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RESEARCH ARTICLE

Tinospora cordifolia - A Future Green Material for Copper Oxide Nanoparticle-based Drug, Reduces the Risk of Diabetes and Cancer

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Abstract

Background: Copper nanoparticles (CuONPs) have garnered significant attention for their unique properties and potential applications in agriculture, medicine, and environmental science. Using eco-friendly methods, the stem extract of *Tinospora cordifolia*, a medicinal plant known for its bioactive compounds, can act as a reducing agent for CuONP synthesis. This approach minimises environmental impact while leveraging the plant's inherent medicinal properties. **Aim:** To synthesise and characterise CuONPs using *T. cordifolia* stem extract and evaluate their agricultural, anti-diabetic, and anticancer applications. **Methods:** Copper nanoparticles were synthesised using *T. cordifolia* stem extract. UV-visible spectroscopy identified the characteristic plasmon resonance peak at 300 nm, while X-Ray Diffraction (XRD) confirmed the crystalline structure of CuONPs. Fourier Transmission Infrared Spectroscopy (FTIR) indicated the stabilization of CuONPs by biomolecules. Scanning Electron Microscopy (SEM) and energy dispersive X-ray (EDX) revealed an approximate size of 100 nm in one dimension. Applications included hydroponic lettuce growth enhancement, anti-diabetic activity (via α-glucosidase inhibition), and anticancer efficacy (via MTT assay on MCF-7 cell lines). **Results:** The CuONPs improved lettuce growth under hydroponics by enhancing moisture content, leaf length, and plant weight at a concentration of 25 mg/L. In biomedical studies, CuONPs exhibited significant anti-diabetic activity with an IC_{50} value of 95.42 µg/mL and potent anticancer activity with an IC_{50} value of 35.51 µg/mL against MCF-7 cells. **Conclusion:** Biogenic CuONPs synthesised using *T. cordifolia* demonstrate promising multifunctional applications. Their eco-friendly synthesis, agricultural benefits, and biomedical efficacy suggest they are a sustainable and versatile nanomaterial for future use.

Major Findings: CuONPs synthesized using *Tinospora cordifolia* stem extract enhanced hydroponic lettuce growth, exhibited anti-diabetic activity (IC_{50}: 95.42 µg/mL), and demonstrated potent anticancer effects (IC_{50}: 35.51 µg/mL) against MCF-7 cells, highlighting their multifunctional potential.

Keywords: Anticancer, Antidiabetic, Copper Nanoparticles, *Tinospora cordifolia*

1. Introduction

Tinospora cordifolia, traditionally used in Indian Ayurveda commonly known as Giloy or Guduchi assures various medicinal features in plant parts. Being indwigenous to the Indian subcontinent and further distinguished by its climbing growth habit having heart-shaped leaves of *T. cordifolia* and easy

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growing ability its further utility always remains the priority in various disciplines. *T. cordifolia* is proven for its immunomodulation, antioxidant capacity, and potential anti-diabetic and anti-cancer effects, providing the rationale for researching *T. cordifolia* as a source for a green copper nano-drug. *T. cordifolia* is positive for the major component as berberine, alkaloids, giloin, and glucosoids. These compounds have many health benefits, such as lowering blood sugar, reducing inflammation, fighting infections, arthritis, spasms, cancer, HIV, leprosy, oxidative stress, allergies, stress, malaria, liver damage, and tumours[1,2]. Therefore, *T. cordifolia* deserves more research potential especially to synthesise bio-nanomaterials[1,3-5].

In recent times use of metal nanomaterials in material science research is gaining new dimensions by being features of minimal size, representing unique properties that make them suitable for numerous applications. Advanced nanotechnology is now utilising green nanoparticles that explore the use of these green nanomaterials in biomedical, catalytic, biosensor, electronic, food, photonic, and other studies[6,7].

Among many oxides- copper oxide is a transition metal oxide with a narrow bandgap and good electrochemical properties. It has high stability in solutions, large surface area and favourable redox potentials and represents its use in biomedical fields[8]. For example, red algae (*Pterocladia capillacea)* synthesised CuO nanoparticles loaded with nedaplatin showed greater anticancer activity[9]. CuO prepared from *Nilgirianthus ciliatus* plant extract able to control breast cancer cell line and lung cancer cell line indicated the parallel use of CuO nanoparticles in nano drug form[10]. The utility of copper oxide nanoparticles finds wide applications in biomedical fields, especially in reducing tumour cell viability by inducing oxidative stress and apoptosis[11-13].

The use of nanoparticles in diabetes has been well documented. Copper-impregnated socks can help diabetic patients prevent skin problems by reducing bacterial and fungal infections, improving blood circulation, and enhancing wound healing[14]. Nanotechnology has enabled the development of various copper oxide nanomaterials, which possess anti-diabetic, anti-cancer and anti-microbial properties. These nanomaterials have made attention and applications in recent years[15-17]. The recent goal is to create a green copper nanoparticle that maximises the bioavailability and targeted delivery of its bioactive compounds while utilising the inherent health benefits of *T. cordifolia*. This strategy aims to boost *T. cordifolia's* therapeutic effects, possibly lowering the risk of diabetes and cancer. Green synthesis techniques are used to reduce toxicity to produce therapeutic products that are safe for the environment and effective for treating diseases.

The present study investigated the potential of CuONPs of *T. cordifolia* for anti-diabetic activity and anticancer features once tested by biological methods confirmed by analytical techniques and later tested by *in vitro* protocols so that its fate in medical biology can be put forward.

2. Materials and Methods

2.1 Extract Preparation

Tinospora cordifolia commonly known as giloy was collected from Nagpur, Maharashtra, India for its stem part. The stems were washed and dried in the shade at ambient temperature (28-35°C). The dried powder (1 g) was used to prepare 1% aqueous extract without boiling the content and further incubated for 24 h. The content was then filtered with muslin cloth and used immediately for nanoparticle synthesis.

2.2 Chemicals

The synthesis of copper oxide nanoparticles made by precursor copper sulphate pentahydrate (Himedia). The 20 mM solution of the $CuSO_4.5H_2O$ was made in sterile distilled water.

