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Research Article

Biogenic Fabrication of CuO Nanoparticles by *Oxalis corniculata L.* to Evaluate Antibacterial and Hypoglycemic Activity on Diabetic Mice

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ABSTRACT

Plant-based synthesis techniques of nanoparticles are interesting because of their cheap production cost, non-toxic nature and eco-friendliness. Metal oxide nanomaterials combined with plant metabolites have synergistic effects on antibacterial and antidiabetic potential. Biogenic fabricated nanoparticles of copper oxide have been accomplished with *Oxalis corniculata* L. leaf extract. For the characterization of nanomaterial X-ray diffraction (XRD), UV-visible spectroscopy and fourier-transform infrared (FTIR) spectroscopy were used. The size and morphology of the NPs were measured using a field-emission scanning electron microscope (FESEM) and high-resolution transmission electron microscopy (HRTEM). The antibacterial potential of synthesized CuO NP has been studied upon gram (+ve) *Staphylococcus aureus* and gram (-ve) *Escherichia coli* bacteria. The ameliorative action of CuO NPs was tested against streptozotocin-induced diabetes in Swiss albino mice. Synthesized CuO NPs were well crystalline and 20 to 36 nm-sized spherical particles. A strong peak at A_{298} using UV-vis was verified the synthesis of CuO NP. Synthesized nanomaterial exhibits satisfactory antibacterial efficacy on both bacterial strains. Data from biochemical, inflammatory and non-inflammatory cytokine profiles of the mice justify its ameliorative action and mode of antidiabetic activity on Swiss albino mice.

INTRODUCTION

In recent years, metal oxide nanoparticles (NPs) gained significant interest in biological applications like antibiotics, drug delivery, anti-inflammatory, antidiabetic, anti-hyperbilirubinemia, and anticancer therapy along with its conventional industrial applications as in photonics, nanodevices, data storage, electronics, sensors and catalysis.[1-3] Copper oxide nanoparticles (CuO NPs) are considered as potent metal oxides for their various beneficial aspects as well as increased surface/volume ratio, surface modification capabilities, superconductivity and electron correlation properties.[4,5] All living beings require a trace amount of copper (Cu). An average adult human requires about 10 mg day $^{-1}$ copper for normal metabolic processes.[6] Dietary sources have to satisfy the requirements to maintain equilibrium. Copper is a key cofactor for many enzymes. Copper-containing enzymes provide a wide range of activities, including antioxidant activity, involvement in the electron transport chain (ETS), and biosynthesis of melanin pigment. Furthermore, copper is a component of ceruloplasmin, the enzyme that catalyzes the oxidation of ferrous to ferric for incorporation into transferrin; hence, the lack of ceruloplasmin causes iron buildup in the liver, comparable to hemochromatosis.[7] Copper is a necessary component of SOD enzymes and is more actively involved in antioxidant reactions than other trace metals.[8] Copper oxide nanoparticle (CuO NP) is a copper compound with several uses. Ancient civilizations in Greece, Rome and others utilized copper or copper-based compounds to cure burns, intestinal worms and bacterial infections in the ears, as well as for general hygiene.[9] Bio-synthesized CuO NP exhibits larvicidal, antibacterial, antifungal and anti-inflammatory potency.[4,10-12] Biosynthesis of CuO NPs has been utilized by several plants such as *Acalypha indica, Thymus vulgaris, Ruellia*

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tuberosa, Carica papaya and *Acanthospermum hispidum*.[13] CuO NPs synthesized using *Moringa oleifera* express very effective antibacterial potential against pathogenic bacterial strains *viz. Bacillus cereus, B. subtilis, Klebsiella pneumoniae, S. haemolyticus, E. coli, Enterobacter aerogenes* and *Salmonella enterica*.[14,15]

In-vivo application of *Bacopa monnieri*-mediated CuO NPs showed a significant decrease in serum glucose levels.[16] Biogenic fabricated CuO and Ag/CuO nanocomposite using Indian curry leaves and ginger showed excellent efficacy in restoring altered enzymes in diabetes *in-vitro*.[17]

