

Abstract

Diabetes, a serious metabolic disorder with elevated blood sugar level holds many complications specifically impaired healing of wounds in diabetic patients. Due to oxidative stress and poor angiogenesis, the wounds become chronic and even lead towards gangrene and ultimately amputation in the patients affected with diabetic foot ulcer (DFU). In current research, Swiss Albino mice were used as an animal model. Alloxan monohydrate was injected intraperitoneally into mice for induction of diabetes. After one week, glucose level was measured using glucometer and then wounds were formed in rectangular shape at dorsal surface of mice foot using scissor and blade. The biomaterial with their conjugated silver nanoparticles were applied to wounded foot of mice to assess healing process. The healing effect of *Bergenia ciliata* (BC) and *Bergenia ciliata* conjugated silver nanoparticles (BCAgNPs) was analyzed by determination of healing time, percent wound contraction, histopathological analysis and by measuring the level of blood biomarkers. Characterization of BCSAgNPs was also done by UV-Vis spectrophotometry and Fourier Transform Infrared Spectroscopy (FTIR) to confirm the formation and stability of nanoparticles. Blood sample was collected through cardiac puncture to evaluate the level of following biomarkers in blood i.e., pro-inflammatory cytokines (TNF- α , IL-6 and IL-8), matrix metalloproteinases (MMPs) (MMP2, MMP7 and MMP9), and tissue inhibitor of metalloproteinases (TIMPs). The excellent result was observed in group treated with *Bergenia ciliata* nanoparticles 10% extract as compared with other treatment groups. As this group showed complete healing in just 12 days and wound contraction was observed up to $93\pm 0.9\%$. While the DC group and the polyfax treated group were healed in 20 and 18 days respectively and their percent wound contraction was observed upto $67.23\pm 1.2\%$ and $84.25\pm 2.40\%$ respectively. The histological analysis also showed best healing in group treated with 10% *Bergenia ciliata* nanoparticles extract as this group was observed having proper formation of epidermis, dermis, subcutaneous layer, large number of sebaceous glands and hair follicles, collagen fibers, fibroblasts, enhanced angiogenesis with reduced ulceration and inflammation after complete healing. The *Bergenia ciliata* nanoparticles with 10% concentration also reduced the level of pro-inflammatory cytokines i.e., TNF- α (19.4 ± 1.5 $\mu\text{g/ml}$), IL-6 (13.8 ± 0.6 $\mu\text{g/ml}$), IL-8 (24.8 ± 1.2 $\mu\text{g/ml}$) with respect to diabetic control group the level was found high i.e., TNF- α (55.0 ± 3.0 $\mu\text{g/ml}$), IL-6 (39.8 ± 1.6 $\mu\text{g/ml}$), IL-8 (70.8 ± 2.8 $\mu\text{g/ml}$) *Department of Zoology, GCU Lahore.* Serum level of MMPs i.e., MMP2 (268.8 ± 3.9 $\mu\text{g/ml}$), MMP7 (266.0 ± 4.4 $\mu\text{g/ml}$), MMP9 (180.8 ± 7.1 $\mu\text{g/ml}$) in group treated with 10% *Bergenia ciliata* nanoparticles was also observed reduced as compare to diabetic control group (MMP2= 591.0 ± 11.9 $\mu\text{g/ml}$, MMP7= 508.8 ± 6.9 $\mu\text{g/ml}$, MMP9= 415.6 ± 5.1 $\mu\text{g/ml}$) ($P<0.001$). The serum level of TIMPs (200.8 ± 5.5 $\mu\text{g/ml}$) was seen elevated in group treated with *Bergenia ciliata* 10% extract as compared with reduced level in diabetic control group (74.2 ± 5.0 $\mu\text{g/ml}$) ($P<0.001$). Hence, it is concluded that silver nanoparticles of *Bergenia ciliata* possess excellent anti-inflammatory, anti-oxidant and anti-microbial properties as they enhanced regeneration, healing process and also reduced the inflammation and ulceration in case of mild and chronic both states of wound. So, *Bergenia ciliata* conjugated silver nanoparticles can be used as an effective and promising treatment for healing of chronic wounds like diabetic foot ulcer (DFU) in normal as well as in diabetic patients with fewer side effects.