2.3 Synthesis of Nanoparticles

The 20mM $CuSO_4.5H_2O$ was mixed with stem extract of *T. cordifolia* (1%) in a 1:1 ratio and by mixing and storing in the umber-coloured bottle. Upon 24 h of incubation at ambient temperature (28°C–35°C), the resultant colour change is an indicator of copper nanoparticle formation has been confirmed. Centrifugation at 15,000 RPM for 30 min was used to pelletise the formed nanoparticles. The pellet was continuously mixed with an ethanol fraction to remove any remaining water content. The preparation was then placed in a 60°C oven for 72 h. The dried nanoparticles were stored in an airtight container for further analysis.

2.4 Characterisation of Nanoparticles

The first confirmation of CuONPs upon synthesis was performed using plasmon resonance in a spectrophotometer by scanning at 200-600 nm. The XRD - X-ray diffraction pattern was also captured using the Braker EcoD8 advance and nickel-filtered CuKa (λ=1.5405 A°) radiation. The average crystalline size (t) was determined by incorporating line broadening and applying Scherrer's relation: t=0.9 λ /ßcos θ, here, the x-ray wavelength is represented by ß is fullwidth of half maximum. Furthermore, using SEM, one glass slide mounted with a powdered sample exposed to capture surface structural details with a resolution of not less than 1 m to 10 nm, the true forms and sizes were documented, followed by EDX analysis. FTIR spectroscopy with KBr pellets Shimazu IR trace 100 revealed a vibration pattern of CuONPs.

2.5 Antidiabetic Activity of Nanoparticles

2.5.1 Alpha–Glucosidase Inhibitor Assay

The following principles are applied in the assay: As demonstrated in (Figure 1), -glucosidase can catalyse the reaction that transforms the substrate 4-nitrophenyl-α–D-glucopyranoside into α–D-glucopyranoside and p-nitrophenol under standard conditions of pH 6.8 and 37°C.

The formation of a yellow colour by p-nitrophenol was then measured at 405 nm. In an assay inhibition of the enzyme α–glucosidase in the presence of an anti-diabetic agent prevents the formation of p-nitrophenol and thereby reduces the yellow colour.

2.5.2 Alpha–Glucosidase Activity Evaluation Mechanism

The effect of CuONPs *T. cordifolia* (TC- CuO NPs) on α – glucosidase activity was determined as per protocol given by Apostolidis[18] using α – glucosidase enzyme. After being synthesised in 100 mM phosphate buffer (pH 6.8), the p-nitrophenyl glucopyranoside (PNPG) was pre-incubated with various doses of CuONPs for 10 min: 10, 20, 40, 80, 160, and 320 µg/ml. Additionally, 400 µl of 5.0 mM (PNPG) was utilised as a substrate and dissolved in 100 mM phosphate buffer (PH 6.8). The ensuing, yellow-coloured reaction mixture, or the amount of 4-nitrophenol released, was measured at 405 nm using a UV-Vis spectrophotometer, after that reaction mixture was incubated at 37°C for 20

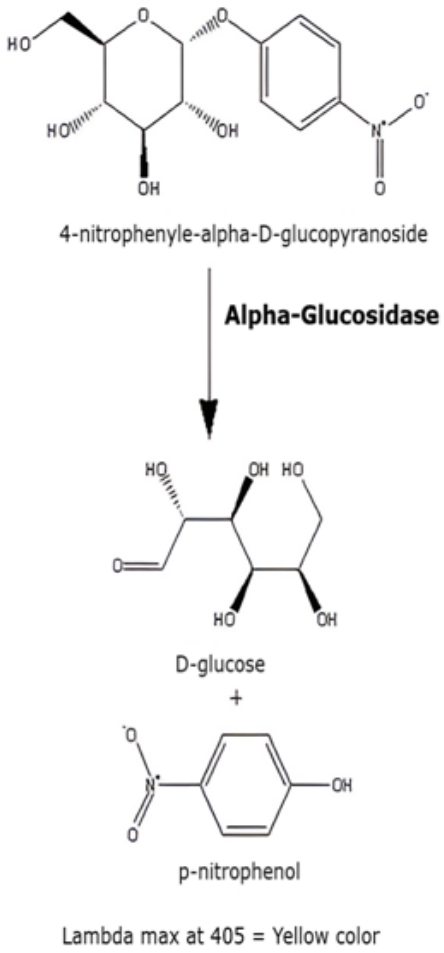


Figure 1. Mechanism of α – glucosidase activity used for assay of α – glucosidase inhibitor.

min. The process was then regulated by adding 1 ml of Na_2CO_3 (0.1M). Voglibose was employed in this study as a positive control, and its inhibitory action on α–glucosidase was also noted, using the formula provided: % Inhibition is calculated as [(Abs Control - Abs Sample) / Abs Control] x 100. The α-glucosidase inhibitor voglibose is widely used to improve postprandial hyperglycemia mainly in Japan. Voglibose is the first oral antidiabetic agent to gain approval in Japan for this indication[19,20].

2.6 Anticancer by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay

2.6.1 Preparation of CuONPs

Tinospora cordifolia CuONPs were two-fold serially diluted in the concentration of 6.25 to 1000 µg/ml using DMSO solvent and tested.

2.6.2 Cell Line and Culture Medium

Epithelial cell line (MCF7) maintained on a modified Dulbecco's modified eagle medium media with 10% FBS, penicillin 100 IU/ml, and streptomycin 100 µg/ml was provided by the cell bank National Centre for Cell Science (NCCS), Pune. The cells were cultured in a CO_2 incubator with 5% CO_2 and a humid environment. The use of Trypsin phosphate versene glucose solution during cell trypsinisation. 0.05% glucose in Phosphate buffer saline, 0.02% EDTA, and 0.2% trypsin are all present in the solution. The viability of the cells was also evaluated. The inclusion of 50,000 cells per well in a 96-well titre plate was carried out. Following that, the plates were placed in an incubator for 24 h at 37°C with 5% CO_2.

2.7 Protocol for Calculating the Growth Inhibition

The obtained monolayer of cell culture was primarily trypsinised and the cell count thereby adjusted to 1.0 x 10^5 cells/ml of media supplemented with 10% Fetal bovine serum and the process continued with 96 well plates. Then each well of the plate seeded with 100 µl of the diluted cell suspension containing 50,000 cells. Upon 24 h of incubation monolayer formed was washed by removing supernatant. After that 100 µl of different concentrations of CuONPs/ Standard drug compound were added to each well and it was incubated in a CO_2 incubator for the period of 24 h. The test solution in each well was separated upon completion of incubation. Then 100 µl of MTT dye with 5mg/10ml of MTT in PBS was added to every well of the microtiter plate and incubated for the next four h in a CO_2 incubator containing 5% CO_2 at 37°C. Upon incubation, the supernatant was decanted and 100 µl of DMSO was added to each well and shaken to solubilise formazan formed in a reaction. The absorbance was recorded using a microplate reader at 570 nm. The percentage of growth inhibition is calculated as per the formula: % viability of cells = (sample absorption / control absorption) x 100

The IC_{50} value of CuONPs and standard drugs was then calculated by using a sigmoid dose-response curve for cytotoxic tests.