Oxalis corniculata L. (Family: Oxalidaceae) or creeping woodsorrel, is an edible herb that has a sourish taste, grown wildly in moist climates.[18] This plant is commonly used in Ayurvedic medicine to balance *vata* and *kapha*. It also has anti-inflammatory, digesting, diuretic, antibacterial, antiseptic, and antidiabetic properties and is used to treat cardiovascular diseases.[19,20] It is one of the sanative herbs against hepatic injury-causing diabetes.[21] Several secondary metabolites for example, tartaric acid, citric acids, flavones, glycoflavones, flavonols and phenolic acid present in this herb, can potentially act as reducing cum stabilizing agents.[22,23]

In this research work leaf extract of *O. corniculata* was utilized as a capping-cum-reducing material for biogenic synthesis of CuO NP. XRD, UV-vis, FTIR, FESEM and HRTEM performed the characterization of particles. Pharmaceutical advantages of the synthesized nanoparticle coupled with secondary metabolites of *O. corniculata* were explored *in-vitro* on *S. aureus* and *E. coli* bacteria for bactericidal potential. *In-vivo* antidiabetic efficacy was tested on streptozotocin-induced diabetic Swiss albino mice. Biochemical and haematological parameters measured cytotoxicity associated with diabetes. Results were compared to investigate the pharmaceutical superiority of biogenic CuO NPs over raw plant extract of *O. corniculata*.

MATERIALS AND METHODS

Materials

Fresh *O. corniculata L.* was collected from the Sreegopal Banerjee College Campus (Fig. 1). Copper (II) sulphate (Sigma Aldrich, India), KOH, streptozotocin (STZ) and other chemical materials utilized in this study were procured from Merck (India) and used without additional purification. *E. coli DH5α* (MTCC 1652) and *S. aureus* (MTCC 96) bacteria were procured from IMTECH, Chandigarh, India. About 20 to 25 days old male Swiss albino mice (32 ± 5 g) were collected from a CPCSEA-registered animal house.

Methods

Plant extract preparation

Freshly harvested leaves (10 gm) of *Oxalis corniculata* L. were washed properly. These leaves were subsequently heated in 100 mL of water for 30 minutes at 80°C. The extract was filtrated and stored at 4°C.

Biogenic fabrication of CuO NP

CuO NPs were synthesized by mixing copper (II) sulfate to plant extract, following the standard method reported earlier.[24] Briefly, 100 mL aqueous copper sulfate solution (0.01 M) was mixed with 25 mL of plant extract, stirring continuously at 50°C for 2 hours. This reaction mixture was kept for settle down the precipitate. After centrifugation at 10,000 rpm, the precipitate was rinsed thrice with distilled water. The purified sample was dried at 60°C for 24 hours.

Characterization of CuO NP

CuO NP formation was primarily detected by visual insight into the precursor's color shift. UV-visible spectroscopy (λ25, Perkin Elmer, Germany) was used to characterize the nanostructures. The XRD of these particles has been recorded in a powder XRD (D8, Bruker Axs, Germany) using nickel filtered Cu-Kα radiation operating under a voltage of 40 KV (2θ from 25°–80°). FTIR spectroscopy of the KBr plate containing about 1% CuO NP was utilized for the determination of molecular groups attached to biogenic CuO NPs in JASCO FTIR instrument 410, USA. FESEM (Inspect 50, FEI, Netherland) and HRTEM (JEM-2100 HRTEM, JEOL, Japan) were used to inspect the morphology and size of CuO nanomaterials.

Antibacterial Activity Study

Antimicrobial activity of biogenic fabricated CuO NP was carried out against gram -ve *E. coli* DH5α and gram +ve *S. aureus*. The standard technique was used to study the interaction between NPs and bacteria. 0 to 400 µg mL^{-1} of biogenic CuO NP powder was introduced to bacterial suspension (10^7 CFU/mL) in standard nutrient broth and incubated overnight. Individual colonies were recorded as colony-forming units on nutrient agar plates inoculating 20 µL of the treated culture after 24 hours. Standard techniques were followed to measure MIC and MBC.[4]

Viability assay

Methylthialazole tetrazolium (MTT) has been utilized to test bacterial viability. The overnight growing bacterial suspension culture was washed with PBS (pH 7.4) and separated by centrifuging at 7500 rpm. About 15 mg of biogenic NPs was added to the 10000 CFU mL^{-1} bacterial cells and incubated for 4 hours. The cultures were then cleaned and suspended in phosphate buffered saline. MTT Stock solution was added in a 1:10 dilution, additionally, the cells were cultured for 1-hour. After centrifugation (10,000 rpm), the precipitates were mixed with 0.5 mL of DMSO and measured at A_{540} nm.[4]

