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Green-Synthesized *Boerhavia Diffusa* Nanoparticles Against Cisplatin-Induced Nephrotoxicity in *Wistar Albino Rats*

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ABSTRACT

Cisplatin remains one of the most widely used chemotherapeutic agents for the treatment of solid tumours, but its clinical utility is substantially limited by dose-dependent nephrotoxicity, mediated through oxidative stress, mitochondrial dysfunction, inflammatory cytokine activation, and apoptotic tubular cell death. Boerhavia diffusa Linn. (Punarnava), a plant with a long-standing traditional reputation as a diuretic and nephroprotective agent, is rich in phenolic, flavonoid, and rotenoid phytoconstituents that are also capable of mediating green, plant-based nanoparticle synthesis. The present study was designed to develop green-synthesised copper oxide nanoparticles from Boerhavia diffusa root extract and to evaluate their nephroprotective activity against cisplatin-induced nephrotoxicity in Wistar rats. Boerhavia diffusa root was authenticated and extracted by aqueous decoction, and the resulting extract was used to green-synthesise copper oxide nanoparticles by bioreduction of a copper precursor salt, with nanoparticle formation confirmed by UV-visible spectroscopy across a 200–800 nm scanning range. Nephrotoxicity was induced in Wistar rats using cisplatin, and animals were randomly allocated into six groups of six animals each: a normal control, a cisplatin (disease) control, a standard reference treatment (taurine and N-acetylcysteine), two test doses of the nanoparticle preparation (500 and 1000 mg/kg), and a toxicity-control group receiving the test preparation alone. Following a 14-day treatment period, renal function was assessed biochemically through estimation of blood urea nitrogen, serum creatinine, albumin, uric acid, and total protein, corroborated by histopathological examination of kidney tissue for tubular necrosis, glomerular damage, interstitial inflammation, tubular degeneration, and cellular swelling. The findings of this evaluation, once obtained, are intended to establish whether the green-synthesised Boerhavia diffusa-derived copper oxide nanoparticles confer dose-dependent protection against cisplatin-induced renal injury, offering a plant-based nanoparticle strategy for mitigating a major dose-limiting toxicity of cisplatin chemotherapy.

Keywords: Boerhavia diffusa, Punarnava, green synthesis, copper oxide nanoparticles, cisplatin, nephrotoxicity, nephroprotective activity, Wistar rats

1. Introduction

Cisplatin remains a cornerstone of chemotherapy for many solid tumours, but its clinical use is limited by cumulative nephrotoxicity affecting the renal proximal tubules [5]. Acute kidney injury has been reported in roughly 30% of patients receiving cisplatin, with loss of the proximal tubule brush border, tubular damage, cast formation and basement membrane thickening [6]. Experimental work has implicated oxidative stress, mitochondrial dysfunction, inflammatory cytokine signalling and apoptotic and ferroptotic cell death in this injury, and several phytochemicals, including daidzein, ginsenoside Rg5 and kaempferol, attenuate it in mice by modulating these pathways [7–9]. Agents that protect the kidney without compromising antitumour activity therefore remain of considerable interest.

Boerhavia diffusa Linn. (family Nyctaginaceae; Sanskrit: Punarnava) is a pantropical herb used in Ayurveda as a diuretic and *rasayana*, and for hepatic and renal disorders [1,2]. Its roots contain rotenoids (boeravinones), the alkaloid punarnavine, flavonoids (including quercetin and kaempferol), phenolic glycosides, lignans and ecdysteroids [1]. Nephroprotective activity has been reported for the plant against experimentally induced renal injury [3,4], and a *B. diffusa–Tinospora cordifolia* combination reduced diclofenac-induced nephrotoxicity ($p < 0.05$), an effect attributed to reduced renal oxidative and inflammatory stress [3].

Green nanotechnology uses biological reducing and capping agents in place of hazardous chemicals, high temperature or toxic solvents, and has been reported to lower the toxicity associated with conventionally prepared nanoparticles, although batch-to-batch reproducibility and scale-up remain challenges [10–13]. The polyphenols, flavonoids and alkaloids that make plant extracts effective reductants are prominent in *B. diffusa*, and its extract has been used to prepare tin oxide, copper and silver nanoparticles with antibacterial, photocatalytic, cytotoxic and antioxidant properties [14–16]. Because oxidative stress is central to cisplatin renal injury, a *B. diffusa*-capped CuO nanoparticle is a plausible nephroprotective candidate. To our knowledge, its activity in a cisplatin nephrotoxicity model has not been reported.