2.8 Nanoparticles Treatment to *Lactuca sativa*

In the present study, the effect of different concentrations of CuONPs on lettuce under hydroponics conditions was investigated once those were synthesised via a green approach. The lettuce plantation was carried out in cocopeat till 20 days after germination. Thereafter plants were transformed into the hydroponics system (deep-water culture) and then maintained for adaptability. Upon three days of adaptation with four sets together for conditioning the plants were considered ready for treatment.

2.9 Experimental Groups

In the study, lettuce plants were exposed to copper nanoparticles at three concentrations (25 mg/L, 50 mg/L and 75 mg/L) along with control once kept at two light conditions as 500 lux under artificial light during the complete study.

2.10 Data Recording

In the process of analysis, the possible change in the plant's overall growth was analyzed in terms of 500 lux light and the effect of CuONPs as variable concentrations exposed to the lettuce. Here the statistical analysis was carried out at $p<0.05$ set with one-way ANOVA analysis using graph and prism software.

3. Result and Discussion

3.1 Spectrophotometric Analysis

The successful synthesis of TC-CuONPs was recorded upon 24 h of incubation as per spectrophotometric analysis (Figure 2). As per plasmon resonance, TC-CuONPs were found to be absorbing maximum at 320 nm with an absorbance of 0.971 at 0 h and then it has been recorded with 0.516 absorbance at 300 nm. In addition, control *T. cordifolia* stem extract absorption was noted at 300 nm with an O.D. of 0.812 and pure $CuSO_4$. $5H_2O$ (2mM) at 290 nm with 0.318 absorption. In a related study leaf extract of *Catha edulis*-based Cuo nanoparticles was found to be absorbing at 333nm which is very much like the present study when common $CuSO_4.5H_2O$ was used as a precursor for synthesis[21]. Similarly, *Prunus africana* bark Extract (PAE) green synthesised Copper oxide nanoparticles (CuONPs) by UV vis absorbance spectra registered surface plasmon resonance peaks between 272 and 286 nm, confirming the presence of CuONPs similar to the present study[22]. In a close value for plasmon resonance recorded in the present for CuONPs, *Trichoderma asperellum* has been used to synthesise copper oxide

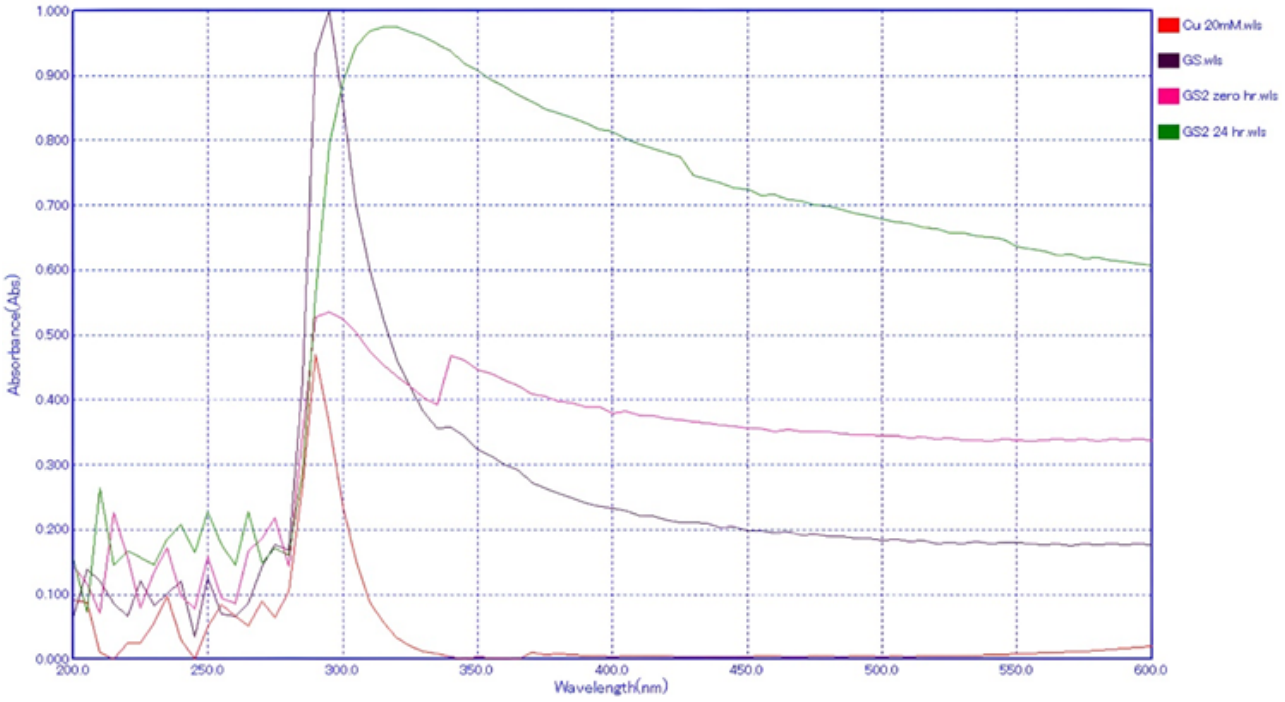


Figure 2. Spectroscopic examination of TC- CuONPs along with pure copper solution (Red), Giloy stems extract (Violet), and 0-hour preparation (Pink) and 24-hour readings (Green) at 200-600nm using absorption data.

nanoparticles giving plasmon resonance of 303 nm[23]. Exhibition of a distinct absorption peak in the region of 238-331 by CuONPs has also been reported by Fawzy *et al.*,[24] when investigating with green synthesis approach.