FESEM analysis of bacterial samples

Morphological analysis of bacterial samples using FESEM was done following standard protocol.[2] Briefly, biogenic

CuO NP was mixed with bacterial growth mediums containing equal cell density in the mid-exponential phase at 37°C for 6 hours. The bacteria were rinsed properly and fixed with glutaraldehyde (2%) and transferred to silicon supports (Plano, Wetzlar, Germany). The sample was dehydrated in ethanol and then stained with an ethanolic solution of 3% uranyl acetate. Samples were cleaned with 100 mM PBS (pH 7.2) and analyzed employing a FESEM.

Antihyperglycemic study

Five groups (n=6) of mice were kept under typical laboratory conditions (with a day/night cycle of 12 h/12 hours at 25 ± 2°C). Water and food were freely given to the mice. The tests were conducted following CPCSEA guidelines with IAEC permission (Appr. No. RCB/IAEC/MBIHS/67 dated 29.01.2024). Mice were given an intraperitoneal dose of STZ (0.1 g kg^{-1} body weight) to induce hyperglycemia.[2] Mice were provided with a high glycemic index diet and mice with FBS higher (≥ 250 mg dl^{-1}) have been designated as diabetic. Every day, blood sugar is recorded from tail vein using a kit (AccuCheck, Germany). Animals in control and auto recovery were administered a 0.5 mL subcutaneous injection of saline water. Diabetic mice from herbal control, CuO NP treated and positive control groups received subcutaneous injections of plant extract, CuO NP and metformin, respectively. The studies lasted for 4 weeks. The mice were starved overnight, anesthetized with ether and executed via cervical dislocation. For hematological studies, blood was drawn from the heart after execution and kept in containers having anticoagulants (heparin). The serum has been extracted from the blood and kept at -20°C. The pancreas was dissected for quantification of cytokines and preserved in a desiccator. A summary of the treatment protocol is briefly described in Table 1.

Estimation of cytokine profile

The quantification inflammatory (TNF- α, IL-6 and IL-1β) and non-inflammatory (IL-4 and IL-10) cytokines have been assessed with the help of Quantikine Immunoassay Kit (Minneapolis, USA) from the pancreas following standard instructions.

Biochemical estimation

The blood used in biochemical studies was drawn from the retro-orbital plexus in sterile tubes and permitted to coagulate for 45 minutes. The serum sample has been collected after centrifugation at 6000 rpm. Quantification of liver function enzymes (ALT, ALP, AST and GGT) and total protein were used to evaluate liver damage.[25]

Table 1: Treatments (kg^{-1}) for hypoglycemic activity

Group	*Treatment with STZ (g)*	*Drug administration*
Control (I)	Nil	Nil
Auto recovery (II)	0.1	Nil
Herb control (III)	0.1	0.025 g of the herb extract
CuO NP (IV)	0.1	5 mg of the CuO NP
Positive control (V)	0.1	5 mg of the metformin

Hematological study

For hematological studies, blood cells, hemoglobin, Mean corpuscular volume, hematocrit and mean corpuscular hemoglobin were measured from blood stored in a heparin-zed tube.[26]

Statistical Data Analysis

Every quantifiable data was presented as mean ± SD. ANOVA was utilized for data comparison in GraphPad Prism (California, USA).

Result and Discussion

Characterizations of Biogenic Fabricated CuO NP

The XRD technique was used to confirm that the biogenic fabricated CuO NPs produced are pure copper oxide nanoparticles. Fig. 2A displays all copper oxide nanoparticle peaks, indicating the crystalline form. 2θ values of biogenic CuO NPs and their corresponding planes coincided with previously published works of Bala *et*

Fig. 1: Image of *Oxalis corniculata L.* plant with a flowering twig (inset)