The present study therefore aimed to (i) prepare CuO NPs from *B. diffusa* root extract and characterise them by UV–visible, SEM and FT-IR analysis, and (ii) evaluate their nephroprotective effect against cisplatin-induced nephrotoxicity in Wistar rats using biochemical and histopathological endpoints.

2. Materials and Methods

2.1 Plant material and extract preparation

Roots of *B. diffusa* Linn. were collected and botanically authenticated [SIDDHA CENTRAL RESEARCH INSTITUTE]. The roots were washed with distilled water, cut with a sterile blade, shade-dried and powdered. Powdered root (10 g) was heated in 100 mL distilled water at 60–80 °C for 30 min with continuous stirring, cooled and filtered through Whatman No. 1 filter paper. The filtrate was screened qualitatively for phenolics, flavonoids, alkaloids and rotenoids and stored under refrigeration, protected from light.

2.2 Green synthesis of CuO nanoparticles

A 1 mM aqueous solution of the copper precursor was freshly prepared. Ninety millilitres of this solution was mixed with 10 mL of root extract (9:1 v/v) and kept in the dark at room temperature for 30 min to 2 h until a visible colour change to dark brown indicated nanoparticle formation. The mixture was centrifuged at 5000 rpm for 10 min, and the pellet was collected, washed with distilled water, re-centrifuged and dried.

2.3 Characterisation

UV–visible absorption was recorded from 200 to 800 nm . For SEM, dried powder was mounted on a conductive stub and imaged at Kiyo R&D Lab, Chromepet, Chennai particle dimensions were read from calibrated scale bars. FT-IR spectra were recorded as transmittance (%) versus wavenumber (cm^{-1}) ATR or KBr, spectral range, resolution, number of scans.

2.4 Animals and ethical approval

- Female Wistar albino rats 200–250 g, but recorded mean initial body weight in this study was about 135 g] were obtained from a CPCSEA-registered supplier. Animals were housed at room temperature, 55–65% relative humidity and a 12 h light/12 h dark cycle, with standard pellet diet and distilled water ad libitum, and acclimatised for 7 days. The protocol was approved by the Institutional Animal Ethics Committee (IAEC) under CPCSEA guidelines [approval number- CPCSEA : 2308/PO/Re/S/2024/CCSEA]. Animals were randomised to groups to balance baseline body weight and euthanised by CPCSEA-approved methods at study end.

2.5 Dose selection

A separate acute oral toxicity study was not performed, in keeping with the 3Rs principle. Doses of 500, 1000 and 1500 mg/kg b.w. were selected from previously reported acute oral toxicity data for CuO nanoparticles [Padilla-Camberos et al., 2022 OECD guideline (420/423/425) and reported NOAEL/LD50].

2.6 Experimental design

Six groups of six animals (n = 6) were treated for 14 days (Table 1). Cisplatin was administered intraperitoneally to induce nephrotoxicity dose 1.15 mg/kg All other treatments were given orally.

Table 1. Experimental groups and treatment schedule

Group	Treatment	Route	Duration
I	Normal saline (normal control)	Oral	14 days
II	Cisplatin (disease control)	I.P.	14 days
III	Taurine + N-acetylcysteine (Nefrosave) 11.5 + cisplatin (standard)	Oral / I.P.	14 days
IV	CuO NPs 500 mg/kg + cisplatin (low dose)	Oral / I.P.	14 days
V	CuO NPs 1000 mg/kg + cisplatin (medium dose)	Oral / I.P.	14 days
VI	CuO NPs 1500 mg/kg + cisplatin (high dose)	Oral / I.P.	14 days

2.7 Body weight and biochemical analysis

Body weight was recorded on days 0, 7 and 14. At study end, blood was collected from the lateral tail vein and serum separated. BUN (urease method), creatinine (Jaffé alkaline picrate), albumin (bromocresol green), uric acid (uricase) and total protein (biuret) were measured with commercial diagnostic kits on a semi-automated biochemistry analyser