3.2 XRD Study of Nanoparticles

The crystalline nature of nanoparticles and their corresponding phases were validated by XRD-based measurement. TC-CuONPs measured by XRD diffraction pattern 2θ= 32.254, 35.258, 38.518, 48.463, 53.264, 53.419, 58.176, 58.079, 61.165, 61.272, 61.363, 65.363, 65.758, 65.959, 67.898, 72.133, 74.944 and 75.104 were assigned to 307, 2341, 2755, 632, 213, 211, 270, 279, 338, 341, 351, 350, 630, 462, 172, 181 and 231 net intensities, respectively of monoclinic CuONPs (Figure 3). Therefore, after CuONPs were synthesised from *T. cordifolia* and considered for previous reports, it was clear from the XRD spectrum that they had a crystalline character[25,26]. According to Balaji *et al.*,[27] *Padina boergesenii*-mediated copper oxide nanoparticles represent the typical crystalline nature once confirmed from XRD. Hu *et al.*,[28] also reported *Alpinia officinarum*-mediated copper oxide nanoparticles give high crystallinity and purity with spherical morphology with a mean size of 46.40. It is not always that typical crystalline nature is the feature of CuONps since the cubic face-centred structure of the TA-CuO NPs also been reported when synthesised using Indigenous fungi as stated by Saravanakumar *et al*[29].

3.3 FTIR Analysis

Tinospora cordifolia-mediated CuONPs detected using FTIR and discovered to have a large number of functional groups and chemical bonds are displayed in (Figure 4). The stretching bond of the O atom is related to the promising peaks at 451, 569, and 1060 cm^{-1} [30,31]. The simple transfer of the stretching bond from the tetrahedral space to the octahedral position may be the cause of the weak peak transfer of 451 cm^{-1} to additional regions of 569 and 642 cm^{-1} [32,33]. The 3664 cm^{-1} stretching vibration indicated the presence of the -OH group. As a result, it has been determined that plants were crucial to the production of CuONPs[34]. It must be assumed that the prominent peaks measured at 3664, 1708, 1543, and 1060 cm^{-1} are connected to produce copper nanoparticles, possibly by proteins or polyphenols. Thus, it has been demonstrated that these

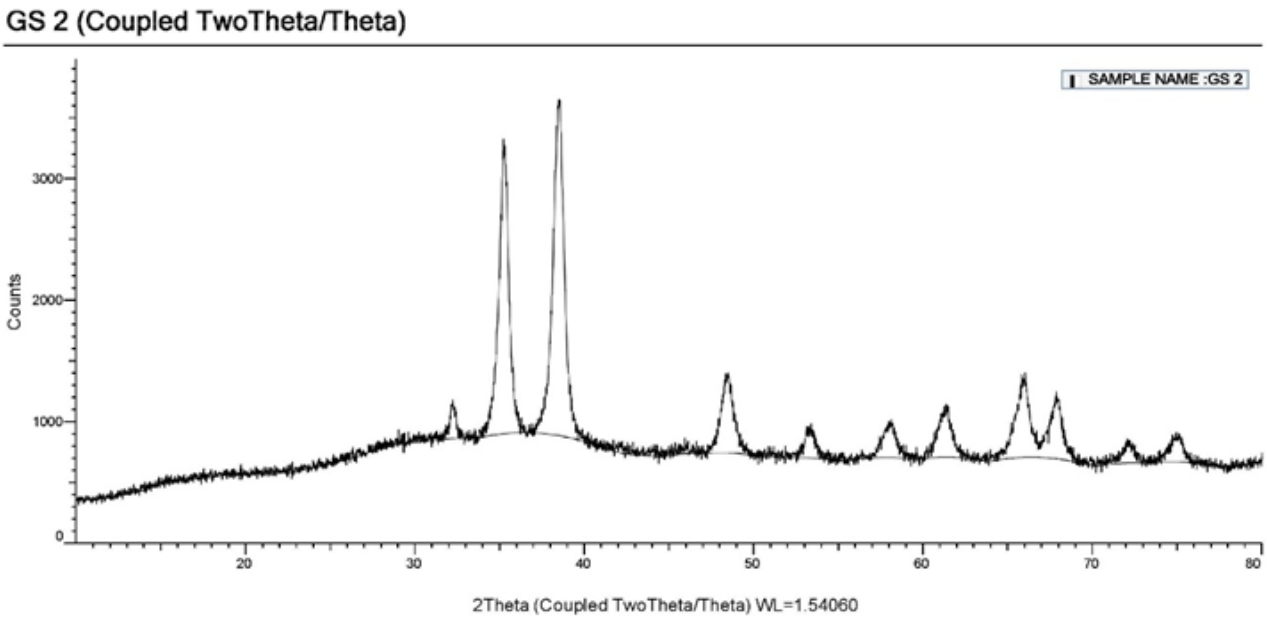


Figure 3. XRD spectrum of copper oxide nanoparticles prepared from *Tinospora cordifolia*.