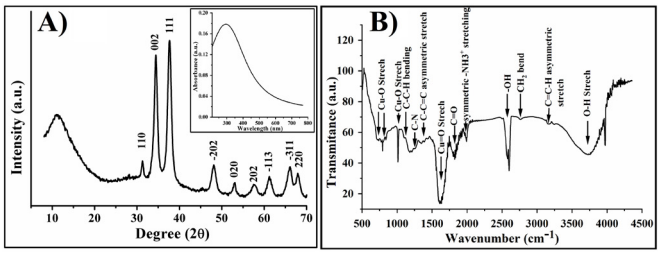

Fig. 2: A) XRD crystallography of biogenic fabricated CuO NP, UV-vis spectra of the same in inset, B) FTIR spectra of CuO NP showing presence of biomolecules of *O. carniculata*

al., 2015.[4] All the monoclinal phases of synthesized NP correspond with JCPDS file no 80-1917. UV-vis spectrum of synthesized NP shows (Fig. 2A inset) a characteristic peak at 298 nm that corresponds to the absorption spectrum of CuO NPs.[27] Many studies on the synthesis of CuO nanomaterials have yielded a combination of CuO and Cu_2O nanostructure.[28,29] In the given condition, the results indicated the production of predominantly CuO phase.

The potential role of bioactive compounds in *O. carniculata* that reduce Cu^{+2} ions and capping agents of CuO NPs was investigated employing FTIR spectroscopy. Fig. 2B displayed infrared transmittance bands at 675, 784 and 1047 cm^{-1} corresponding to Cu-O stretch.[4,30] The characteristic C=O stretch was recorded at 1630 cm^{-1}. These bands confirmed the Cu-O stretching mode, as shown by the matching plane (202) in the XRD study. The FTIR spectrum exhibits wide transmittance bands between 3300 and 4000 cm^{-1}, primarily as a result of phenolic O-H stretch.[2,31] The aromatic C-C-H bend of dihydroxyphenyl is responsible for the vibration at 1,149 cm^{-1}.[32] Asymmetric C-C=C stretch for aromatic ring was observed at 1400 cm^{-1}.[33] The peaks at 1250, 1780 and 2050 cm^{-1} were characterized by the C–N stretch of aromatic amines, C=O in carboxylic group COOH and symmetric -$NH3^+$ stretching vibration of free amino acid, respectively.[34-36] The presence of the hydroxyl group of -COOH, -CH_2 group and C=C-H asymmetric stretch were noted at 2570, 2750 and 3150 to 3200 cm^{-1}.[37,38] FTIR spectrum of biogenic fabricated CuO NP confirms that phenols (para-hydroxybenzoate, syringic acids and vanillic acid), flavonoids, free amino acid etc. present in the leaves can reduce Cu^{+2} ions and acts as stabilizer.[39-41] Carboxyl group (-COOH) of these secondary metabolites dissociated to generate $–COO^-$ and H^+ ions, while its -COO^- donates electrons to reduce Cu^{2+} ions. Phytochemicals present in the plant extract bind to Cu^0 molecules, generating a charge transfer complex. During the ageing process, these molecules become Cu^0 particles. Negative -δ charges from hydroxyl groups (COO^-) of secondary metabolites in *O. corniculata* restrict size of the Cu^0 atoms to nano range. Cu^0 particles undergo additional oxidation to create CuO NPs (Fig. 3).[4]

FESEM micrographs were utilized to study the surface topography of fabricated CuO NPs (Fig. 4A). It was discovered that spherical-shaped nanoparticles were homogenously distributed. Increased magnification reveals aggregation of a collection of smaller spherical particles, with diameters ranging from 25 to 45 nm. The crystallinity of CuO NPs seen in the FESEM has already been verified by XRD (Fig. 2A). HRTEM studies corroborated the FESEM results (Fig. 4B). HRTEM analysis authenticated the form and dimension of the nanomaterials. HRTEM analysis of the NPs revealed isolated tiny particles ranging from 20 to 36 nm.

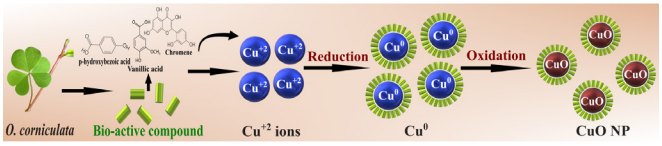

Fig. 3: Schematic representation of *O. corniculata* assisted biogenic fabrigaion of CuO NPs

Antibacterial Activity

Antibacterial properties of CuO NPs have already been explored by several studies.[42-45] It has been reported that gram-ve bacterial strains were comparatively more susceptible to CuO NPs than gram-ve bacterial strains.[4,45] Experiments demonstrated the same but more positive results, indicating that the antimicrobial activity of biogenic fabricated CuO NP is preserved even when synthesized sustainably. It was evident from Fig. 5 that both plant extract and CuO NP showed antibacterial effects on both strains, but the efficacy was higher in biogenic

