exciting hybrid system with the advantages of controlled drug delivery, significant biological activity, and environmental compatibility. Although the *in vitro* data are encouraging, future research will need to be conducted in order to address the mechanism of action and *in vivo* studies (Karra *et al.*, 2026).

CONCLUSION

Overall, the current work has been able to design an environmentally friendly CuO nanoparticle-loaded hydrogel based on a combination of CS/PVA with *Tribulus terrestris* extract. The application of the green synthesis method has led to the preparation of stable CuO nanoparticles, which have been embedded into the hydrogel matrix. Physical chemical analysis has proved that nanoparticles have been formed, with the identification of different functional groups linked to the polymer matrix and bioactive agents. The synthesized nanohydrogel has shown a high level of biological activity by displaying potent dose-dependent cytotoxic effects against A549 cancer cell line, alongside with morphological alterations and apoptosis via fluorescence method. Moreover, the preparation exhibited remarkable anti-diabetic properties owing to the enzyme inhibition of α-amylase and α-glucosidase, indicating its role in controlling postprandial glucose level regulation. It is evident that the combined use of copper oxide nanoparticles, bioactive molecules from *T. terrestris*, and the hydrogel matrix of CS/PVA provides a unique combination for effective delivery of drugs along with biocompatibility. In conclusion, it is seen that the present work emphasizes the feasibility of using environmentally friendly nanocomposite based hydrogels as efficient platforms for cancer treatment and controlled drug release in metabolic disorders. Further research is required to understand the mechanisms of action along with *in vivo* studies and safety considerations in detail.

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ABBREVIATIONS

AO: Acridine Orange; **CS:** Chitosan**; CS/PVA:** Chitosan/ Poly (vinyl alcohol); **CuO:** Copper Oxide; **DMEM:** Dulbecco's Modified Eagle Medium; **EtBr:** Ethidium Bromide; **FBS:** Fetal Bovine Serum; **FTIR:** Fourier Transform Infrared Spectroscopy; **IC_{50}:** Half Maximal Inhibitory Concentration; **MTT:** 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide; **NPs:** Nanoparticles; **PBS:** Phosphate Buffered Saline; PVA: Poly (vinyl alcohol); **ROS:** Reactive Oxygen Species; **TEM:** Transmission Electron Microscopy; **T. terrestris:** *Tribulus terrestris*; **UV:** Ultraviolet; **EDS:** Energy dispersive X-ray spectroscopy; **MW:** Molecular Weight; **v/v:** Volume/volume; **w/v:** Weight/volume; **ANOVA:** Analysis of variance; **SD:** Standard deviation; **EDTA:** Ethylenediaminetetraacetic acid.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

Akshaya Viswanathan: Conceptualization, Methodology, Investigation, Formal Analysis, Visualization, Writing - Original Draft Preparation. Avinash P.V: Methodology, Writing - Review & Editing. Kedari G. S. R: Formal Analysis, Validation, Data Curation, Visualization. Vimal S: Supervision, Conceptualization, Writing - Review & Editing. T. S. Mohamed Saleem: Supervision, Validation. All authors have read and approved the final version of the manuscript and agree to be accountable for all aspects of the work.

SUMMARY

This study successfully developed a green-synthesized multifunctional nanocomposite hydrogel by incorporating *Tribulus terrestris*-mediated Copper Oxide (CuO) nanoparticles into a Chitosan/Poly (Vinyl Alcohol) (CS/PVA) hydrogel matrix. The eco-friendly synthesis approach utilized plant-derived phytochemicals as natural reducing and stabilizing agents for CuO nanoparticle formation. TEM analysis confirmed the successful formation and homogeneous distribution of CuO nanoparticles within the hydrogel, while FTIR characterization demonstrated the presence of functional groups associated with the polymer matrix and bioactive compounds. The nanohydrogel exhibited significant anticancer activity against A549 lung cancer cells, with an IC_{50} value of 8 μg/mL, inducing morphological

changes and apoptosis as confirmed by fluorescence staining. Furthermore, the formulation displayed a biphasic drug release profile with an initial burst release followed by sustained release for 24 h. In addition, the hydrogel showed promising antidiabetic activity through inhibition of α-amylase and α-glucosidase enzymes. These findings suggest that the developed CuO nanoparticle-loaded CS/PVA hydrogel is a promising multifunctional platform for controlled drug delivery, cancer therapy, and diabetes management.

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