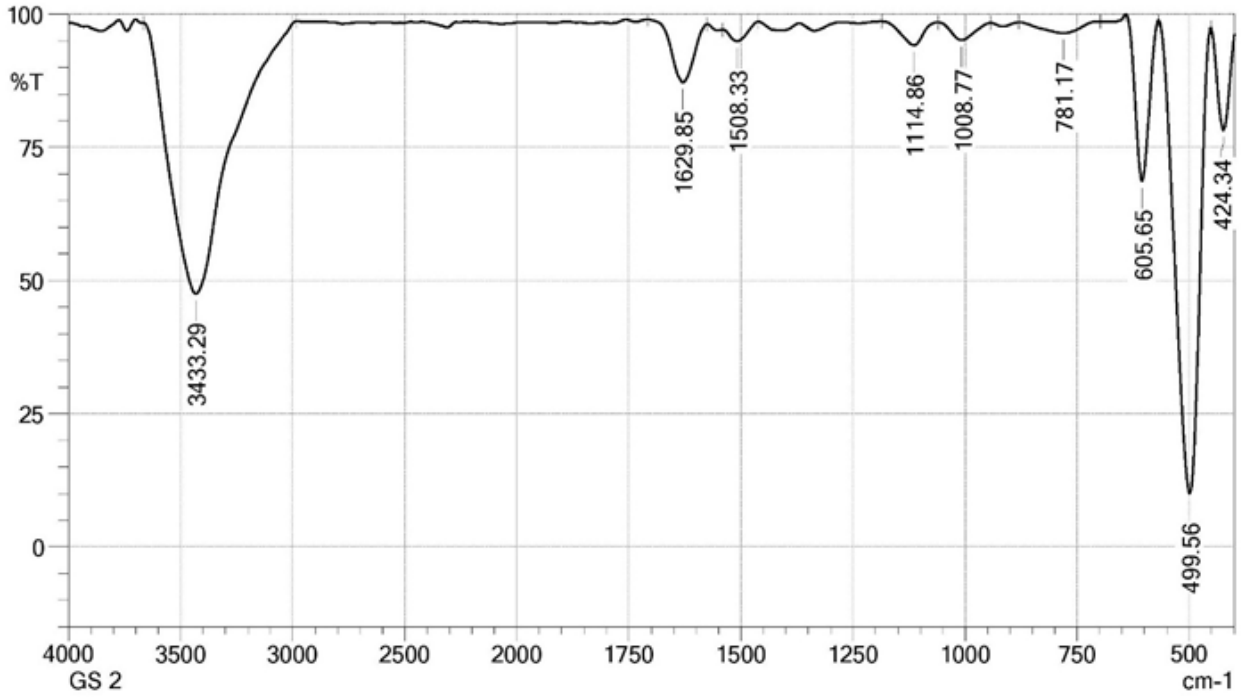


Figure 4. FTIR absorption spectra of copper oxide nanoparticles prepared from *Tinospora cordifolia*.

biomolecules cause the stabilisation of nanoparticles as previously seen[35-37]. According to Pérez-Alvarez *et al.*,[38] stretching of 499.56 cm^{-1} corresponds to CuNps via cotton which is also in agreement with the present study also where we have received a peak which confirms the synthesis of nanoparticles.

3.4 SEM and EDX Analysis

As seen in (Figure 5), the TS-CuONPs were examined to be amorphous in form and were subsequently found to have aggregated. According to Indhira *et al.*,[39] formations of CuO NPs by plants like *Elaeagnus indica* leaf extract can produce the formation of aggregated

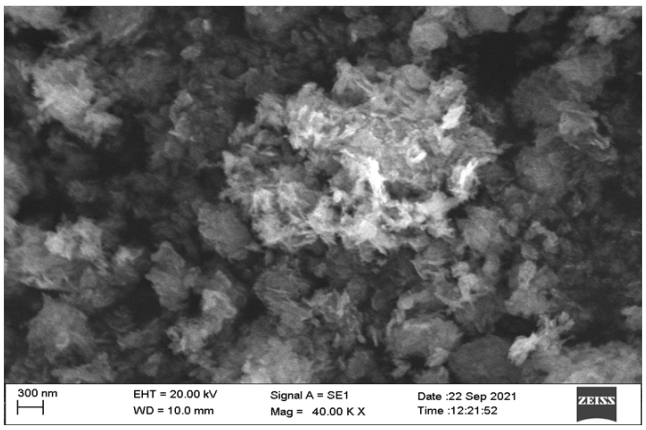
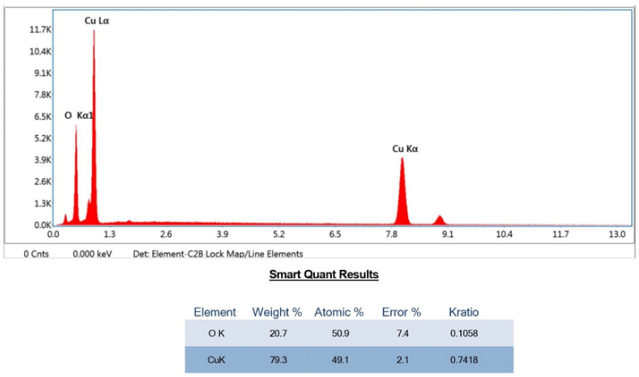


Figure 5. SEM of *Tinospora cordifolia* reduced CuONPs at 300 nm scale resolution.



Smart Quant Results

Element	Weight %	Atomic %	Error %	Kratio
O K	20.7	50.9	7.4	0.1058
CuK	79.3	49.1	2.1	0.7418

Figure 6. *Tinospora cordifolia* reduced copper nanoparticles' EDX spectra were captured while copper and oxygen atoms were present.

CuO NPs with phytochemicals acting as reducing and stabilizing agents which confirms that the present study also represents an aggregation of TC- CuONPs with plant extract.

However, the EDX analysis demonstrated elemental copper nanoparticles with the Kα line at 7.8 and Lα line at 0.08 (copper X-ray energies) as shown in (Figure 6). Here, Kα1 has a 0.05 linkage to oxygen. These substances are frequently identified with phytochemicals that are high in copper and are still enclosed in the nanomaterial. The lack of contaminants in the sample is confirmed by the peak pattern as previously mentioned by Das *et al.,*[40] and confirmed by SEM examination, nanoparticles were found to be smaller than 100 nm in at least one dimension, which further reinforces the findings of our study.

3.5 Anti-cancer Activity

As per the MTT assay MCF7 cell line was found to be growing prominently with DMEM medium supplemented with fetal bovine serum. Here as per standard drugs (Cisplatin) when tested with 6.25–100 µg/ml. Its IC_{50} value was recorded as 4.53 µg/ml ($p<0.01$) (Figures 7 and 8) (Table 1). In comparison to standard, experimental CuONPs of *T. cordifolia* reported with higher IC_{50} value of 35.51 µg/ml ($p<0.01$) with a significant difference. As per previous research IC_{50} value of CuONPs on kidney cell line recorded as 65.5 µg/ml which is attributed to be oxidative stress-based inhibition Reddy and Lonkala[41] which indicates that TC-CuONPs studied in the present research able to control the cancer cell growth better way at the lower dose concentration. In another study CuONPs ranging from 50-60 nm also noted with IC_{50} rates of 16.4 µg/ml Al-zharani *et al.,*[42] which suggests that the smaller the nanoparticles better are the MTT IC_{50} values as with our 100nm CuONPs IC_{50} achieved as 35.51 µg/ml. According to Kolahalam *et al.,*[43] bio-inspired copper oxide nanoparticles noted with IC_{50} value as low as 4.14 µg/ml and equivalent to Cisplastin (standard) drug tested in our study which showcased the success of the copper nanoparticles in anticancer activity.