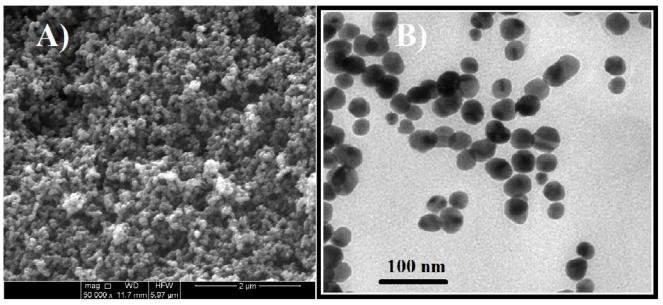

Fig. 4: EM studies of synthesized CuO NP A) FESEM and B) HRTEM

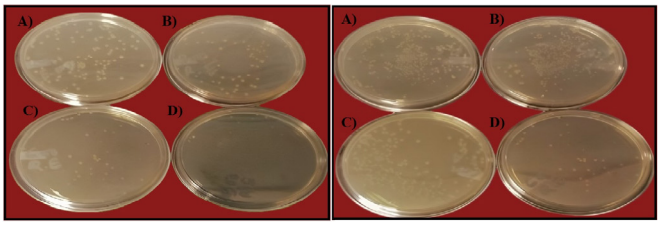

Fig. 5: Antimicrobial effects of green CuO NP, *E. coli* (at left) and *S. aureus* (at right) both treated with A) Control B) 50 µL mL^{-1} plant extract, C) 0.1 g mL^{-1} CuO NPs and D) 0.2 g mL^{-1} CuO NPs individually cultured on agar plate

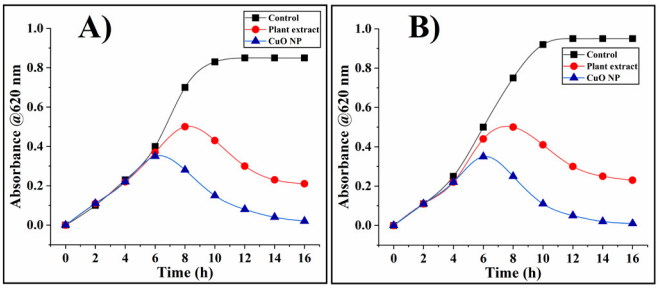

Fig. 6: Time dependent antibacterial potential analysis by monitoring absorbance at A_{620}. (A) *E. coli* and (B) *S. aureus*

fabricated CuO NPs. Synthesized NPs contain bioactive constituents like phenols and flavonoids of *O. corniculata* that enhance the bactericidal efficacy of CuO NP.[46] Time-killing assays were utilized to evaluate potential bactericidal dynamics on exponentially multiplying bacterial strains (Fig. 6). CuO NPs have excellent antibacterial action against both bacterial species. After 8 hours of CuO NP treatment, *E. coli* and *S. aureus* bacteria populations had been decreased by 99%. The experiment mentioned above shows that biogenic CuO NP is efficient in killing/inhibiting bacterial growth.

CuO nanoparticles have MIC values for *E. coli* and *S. aureus* bacteria were 100 and 150 µg mL^{-1}, respectively (Table 2). Biogenic NPs has MBC 200 and 100 µg mL^{-1} to *S. aureus* and *E. coli*, which correlated to the MIC. In biogenic CuO NPs treatment, the MBC is below four times to that of the MIC, indicating a bactericidal instead of bacteriostatic effect of the nanoparticles. Very little concentration of the NPs can eliminate gram (-ve) bacteria (≥99%) since gram (+ve) bacterial strains were more resilient to growth inhibitory function than gram (-ve) bacterial cells (Table 2). In the MTT assay (cell viability assay), CuO NP treatment of bacterial cells results in much-decreased formazan production compared to control and plant extract-treated cells (Fig. 7). The decreased cellular viability suggests that CuO NP is an efficient antimicrobial agent.