2.8 Histopathology

Kidneys were excised, trimmed of perirenal fat and fixed in 10% neutral buffered formalin, processed through graded alcohols and xylene, embedded in paraffin, cut at 4–5 µm and stained with haematoxylin and eosin. Sections were graded for tubular necrosis, glomerular damage, interstitial inflammation, tubular degeneration and cellular swelling on a 0–3 scale (0 = absent, 1 = mild, 2 = moderate, 3 = severe)

2.9 Statistical analysis

Data are expressed as mean ± SEM (n = 6). Groups were compared by one-way ANOVA followed by Dunnett's test against the cisplatin control (Group II); $p < 0.05$ was considered significant.

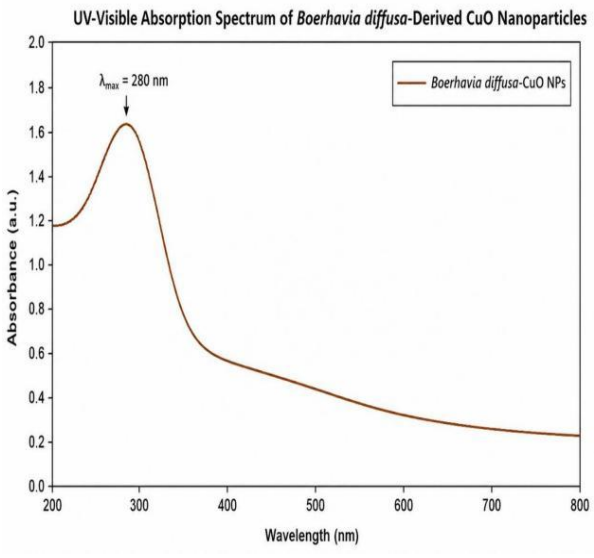
3. Results

3.1 UV–visible spectroscopy

The synthesised CuO NPs showed a single broad absorption band in the UV region with λmax at about 280 nm (absorbance 1.63), supporting nanoparticle formation.

Sample	**λmax (nm)**	**Absorbance (a.u.)**	**Remarks**
Boerhavia diffusa extract	—	—	Plant extract used as reducing/stabilising agent
Copper precursor solution	—	—	Precursor before nanoparticle formation
Synthesised *Boerhavia diffusa*-CuO NPs	**280**	**1.63**	**Broad absorption band in the UV region; indicates formation of CuO nanoparticles**

Figure 1. UV–visible absorption spectrum of *B. diffusa*-derived CuO nanoparticles.

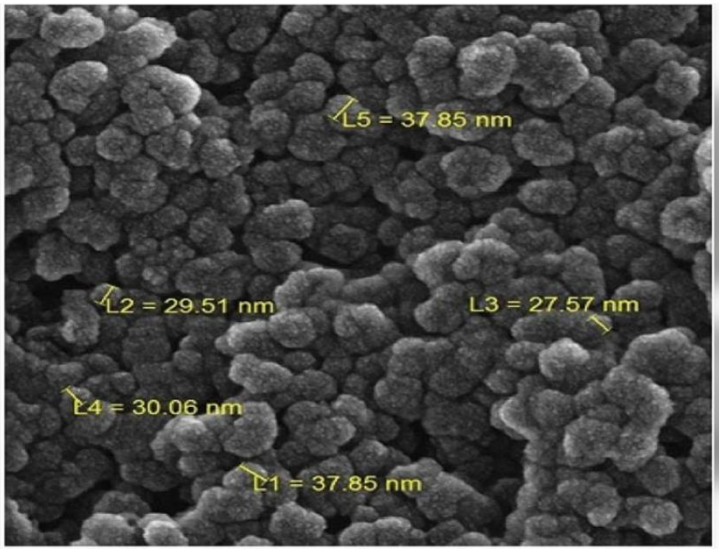


3.2 SEM analysis

SEM showed closely packed particles of rounded to irregular shape with rough, granular surfaces and clusters indicating aggregation (Fig. 2). Five annotated dimensions (37.85, 29.51, 27.57, 30.06 and 37.85 nm) ranged from 27.57 to 37.85 nm, with an arithmetic mean of 32.57 nm. This describes only the five annotated features and is not a representative size distribution.

