

Abstract

The current study was designed to evaluate the diabetic and burn wound healing capacity of *Bergenia ciliata* (BC) extract, BC-loaded *Salvia hispanica* hydrogel (CH-BC) and *Bergenia ciliata* nanoparticles (NPs) loaded *Salvia hispanica* hydrogel (CH-BC AgNPs/NPs loaded hydrogel), *Bergenia ciliata* loaded Carboxymethyl cellulose (CMC-BC) hydrogel, and *Bergenia ciliata* nanoparticles loaded CMC hydrogel (CMC-BCNPs) using *in vitro* and *in vivo* studies. The prepared hydrogel and its components were analyzed using UV-visible spectrophotometry (UV-Vis), Scanning electron microscopy (SEM), Particle size analyzer (PSA)/Dynamic light scattering analysis (DLS), Zeta potential, Fourier transform infrared spectrometry (FT-IR), X-Ray diffraction (XRD), and GC-MS (Gas chromatography-mass spectrometry). Peaks and peak shifts at 450-460nm, 250-260nm, and 270-280nm in UV-Vis and FT-IR (500-4000cm⁻¹) confirmed extract loaded and various functional groups presence while SEM assessed average size of hydrogel-loaded *Bergenia ciliata* mediated nanoparticles (CH-BC AgNPs) ranging from 2-22.3nm and XRD peaks at various 2 θ (10-70) confirmed topographical and crystalline and porous nature of present study materials.

Hydrogel swelling increased when added to the phosphate buffer of pH 7.4 and decreased in solution with pH 1.2. Similarly, the swelling was observed in deionized water, while deswelling was analyzed in an ethanolic solution. Moreover, the hydrogel weight increase in a fashion pH 1.2 < water < pH 7.4 < pH 7.4, was assessed. The highest swelling was analyzed in the KCl solution, followed by NaCl, MgSO₄, and AlCl₃, indicating the wide range of ionic interaction among the salts and the hydrogel components. *Bergenia ciliata* extract showed higher radical scavenging potential (74.19%±1.84) and iron chelation activity (93.70±2.20) at 50 μ L/mL and 25 μ L/mL, respectively, while CH-BC AgNPs produced the lowest (48.97%±3.0) at 25 μ L/mL and 75 μ L/mL (54.35±3.24). Moreover, Chia hydrogel (CH) synergistically enhanced (1.30%) DPPH scavenging.

The bactericidal activity of CH-BC AgNPs was found to be concentration dependent, *i.e.*, 6mg/mL > 4mg/mL > 2mg/mL. The largest zone of inhibition (ZOI) (22.9±2.25mm) was measured against the *E. coli* at 6mg/mL of BC AgNPs, while the smallest (01±.50mm) (ZOI) was observed for 2mg/mL BC extract against *P. fluorescens*. Similarly, the highest (22.7±1.53mm) ZOI was observed at pH7 against *P. aeruginosa* for BC AgNPs, while the smallest (15.3±1.47mm) was against *S. aureus* at pH11. Furthermore, the highest

(23.20±1.11mm) ZOI at 20°C was assessed for BC AgNPs against *E. coli*, and the smallest (14.90±2.34mm) zone was measured for *S. aureus* at 10°C.

Furthermore, compared to a positive control (diclofenac sodium) (paw inflammation was normalized at 4th hour), the inflamed paw diameter in CH-BC AgNPs group, multiple treatments of BC extract (200mg/kg) and 300mg/kg group were restored to normal (3.45±0.16cm) at 3rd and 12th-hour post carrageenan induction (1g/100mL), respectively. At the same time, CH-BC AgNPs produced the lowest (48.97%±3.0) activity at 25µg/mL and 75µg/mL (54.35±3.24). On the other side, the fastest restoration of paw edema to normal was observed in the positive control (diclofenac sodium) group, followed by multiple treatments of BC extract and the CMC-BCNPs group at the 4th and 12th hour of post-carrageenan induction, showcasing the variable anti-inflammatory potential of the hydrogel and its components.

The CH-BC AgNPs augmented wound contraction percentage as a potent candidate for wound healing (14th day). These findings were further supported by significant (P<0.001) restoration of levels of MMP2 (184.6±11.7pg/mL), MMP7 (247.6±25.2pg/mL), MMP9 (148.8±19.6pg/mL), TIMPs (184.8±15.7pg/mL), IL-6 (10±1pg/mL), IL-8 (19.8±3.3pg/mL), TNF-α (16.8±2.6pg/mL), Glutathione peroxidase (GPx) (153.4±11.5U/L), Glutathione (GSH) (5.8±0.4µmol/L), and Catalase (CAT) (14.7±0.5mmol/L) in treatment groups than diabetic negative control (DNC) in the diabetic wound healing of the mice.

Similarly, the levels of MMP2 (215.4±11.7pg/mL), MMP7 (241.6±12.2pg/mL), MMP9 (151±13.1pg/mL), TIMPs (223.6±8.2pg/mL), GPx (179.4±17.53 U/L), IL-6 (15.8±3.4pg/mL), IL-8 (18.4±4.2pg/mL), TNF-α (19.8±5pg/mL), Glutathione (5.0±0.7 µmol/L), SOD (194.6±9.0 U/mL), CAT (6.2±0.8 mmol/mL), and MDA (3.5±0.4mmol/L) were also restored significantly in the treatment groups at varying degrees but best was seen in CMC-BC AgNPs treated group as compared to the control group in burn wound healing of the mice.

Furthermore, the histopathological findings of the diabetic wound tissues showed normal reepithelialization, angiogenesis, collagen synthesis, and maturation of wounds in treatment groups, further strengthening the supposed rationale that CH, along with BC extract and CH-BC AgNPs, can act synergistically to improve therapeutic results in wound healing applications. Similarly, fibroblast infiltration, keratinocyte migration, hair follicle presence, collagen synthesis, intact epidermis, and dermis formation were observed in the treatment groups of burn wound healing, especially the CMC-BCNPs group. xi

Hence, CH-BC appeared as a sustainable, biocompatible, and non-toxic agent for wound healing (both in diabetic and burn) and paved the way for futuristic biomedical investigations. In comparison to the already available and commercially used alginate or chitosan-based dressings, which offer certain moisture retention but limited intrinsic compatibility, they can also raise allergenicity and sustainability concerns. In this regard, the fully plant material-based green synthesized hydrogel and its components delivered superior antibacterial, antioxidant, and anti-inflammatory activity, thus underscoring its competitive nature as a greener, viable, and multifunctional wound healing system. Moreover, findings of the antibacterial, antioxidant, iron chelation, and anti-inflammatory activity further strengthened the fact that the present study hydrogel and its components can heal wounds in an accelerated way with a hint of biocompatibility. However, further optimization could be a better way forward for more insights regarding evaluating the diverse biomedical efficacy of such hydrogels.