Fig. 8 depicts the morphology of bacterial cells treated with biogenic fabricated CuO NPs as observed by FESEM. FESEM

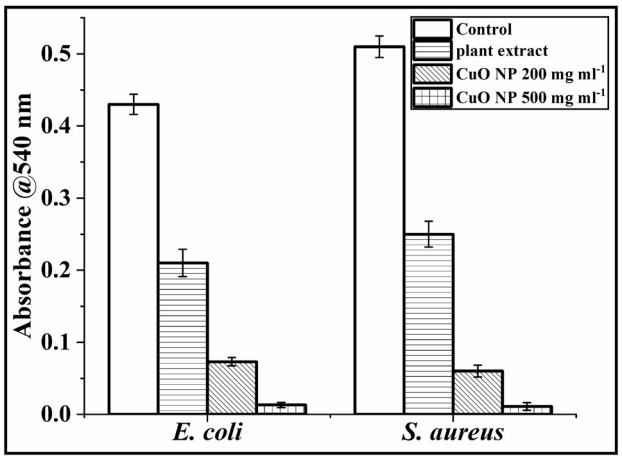

Fig. 7: Bacterial cell viability (MTT) assay

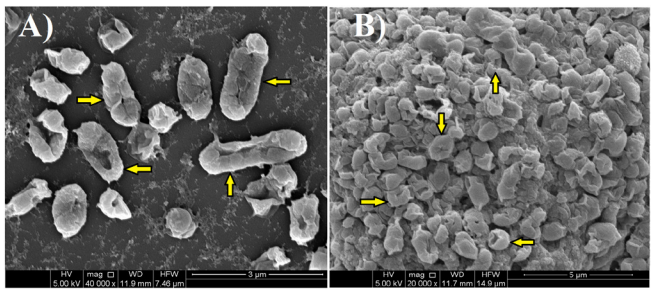

Fig. 8: FESEM micrograph of CuO NP treated A) *E. coli* and B) *S. aureus* bacteria

Table 2: MIC and MBC of bacteria treated with biogenic CuO NPs

Bacteria	*CuO NP (µg mL^{-1})*	
	MIC	*MBC*
E. coli	100	150
S. aureus	150	200

micrographs showed CuO NP-treated *E. coli* cells (Fig. 8A) lost their cellular integrity due to ruptured cell walls. The same thing was observed in the CuO NPs treated *S. aureus* (Fig. 8 B) cell. The thickness of peptidoglycan differs in their cell wall for differential gram-stained bacterial strains. Changes in cellular structure, metabolic processes, or response to NPs may clarify variations in susceptibility to differential gram-stained bacterial samples. Gram (+ve) *S. aureus* is more susceptible to biogenic CuO NP due to their higher concentration of functional groups like -NH_2 and -COOH on its cell membrane, which showed more affinity for copper.[47] Gram (-ve) *E. coli* bacteria have an exceptional bacterial wall organization that helps them withstand antimicrobial agents.[48] Cu^{2+} ions penetrate bacterial cell membranes and inhibit enzyme activity.[49] High concentrations of Cu^{2+} ions can form ROS that cause oxidative disruption of nucleic acid and protein synthesis machinery, ultimately, cell death.[4,44,45,50] Based on these findings, it is possible to conclude that the *O. corniculata* assisted biogenic fabricated CuO NPs have exceptional antibacterial potential on both bacterial strains.

Antidiabetic Study

Many metallic and metallic oxides NPs have been used in medicinal and biological applications.[51, 52] Hyperglycemia is caused by hepatic glycogenolysis and gluconeogenesis, which promote excessive glucose production while inhibiting tissue usage.[53] Blood glucose is a critical biomarker utilized in the diagnosis and prognosis of diabetes Mellitus (DM). This study demonstrates that administering biogenic CuO NP into STZ induced diabetic Swiss albino mice decreases blood glucose significantly (Fig. 9). Blood glucose level is highest in auto recovery (Gr II) which was reduced by 32.85, 48.21 and 44.64% after administration of plant extract, CuO NP and metformin, respectively (positive control). The best hypoglycemic efficacy was performed by CuO NP (Gr IV) followed by metformin (Gr V) and plant extract (Gr III). Similar kind of results was observed in other biochemical parameters like cholesterol, triglyceride and lipid peroxidation. CuO-NP is an excellent therapeutic agent in type 2 diabetes management. Natural remedies are perhaps the most intriguing alternatives to treating diabetes.[54] Several trace elements, including Cr, Se, V, Mo and Mg are effective therapeutic agents for decreasing blood glucose because of their insulin mimic action, which includes hypoglycemic properties.[55, 56]