Parameter	Result
Number of annotated measurements	5
Minimum annotated dimension	27.57 nm
Maximum annotated dimension	37.85 nm
Mean of annotated dimensions	32.57 nm

Figure 2. SEM micrograph of the CuO nanoparticles with five annotated dimensions.



3.3 FT-IR analysis

FT-IR bands appeared at about 3439, 1628, 1107, 784, 624 and 529 cm^{-1}

Figure 3. FT-IR spectrum of the CuO nanoparticles.

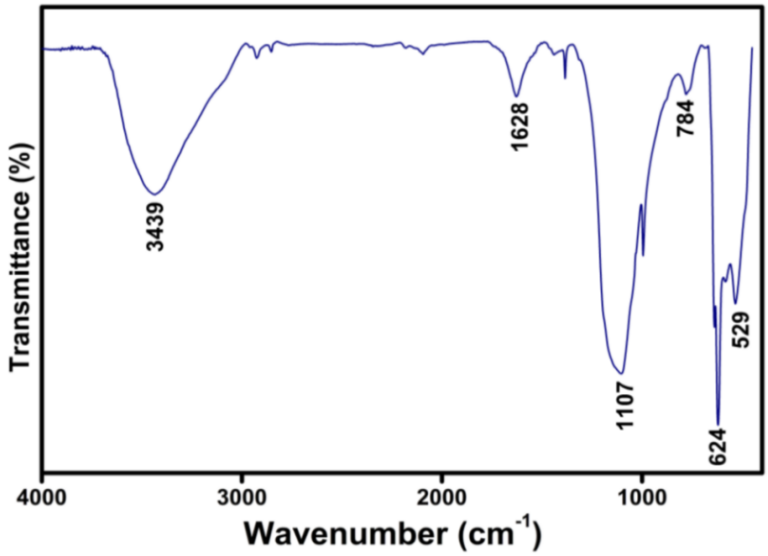


Table 2. FT-IR band assignments (tentative)

Band (cm^{-1})	Assignment	Interpretation
3439	O–H stretching	Hydroxyl groups and/or adsorbed moisture
1628	H–O–H bending	Adsorbed water
1107	C–O stretching	Possible phenolic/alcoholic residues from the extract
784, 624, 529	Cu–O-related vibrations	Consistent with copper–oxygen bonding

3.4 Body weight

All groups gained weight and none showed weight loss (Table 3). Gain was smallest in the cisplatin control (2.98%) and largest in the standard group (7.72%); CuO NP groups gained 5.32–6.41%.

Table 3. Body weight (g, mean ± SEM, n = 6)

Group	Day 0	Day 7	Day 14	Change (%)
I Normal control	135.30 ± 3.27	139.42 ± 3.21	143.52 ± 3.18	+6.08
II Cisplatin control	136.12 ± 2.47	138.05 ± 2.74	140.18 ± 2.91	+2.98
III Standard	135.23 ± 2.39	140.12 ± 2.65	145.67 ± 2.84	+7.72
IV CuO NPs 500 mg/kg	137.50 ± 1.71	141.18 ± 2.42	144.82 ± 2.63	+5.32
V CuO NPs 1000 mg/kg	137.50 ± 3.35	142.03 ± 2.91	146.31 ± 2.76	+6.41
VI CuO NPs 1500 mg/kg	136.40 ± 1.54	140.17 ± 2.38	143.92 ± 2.58	+5.51

3.5 Renal biochemical parameters

One-way ANOVA showed significant group effects for BUN ($F(5,30) = 28.64$), creatinine (24.87), albumin (11.52), uric acid (19.76) and total protein (9.84), all $p < 0.001$. Relative to normal control, cisplatin increased BUN, creatinine and uric acid and decreased albumin and total protein (Table 4; Fig. 4). The standard treatment largely restored all five parameters. CuO NPs improved them in a dose-related manner (Table 5): at 500 mg/kg, albumin and total protein did not differ significantly from cisplatin control and creatinine improved only at $p < 0.05$, whereas at 1000 and 1500 mg/kg all parameters improved significantly, with the high dose closest to the standard group.