3.6 Antidiabetic Assay

Diabetes mellitus is a chronic, complex metabolic disorder that is becoming more severe every day[44]. To report better-diabetes-controlling enzyme Alpha (α) glucosidase (GAA) highlighted its effectiveness as the given enzyme can convert oligosaccharides and disaccharides to glucose and the resultant monosaccharides are absorbed by the small intestine, which elevates final blood glucose level[45]. Therefore, GAA is used as a marker enzyme for the prevention and treatment of type 2 Diabetes[46]. In search of a better α – glucosidase inhibitor which can control post-prandial blood glucose levels in diabetic patients by keeping the blood level up to normal by showing delayed mechanisms of digestion of carbohydrates and lowering the absorption of monosaccharides, rigorous research is going on[47].

As per α – glucosidase inhibitor assay inhibition of α – glucosidase by a promising drug with minimum IC_{50} value could be promising. Here in a result mean value of % inhibition by CuONPs *T. cordifolia* (GS)

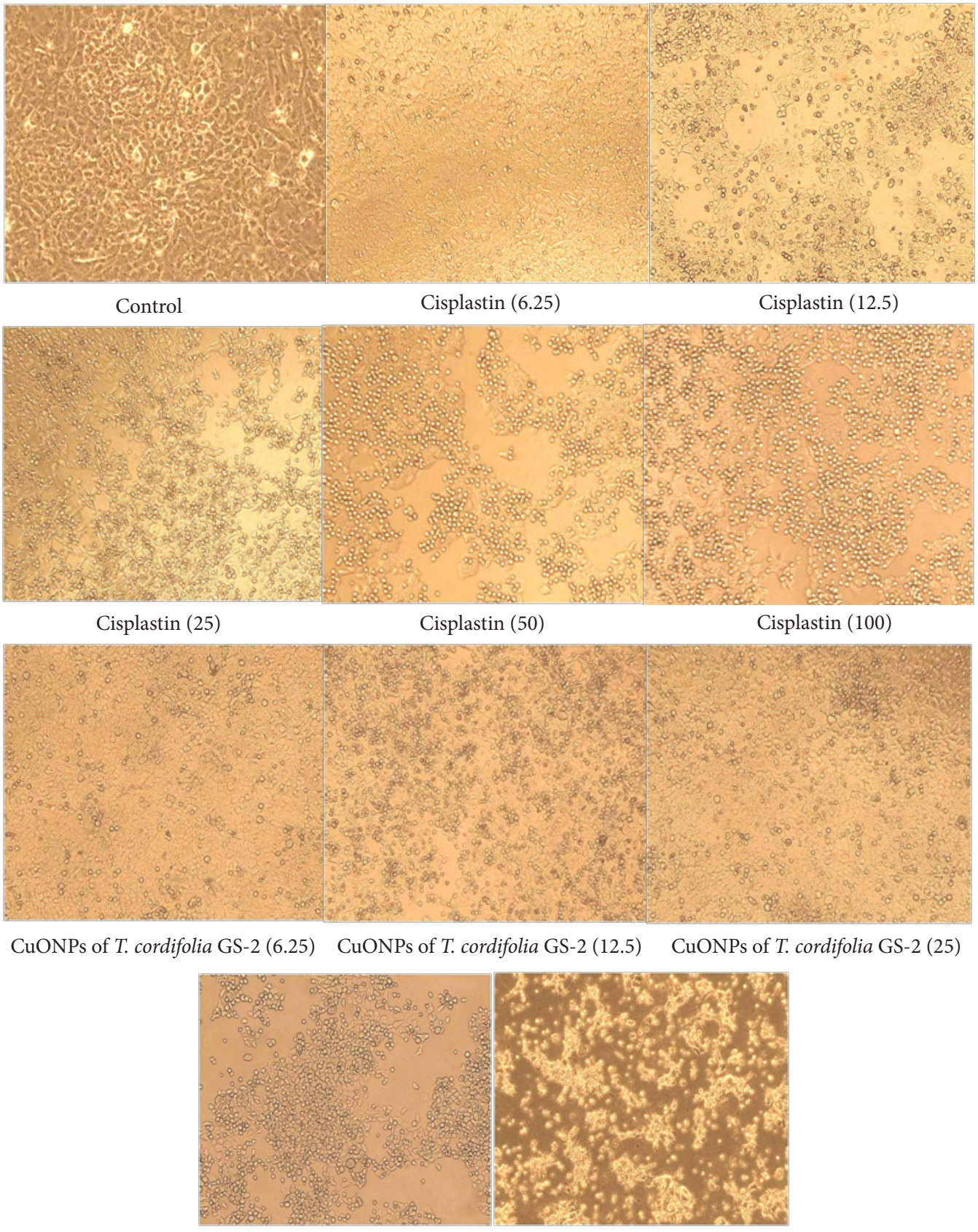


Figure 7. MTT assay on MCF7 cell line of standard drug (Cisplastin) and of CuONPs of *T. cordifolia*.

and voglibose was recorded for 10-320 µg/ml. (Table 2) (Figures 9, 10). As per linear regression, the IC_{50} value of CuONPs of *T. cordifolia* was recorded to be 95.42 µg/ml ($p<0.01$) and that of voglibose recorded as 28.64 µg/ml ($p<0.05$) with a significant difference in activity. In the previous studies, the aqueous leaf extract of *Cocculus hirsutus* synthesised copper nanoparticle possess α – glucosidase inhibitor assay value of 68.5 µg/ml which is found to be close to our nanoparticles also[48]. In the previous study quenching effect of 4-nitrophenol on 1-dodecanethiol – capped Cu nano-clusters was found to be suitable for screening α – glucosidase inhibitors with high sensitivity and reported to use in the study for antidiabetic assay[49]. The use of copper nanoclusters for α – glucosidase activity was recorded with a detection limit of 0.43 U/L and its developed ultra-sensitive fluorescence sensing approach facilitates easy screening of α – glucosidase inhibitors[50]. Overall study put forward that the use of *T. cordifolia* reduced CuONPs proving to be useful to control cancer growth as well as to reduce type 2 diabetes under *in vitro* studies and hence can be recommended for future studies.