Table 3: Profiling of total protein (g dl^{-1}) and enzymes (IU L^{-1}) for liver function

Group	*AST*	*ALT*	*ALP*	*GGT*	*Total protein*
I	85.45 ± 7.21	30.01 ± 1.41	28.30 ± 2.63	2.60 ± 0.42	6.35 ± 0.55
II	142.45 ± 22.32**	58.45 ± 3.72**	53.38 ± 5.48**	5.49 ± 0.66**	5.80 ± 0.40*
III	97.98 ± 17.19*	41.72 ± 2.28*	44.30 ± 4.88*	4.78 ± 0.48*	5.85 ± 0.44
IV	79.45 ± 8.32	32.38 ± 0.81	33.30 ± 2.88	3.72 ± 0.37	5.94 ± 0.34
V	75.43 ± 9.44	35.79 ± 3.89	34.50 ± 4.47	4.15 ± 0.47*	5.70 ± 0.34*

Table 4: Hematological parameters in mice

Hematological Parameters	*Hb (gm dl^{-1})*	*RBC (10^6 mL^{-1})*	*HCT (%)*	*MCV (ft)*	*MCH (pg)*	*WBC (10^3 mL^{-1})*	*Platelets (10^5 mL^{-1})*
Group-I	10.9 ± 0.5	6.37 ± 0.3	56.1 ± 1.1	61.8 ± 2.3	18.8 ± 0.7	6.65 ± 0.11	636 ± 17
Group-II	12.95 ± 0.7	6.20 ± 0.25	57.8 ± 0.9	62.41 ± 2.0	21.5 ± 1.1	7.3 ± 0.15	662 ± 12
Group-III	12.34 ± 0.5	6.84 ± 0.31	58.32 ± 1.2	63.01 ± 1.9	20.49 ± 0.9	7.6 ± 0.12	653 ± 15
Group-IV	12.84 ± 0.6	6.48 ± 0.45	61.72 ± 1.1	63.18 ± 2.1	20.61 ± 0.8	7.7 ± 0.15	667 ± 11
Group-V	11.7 ± 0.7	6.61 ± 0.35	59.62 ± 1.3	63.25 ± 2.1	19.36 ± 0.8	6.9 ± 0.11	669 ± 14

