Abstract

Diabetes is an insulin-sensitive chronic disorder, characterized by hyperglycemia due to improper functioning of beta-pancreatic cells. Diabetes has become a complex medical concern of 21st century, impacting public health significantly due to its magnified prevalence. Among the severe complications that diabetic patients experience, impaired wound healing is a major concern. Throughout history, natural products containing bioactive compounds have demonstrated remarkable effectiveness in treating various diseases including wound healing. The aims of current research was to analyze the wound healing potential of *Psidium guajava* (PG) and *Ocimum sanctum* (OS) leaf extract and their conjugated silver nanoparticles (AgNPs) against alloxan monohydrate induced diabetes in mice model. The characterization of nanoparticles was performed by UV-Vis spectrophotometer and Fourier-transform infrared spectroscopy (FTIR). The UV-Vis spectrum analysis of PGNP and OSNP showed an absorption peak at 432nm and 428nm, respectively. The FTIR analysis of PGNP and OSNP showed a band shifting due to Ag+ conjugation. In this study, alloxan monohydrate was used to induce diabetes in Swiss albino mice. Following the successful diabetes induction in mice, a biopsy puncture (6 mm) was used to create excision wounds. The diabetic wounds were subjected to treatment with various biomaterials and the healing progress was evaluated through various parameters. Wound healing effects of extracts 5% *Psidium guajava* (PG) and 7% *Ocimum sanctum* (OS) leaf extract were assessed individually and in combinations (5% PG + 7% OS) along with their nanoparticles individually (PGNP and OSNP) and combined form (PGNP+OSNP). This assessment involved parameters including the percentage wound contraction, healing duration, and histological analysis. The study involved the analysis of serum levels of various biochemical markers, i.e., matrix metalloproteinases (MMP 9, MMP7, MMP 2), pro-inflammatory cytokines (TNF-α, IL-8, IL-6), and tissue inhibitors matrix metalloproteinases (TIMPs). The most favorable results were observed with the combination (PGNP+OSNP), demonstrating wound healing within 12 days and achieving a remarkable wound contraction of 100.0±0.0%. Correspondingly, the wounds in the Polyfax group and diabetic control group showed wound contractions of 96.3±1.5% and 95.8±1.4%, respectively, corresponding to the 16th and 18th day. The histological analysis revealed that the combination of nanoparticles (PGNP+OSNP) resulted in increased number of keratinocytes and fibroblasts, along with enhanced growth of collagen fibers, increase blood vessel formation, and reduced inflammation. The application of these

Department of Zoology, GCU Lahore.

extracts, nanoparticles, and their combined formulation demonstrated the ability to normalize disrupted levels of blood serum biomarkers. The combination of nanoparticles (PGNP+OSNP) demonstrated significant reductions in the serum levels of matrix metalloproteinases, including MMP2 (284.4±5.1 pg/ml), MMP7 (268.0±3.4 pg/ml), and MMP9 (180.8±7.1 pg/ml), when compared to the Diabetic control group (MMP2=591.0±11.9 pg/ml, MMP7=508.8±6.9 pg/ml, MMP9=415.6±5.1 pg/ml) ($P<0.001$). The serum level of pro-inflammatory cytokines i.e., TNF-α (20.0±1.1 pg/ml), IL-6 (14.4±0.7pg/ml), and IL-8 (26.2±1.0 pg/ml) in the same group were significantly lower than those in the Diabetic Control group (TNF-α=55.0±3.0 pg/ml, IL-6=39.8±1.6 pg/ml, IL- 8=70.8±2.8 pg/ml) ($P<0.001$). The serum level of TIMPS (209.6±8.4 pg/ml) in this combination group exhibited a considerable increase compared to the diabetic control (74.2±5.0 pg/ml) ($P<0.001$). In conclusion, these nanoparticles synergistically enhance wound healing potential, offering a promising therapeutic approach for chronic wound healing in non-diabetic and diabetic individuals.

Keywords: Diabetic wounds, *Psidium guajava*, *Ocimum sanctum*, Pro-inflammatory cytokines