Abstract

Wound healing is one of the complex biological processes aiming at restoration of tissue structure and function. In addition to components of the host, wound care agents aims at reducing the time of healing and minimizing the complications associated with healing. Latex, an important plant component is widely used in folk medicine to stop bleeding and to facilitate wound healing. However, information regarding the molecules responsible for wound healing and their mechanisms is limited. In this direction, present work provides the biochemical basis and scientific validation for the folk medicinal usage of latex to promote healing, with special reference to proteases. Screening of latices revealed that Apocyanaceae and Euphorbiaceae plant latices contain serine proteases, whereas, Asclepiadaceae latex contains cysteine proteases. Irrespective of the nature, the proteases exhibit procoagulant effect and facilitate the formation of clot, with distinct mechanisms. Cysteine proteases exhibited ‘thrombin-like’ activity, whereas, serine proteases from Euphorbiaceae latices induced platelet aggregation. Based on the hemostatic property and available literature, protease fractions of latices from Wrightia tinctoria, Synadenium grantii, Calotropis gigantea and Pergularia extensa were selected for wound healing studies using excision wound model in mice. Latex proteases promoted healing (W. tinctoria > P. extensa > C. gigantea > S. grantii), which was supported by wound contraction rate and collagen content in granulation tissues. The results were compared with reference proteases (trypsin and papain) and standard drug. Role of proteases was confirmed by heat inactivation and inhibition studies. Further, the mechanisms of action of latex proteases were evaluated by their MMP-like and plasmin-like activities. Based on the efficacy, non-toxic nature and ethnopharmacological usage, W. tinctoria latex proteases were selected for detailed
study of wound healing by evaluating physical, biochemical and histological parameters. The involvement of proteases in promoting wound healing was confirmed by the inability of inhibitor pre-treated and heat inactivated protease to promote healing. Wound treated with W. tinctoria latex proteases showed significant healing, compared to control wounds. The finding was supported by enhanced collagen content and increased activities of MMP and catalase. Restoration of normal skin structure also supported the findings. Assessment of toxicity of W. tinctoria latex serine proteases was done in comparison with cysteine proteases of P. extensa latex and metalloproteases from Echis carinatus venom. Proteases from either source efficiently hydrolyzed fibrin and fibrinogen. However, latex proteases non-specifically hydrolyzed the sub units of these proteins. Further, latex cysteine proteases and snake venom metalloproteases induced hemorrhage (upon intradermal injection) and myotoxicity (upon intramuscular injection). The findings were supported by histological and biochemical evaluations. In contrast, latex serine proteases did not induce hemorrhage and myotoxicity, even at higher concentrations. Based on these findings, assessment of latex proteases in terms of efficacy, toxicity and route of administration will be an important aspect prior to exploiting their pharmacological properties.