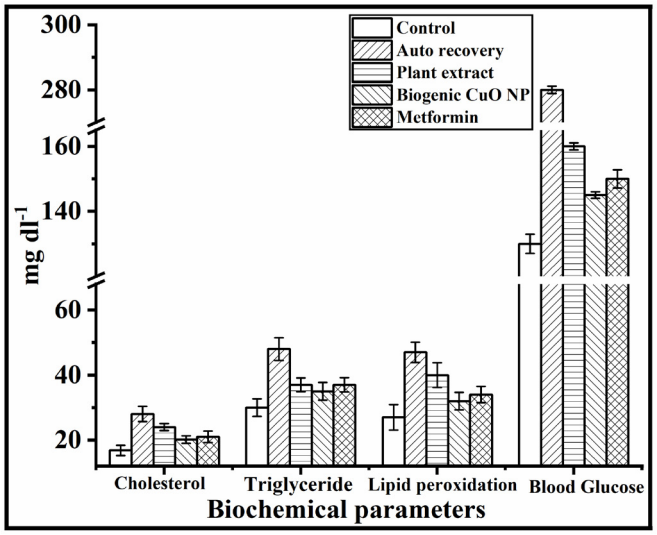

Fig. 9: Biochemical parameters of experimented mice

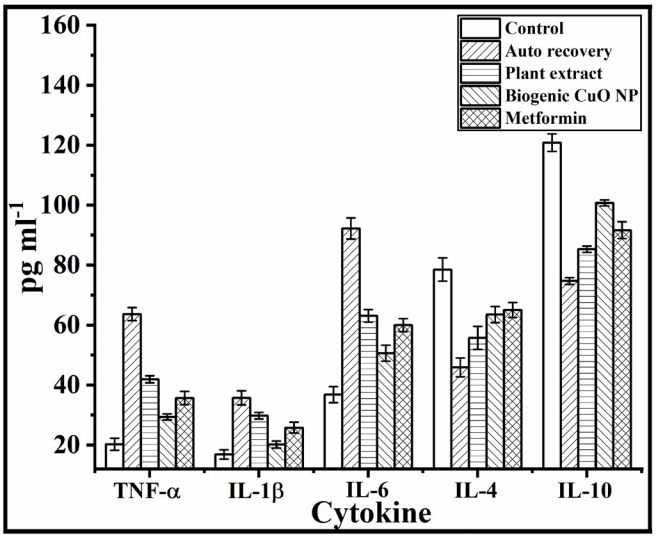

Fig. 10: Cytokine profile of experimented mice

Hepatic glycogenosis and gluconeogenesis cause hyperglycemia by promoting glucose synthesis.[53] STZ drug damages DNA through poly-ADP-ribosylation, causing diabetes.[57] Zinc may promote insulin actions and decrease cytokine formation by potentially killing pancreatic insulin-secreting cells.[58] Inhibition of alpha-amylase and alpha-glucosidase enzyme that reduces the breakdown of polysaccharides and restrain carbohydrate absorption. By inhibiting these enzymes, CuO NPs lower the postprandial glucose levels in diabetic mice.[59] In addition, the hypoglycemic potential of biogenic CuO NPs stabilized by phenols (para-hydroxybenzoate, syringic acids and vanillic acid), flavonoids, glycosides etc., increased peripheral glucose consumption.[39,60,61]

Lipid profile studies demonstrated that diabetes elevated cholesterol, triglyceride, and lipid peroxidation levels, whereas CuO NP-supplemented therapy served to alleviate the adverse impacts (Fig. 9). In STZ induced diabetes, there are excess fatty acids in the blood, triggering the liver to convert the surplus fat phospholipid and cholesterin. These compounds combine well to generate excess triglycerides in the liver, which are then released into the bloodstream as lipoproteins. Since insulin suppresses the action of hormone-sensitive lipase, the abnormally high quantity of blood lipids in our findings is mostly caused by an upsurge of the mobilization of unrestrained fatty acids from the periphery.[62]

The results of the liver function test indicated an estimate of liver damage. The liver enzymes AST, ALT, ALP, and GGT all elevated considerably in STZ-induced animals. During therapy, these altered liver function enzymes were considerably recovered (Table 3). High levels of AST and ALT may indicate hepatic cholestasis.[63] The marker

enzymes were not elevated after treatment with CuO NP and metformin. Diabetes increases oxidative stress and ROS production, which damages the cellular protein machinery and alters serum levels.[64] The observation might be attributed to micro-proteinuria and high protein catabolism levels, which are major clinical signs of the illness.[65]

STZ-induced diabetes is linked to chronic inflammation from cytokines like IL-6, 1β, TNF-α etc. This results in pancreatic β cell death, neuropathy, endothelial dysfunctioning including nephropathy, retinopathy as well as cardio-vascular damage.[66-68] The disparity among the two thymus cells (Th1 and Th2) was reported from ELISA results of cytokine profile. IL-6, 1β and TNF-α have enhanced promptly after the induction of diabetes, which corresponds to Th1 (Fig. 10), while Th2 corresponding to IL-4 and IL-10 was decreased. Supplementing the mice with plant extract, CuO NP and metformin also reduced these effects. After diabetes induction, IL-6, 1β and TNF-α production levels increased by 2.51, 2.12, and 3.15 fold compared to control groups, respectively. In contrast, IL-4 and IL-10 levels decreased 1.71 and 1.62 fold compared to control groups. The significant best result was observed in CuO NP-treated animals, followed by metformin and plant extract-treated groups. Table 4 represents the hematological profile of mice belonging to groups- I, II, III, IV and V. Differences in hematological parameters were statistically insignificant. The result conclusively reveals that *O. corniculata* fabricated CuO NP does not interact with the hematological system.

Conclusion

The biosynthesis of CuO NP by employing a biogenic method that uses *O. corniculata* leaf extract is non-toxic to the environment, inexpensive and simple. The CuO NP showed well-defined physiochemical properties, spherical shape and a modest size of 20 to 36 nm. Biogenic CuO NPs exhibit wide antibacterial potential on both bacterial strains employed for this study. The CuO NPs have effective antimicrobial capabilities and might be used as a novel medication against harmful microorganisms. Biogenic fabricated CuO NP showed potential antidiabetic application in STZ-induced hyperglycemia in Swiss albino mice by restoring biochemical and cytokine profiles.

Acknowledgment

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Reference

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