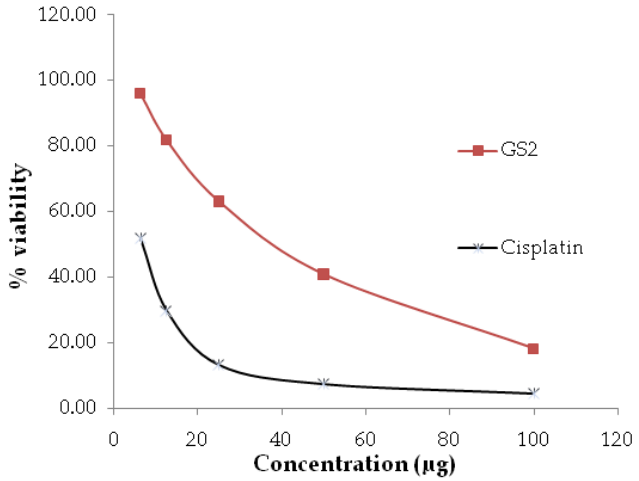


Figure 8. Comparative trend between the cytotoxic effects of CuONPs of *T. cordifolia* (code: GS2) and the reference anticancer drug (cisplastin).

Having a typical size of 63.46 nm. Later, the synthesised CuNPs efficiency was evaluated against bacterial pathogens and was found highly toxic to B. subtilis and S. aureus strains. The synthesised CuNPs were examined through H_2O_2 and PMA assays which demonstrated the highest free radical scavenging activity. Besides, the resulting CuNPs revealed higher anti-diabetic efficacy in both the [Formula: see text]-amylase and [Formula: see text] -glucosidase inhibition assays (64.5% ± 0.11 and 68.5% ± 0.11, respectively). Finally, our findings report that *C. hirsutus* can be exploited as a source for green synthesis of CuNPs, having potent *in vitro* antioxidant, antibacterial, and anti-diabetic properties.

3.7 CuONP Effect on Lettuce Plant Growth

The study put forward the effect of the overall changes in the plant growth once lettuce was exposed to nanoparticles and data was recorded as follows for any significant change:

Table 1. Percent inhibition of MCF cell line as per dose concentration

Standard	Conc. (µg)	Singlet OD	Duplicate OD	Triplicate OD	Mean OD	Mean OD – Blank Mean OD	SD	% Viability
Cisplatin	6.25	1.059	1.052	1.055	1.055	1.035	0.004	51.66
	12.5	0.611	0.620	0.616	0.616	0.595	0.005	29.71
	25	0.289	0.285	0.282	0.285	0.265	0.004	13.23
	50	0.166	0.173	0.170	0.170	0.149	0.004	7.45
	100	0.113	0.115	0.107	0.112	0.091	0.004	4.56
CuONPs of *T. cordifolia* (code: GS2)	6.25	1.946	1.954	1.928	1.943	1.922	0.013	95.94
	12.5	1.688	1.656	1.632	1.659	1.638	0.028	81.77
	25	1.293	1.276	1.282	1.284	1.263	0.009	63.05
	50	0.852	0.834	0.829	0.838	0.818	0.012	40.83
	100	0.378	0.384	0.393	0.385	0.365	0.008	18.20

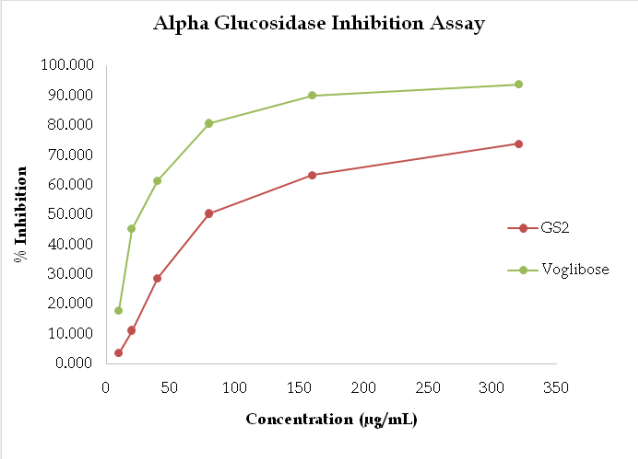


Figure 9. Comparative % inhibition of alpha glucosidase recorded with CuONPs of *T. cordifolia* (code: GS2) and Voglibose standard control.

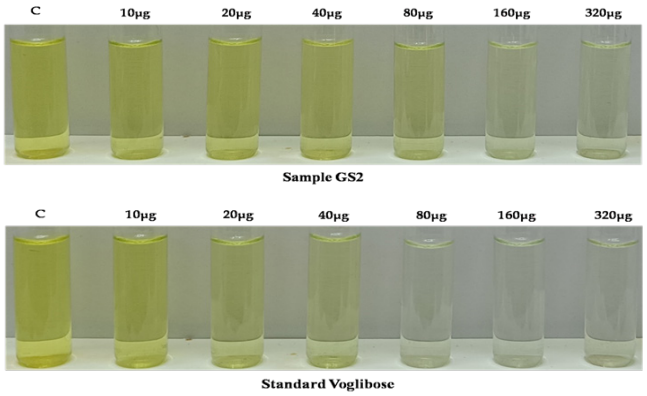


Figure 10. Alpha glucosidase activity of standard voglibose and CuONPs of *T. cordifolia* (code: GS2).

Table 2. Comparative % inhibition of alpha-glucosidase of CuONPs of *T. cordifolia* (code: GS2)

Samples	Conc.(µg)	Singlet	Duplicate	Triplicate	Mean	SD
Voglibose	10	18.836	17.936	17.397	18.056	0.727
	20	45.471	45.831	44.751	45.351	0.550
	40	61.488	62.028	61.128	61.548	0.453
	80	81.464	80.204	80.564	80.744	0.649
	160	90.102	89.562	90.462	90.042	0.453
	320	94.241	93.161	93.881	93.761	0.550
CuONPs of *T. cordifolia* (code: GS2)	**Conc.(µg)**	**Singlet**	**Duplicate**	**Triplicate**	**Mean**	**SD**
	10	3.899	2.819	4.259	3.659	0.749
	20	11.638	11.638	10.198	11.158	0.831
	40	28.374	28.374	29.634	28.794	0.727
	80	50.690	50.690	50.330	50.570	0.208
	160	63.827	63.827	62.567	63.407	0.727
	320	73.545	73.545	74.625	73.905	0.623

3.7.1 Wet Weight of Leaves

Upon 53 days of exposure to the variable concentration of CuONPs of *T. cordifolia,* the overall change of the leaves' weight in moisture-containing conditions was recorded. Here it has been observed that the control set recorded with weight 0.52 ± 0.26 g while with 25, 50 and 75 mg/L CuONPs treated group, it has improved as 1.6 ± 0.23 g, 2.0 ± 0.52 g and 1.9 ± 0.38 g, respectively. According to statistics, a significant increase ($p<0.0001$) was recorded once all treated groups compared with the control set as shown in (Table 3) and (Figures 11 and 12) and further no significant change was recorded among treated groups indicating that concentration i.e. 25 mg/L is sufficient to bring about an increase in weight and not needed with supplementation of a higher dose.

