

IDENTIFICATION, CLONING AND FUNCTIONAL CHARACTERIZATION OF A  
NOVEL DERMONECROTIC TOXIN (PHOSPHOLIPASE D) LiRecDT6 FROM  
BROWN SPIDER (*Loxosceles intermedia*) VENOM.

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Brown spider bites are associated with lesions including dermonecrosis, gravitational spreading and a massive inflammatory response, along with systemic problems that may include hematological disturbances and renal failure. The mechanisms by which the venom exerts its noxious effects are currently under investigation, while it is known that the venom contains a major toxin (dermonecrotic toxin, biochemically a phospholipase D) that can experimentally induce dermonecrosis, inflammatory response, animal mortality and platelet aggregation. Herein, we describe cloning, heterologous expression and purification of a novel isoform of the dermonecrotic toxin: LiRecDT6. The recombinant protein stably expressed in *Escherichia coli* cells was purified in a single step producing a soluble protein of 32kDa. The recombinant toxin was recognized by whole venom serum antibodies and by a specific antibody to dermonecrotic toxin LiRecDT1. Also, recombinant isoform displayed lipase activity, induced experimental skin lesions and caused a massive inflammatory response in rabbit skin dermis. Additionally, LiRecDT6 evoked platelet aggregation, increased vascular permeability as well as caused death in mice. These results corroborate that a family of dermonecrotic toxins exists, and includes a novel member that can be useful for future structural and functional studies.