Table 4. Serum renal function parameters (mean ± SEM, n = 6)

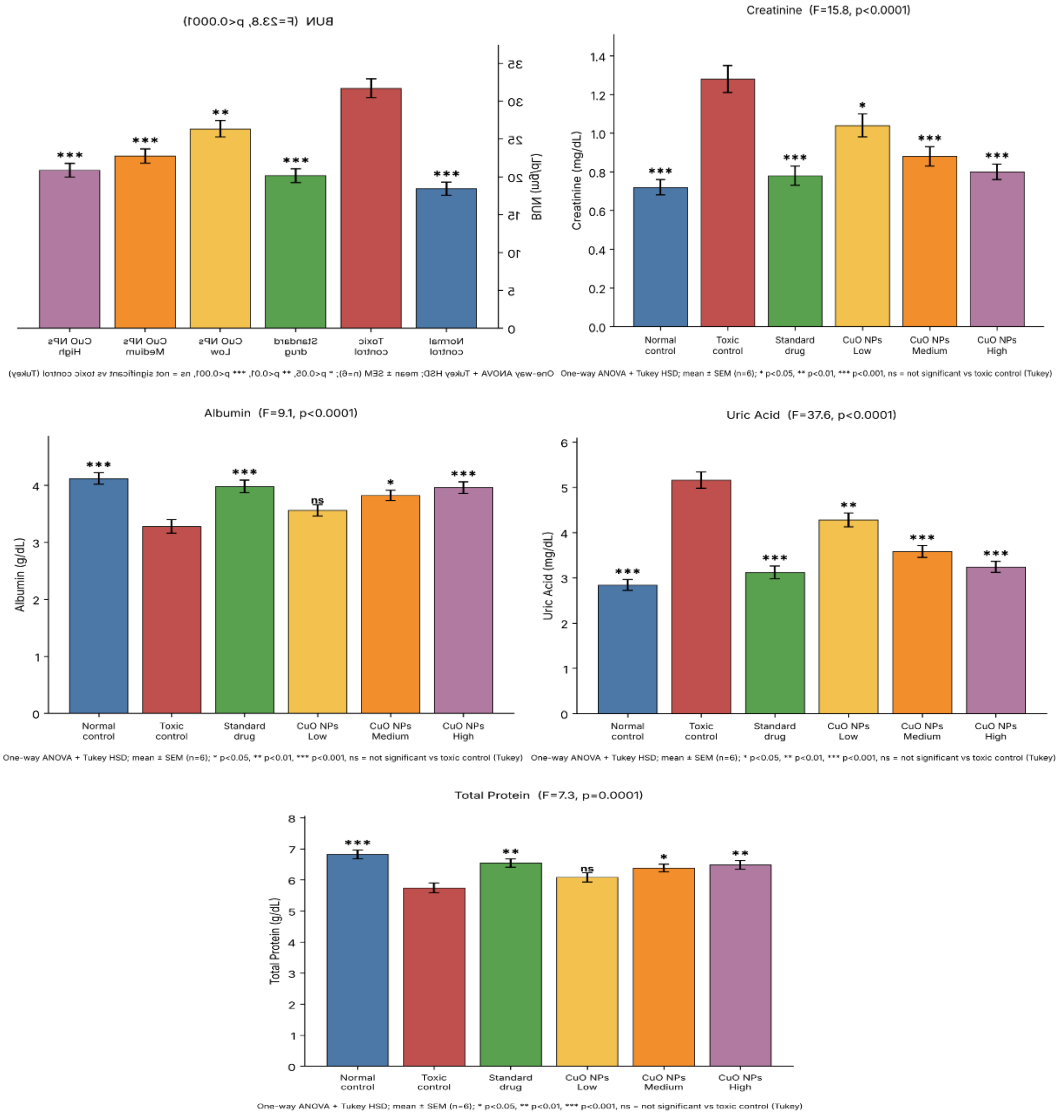
Group	BUN (mg/dL)	Creatinine (mg/dL)	Albumin (g/dL)	Uric acid (mg/dL)	Total protein (g/dL)
I Normal control	18.42 ± 0.86***	0.72 ± 0.04***	4.12 ± 0.10***	2.84 ± 0.12***	6.82 ± 0.14***
II Cisplatin control	31.68 ± 1.24	1.28 ± 0.07	3.28 ± 0.12	5.16 ± 0.18	5.74 ± 0.16
III Standard	20.14 ± 0.92***	0.78 ± 0.05***	3.98 ± 0.11***	3.12 ± 0.14***	6.54 ± 0.13**
IV CuO NPs 500 mg/kg	26.32 ± 1.08**	1.04 ± 0.06*	3.56 ± 0.10 ns	4.28 ± 0.15**	6.08 ± 0.15 ns
V CuO NPs 1000 mg/kg	22.74 ± 0.96***	0.88 ± 0.05***	3.82 ± 0.09*	3.58 ± 0.13***	6.38 ± 0.12*
VI CuO NPs 1500 mg/kg	20.86 ± 0.90***	0.80 ± 0.04***	3.96 ± 0.10***	3.24 ± 0.12***	6.48 ± 0.14**