3.7.2 Dry Weight of Leaves

Upon 53 days of exposure to CuONPs, the leaves were dried for 4 days under controlled conditions and their dry weight was recorded. Here in the control set the leaves' dry weight was 0.12 ± 0.07 g, while for 25, 50

Table 3. Comparative change in the wet weight of leaves upon day 53 of lettuce treated with CuONPs (mg/lit) under 500 lux

	Control	25mg	50mg	75mg
Mean±Std. deviation	0.52±0.26a	1.6±0.23be	2.0±0.52ce	1.9±0.38de

The same alphabet in superscript in a row indicates the data is non-significant and vice versa.

Table 4. Comparative change in the dry weight of leaves upon day 53 of lettuce treated with CuONPs (mg/lit) under 500 lux

	Control	25mg	50mg	75mg
Mean ± Std. deviation	0.12±0.07a	0.12±0.04a	0.10±0.04a	0.12±0.05a

The same alphabet in superscript in a row indicates the data is non-significant and vice versa.

Table 5. Comparative change in the leaf length of leaves upon day 53 of lettuce treated with CuONPs (mg/lit) under 500 lux

	Control	25mg	50mg	75mg
Mean±Std. deviation	10±2.9^{a}	16±2.4^{be}	15±0.5^{ce}	15±1.6^{de}

The same alphabet in superscript in a row indicates the data is non-significant and vice versa.



Figure 11. The lettuce growth showcased upon treatment with 25, 50 and 75 mg/lit of CuONps of *T. cordifolia* and control (Left to Right).

and 75 mg/L CuONPs sets, it was 0.12 ± 0.04 g, 0.10 ± 0.04 g and 0.12 ± 0.05 g, respectively. This statistical analysis indicated a non-significant change ($p>0.05$) among the control set and treated groups as well as among the treated group as shown in (Table 4) and (Figures 11 and 12).

3.7.3 Leaf Length

The lettuce plant once treated with CuONPs the positive effect or increase in leaf length was found to be realised. Here in a control set the 53-day-old leaf length was 10 ± 2.9 cm while with 25, 50 and 75 mg/L. it has improved significantly ($p<0.05$) once compared to control with

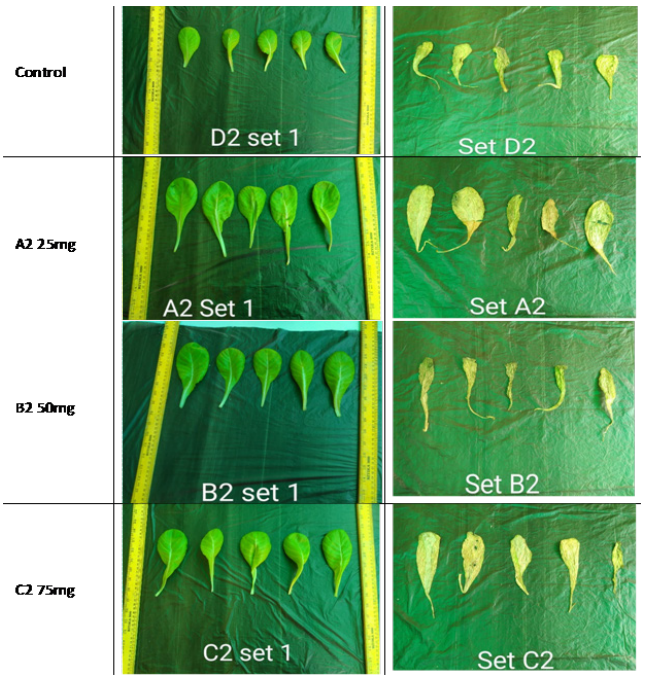


Figure 12. The lettuce leaf length and its weight and dry state showcased upon treatment with 25, 50 and 75 mg/lit and control of CuONps of *T. cordifolia*.

mean values as 16 ± 2.4 cm, 15 ± 0.5 cm and 15 ± 1.6 cm as shown in (Table 5) and (Figures 11 and 12).

In the present study it has been observed that lettuce once exposed to CuONPs imparts a positive effect on

the overall plant growth of lettuce especially found to increase their moisture contents and thereby the overall weight of lettuce leaves found to be improved. A similar trend recorded earlier by many researchers as Pelegrino *et al.*,[51] mentioned CuONPs especially of biosynthesised type at ≤ 20 µg/ml concentration found to be beneficial for seeding growth, radical growth and enhanced overall plant growth, especially with lettuce (*Lactuca sativa*). Shang *et al.*,[52] informed that lettuce is prone to get infected with *Fusarium oxysporum* sp. *Lactucae* which can be better controlled by CuONPs embedded hydrogels. Here treated plants can improve biomass significantly by at least 27% to a maximum of 40.5%. According to Xiong *et al.*,[53] CuONPs impart positive as well as negative responses and its treatment on lettuce imparts variable gene expression profiling. The genes of the transporter family, Heavy metal–associated Isoprenylated Plant Proteins (HIPPS) and endocytosis-related plant proteins are found to be responsive. Overall study put forward that these green synthesised nanoparticles were better for the plants, especially of copper oxide type at a given concentration.

4. Conclusion

The ability of plants to reduce $CuSO_4.5H_2O$ into promising nanoparticles which are amorphous 100 nm sized found to be effective to be antidiabetic and anticancer. In the study, it is also observed that the use of CuONPs was found to be promising to increase the moisture content along with leaf length in the lettuce plant and thus found to be imparting positive responses in the plant growth and thus finds utility in agriculture. In the study by using various analytical techniques, it has been confirmed that CuONPs were formed successfully and their purity and integrity make them stable nanomaterials useful in the medical field. The *in vitro* assay by alpha-glucosidase inhibition and by MTT proved that the formed CuONPs are promising nano drugs which can be optimised if size-based efficacy is investigated.

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6. Author Contribution

Kundan Kumar: Investigation, analysis and writing; Ravi Kant Singh, Dilip Gore: Validation, review and editing; Pankaj Kumar Tyagi: Review and editing; Varaprasad Kolla and Ravikant Singh: Final review and editing. All authors have read and agreed to the published version of the manuscript.

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