*p < 0.05, **p < 0.01, ***p < 0.001 versus Group II (one-way ANOVA, Dunnett's test); ns, not significant.

Table 5. Change relative to cisplatin control (%)

Group	BUN	Creatinine	Albumin	Uric acid	Total protein
I Normal control	−41.86	−43.75	+25.61	−44.96	+18.82
III Standard	−36.43	−39.06	+21.34	−39.53	+13.94
IV CuO NPs 500 mg/kg	−16.92	−18.75	+8.54	−17.05	+5.92
V CuO NPs 1000 mg/kg	−28.22	−31.25	+16.46	−30.62	+11.15
VI CuO NPs 1500 mg/kg	−34.15	−37.50	+20.73	−37.21	+12.89

Figure 4. Effect of treatments on serum (a) BUN, (b) creatinine, (c) albumin, (d) uric acid and (e) total protein.



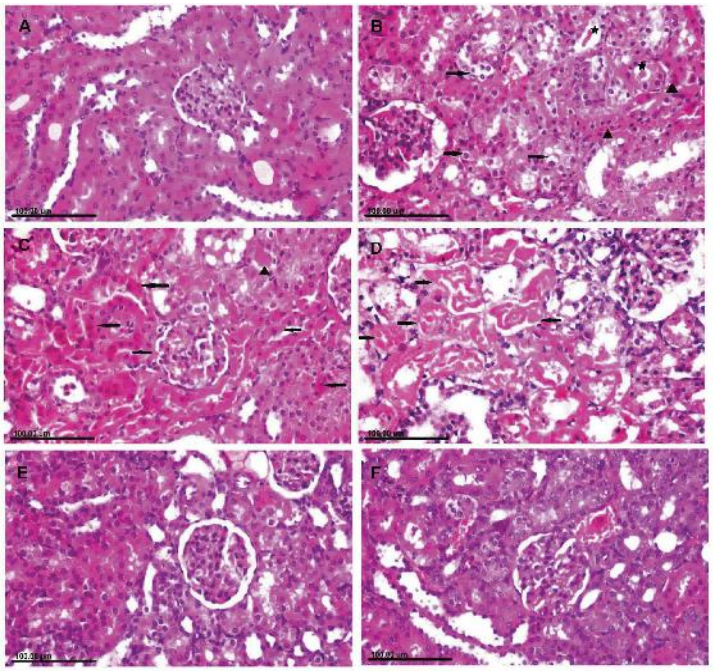
3.6 Histopathology

Normal control kidneys showed preserved architecture (score 0 for all parameters). The cisplatin control showed the maximum score (3) for tubular necrosis, glomerular damage, interstitial inflammation, tubular degeneration and cellular swelling. The standard group and the 1000 and 1500 mg/kg CuO NP groups scored 1 for every parameter, and the 500 mg/kg group scored 2 (Table 6; Fig. 5).

Table 6. Histopathological severity scores of kidney sections (0 = absent, 1 = mild, 2 = moderate, 3 = severe)

Group	Tubular necrosis	Glomerular damage	Interstitial inflammation	Tubular degeneration	Cellular swelling
I Normal control	0	0	0	0	0
II Cisplatin control	3	3	3	3	3
III Standard	1	1	1	1	1
IV CuO NPs 500 mg/kg	2	2	2	2	2
V CuO NPs 1000 mg/kg	1	1	1	1	1
VI CuO NPs 1500 mg/kg	1	1	1	1	1

Figure 5. Representative H&E-stained kidney sections (×40): (A) normal control; (B) cisplatin control; (C) standard; (D–F) CuO NPs 500, 1000 and 1500 mg/kg with cisplatin



4. Discussion

The UV–visible spectrum, SEM images and FT-IR bands together support formation of a Cu–O material capped by plant-derived organic species. A broad UV absorption at 280 nm is not far from the 286–420 nm range reported for green CuO nanoparticles prepared with other plant and biological reductants [17–20], though it lies below the 410 nm originally anticipated for this system. The FT-IR bands at 784, 624 and 529 cm^{-1} fall in the region where Cu–O vibrations occur, and the 1107 cm^{-1} and 3439 cm^{-1} bands are compatible with phenolic or alcoholic residues and hydroxyl groups from the extract, consistent with a phytochemical capping layer [13]. However, these assignments are tentative: UV–visible and FT-IR data cannot establish crystal phase, purity, or size distribution, and the SEM dimensions (mean 32.57 nm) derive from only five particles in an aggregated field. XRD, EDX, DLS/zeta-potential and multi-field TEM or SEM sizing are needed for definitive identification.

Cisplatin produced the expected pattern of renal injury: higher BUN, creatinine and uric acid, lower albumin and total protein, reduced weight gain, and severe tubular and glomerular histopathology. CuO NP treatment attenuated each change in a graded way across 500, 1000 and 1500 mg/kg, and the biochemical and histological responses were concordant. At 1500 mg/kg, BUN and creatinine fell by 34.15% and 37.50% relative to cisplatin control, approaching the standard group (36.43% and 39.06%), and histopathological scores fell from 3 to 1. Body weight rose in all groups, which suggests the nanoparticle doses did not impair general growth over 14 days.

The findings are consistent with reports that *B. diffusa* preparations and phenolic/flavonoid-rich phytochemicals reduce chemically induced renal injury [3,4,7–9]. A plausible explanation is that antioxidant and radical-scavenging phytoconstituents of *B. diffusa* associated with the particle surface counter the oxidative and inflammatory injury that underlies cisplatin nephrotoxicity [5,6]. This remains a hypothesis: oxidative-stress, inflammatory, apoptotic and tubular-injury markers (MDA, GSH, SOD, catalase, GPx, TNF-α, KIM-1, NGAL) were not measured.

4.1 Limitations

Several limitations constrain interpretation. First, the design had no extract-only group, so the protection cannot yet be attributed to the nanoparticle rather than to the *B. diffusa* extract itself. Second, there was no nanoparticle-alone group, and copper levels in serum and kidney were not measured; given that oral copper-based nanoparticles have been reported to be nephrotoxic in rodents in some studies, safety at 500–1500 mg/kg needs direct demonstration and the dose rationale needs a primary reference. Third, characterisation was limited to UV–visible, SEM and FT-IR. Fourth, groups were small (n = 6), the study lasted 14 days, and histopathological scores are reported as group-level ordinal values without a stated statistical test. Fifth, renal endpoints were limited to five conventional serum markers.

5. Conclusion

CuO nanoparticles prepared from *B. diffusa* root extract attenuated cisplatin-induced increases in BUN, creatinine and uric acid, restored albumin and total protein, and reduced renal histopathological injury in a dose-related manner in Wistar rats, with the highest dose approaching the standard taurine–N-acetylcysteine treatment. These results are preliminary and support further work with extract-only and nanoparticle-alone controls, comprehensive nanoparticle characterisation, copper biodistribution, and oxidative-stress, inflammatory and tubular-injury biomarkers.

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Author contribution: Mohanapriya. N had principal responsibility for the conception, design of the study and drafting of manuscript. Santhosh Kumar.G was responsible for the revision of the manuscript for important intellectual content Dr. J. Karthi, Dr. S. Swarnalatha contributed towards reviewing of the literature. All authors agreed on the final version of manuscript.

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