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Title: Antiplasmodial Screening of Snake Venom Fractions: Efficacy and Cytotoxicity Assessment

Abstract: The spread of multidrug-resistant *Plasmodium falciparum* poses a major challenge to malaria control, underscoring the urgent need for novel therapeutic agents. Snake venoms (SVs) contain diverse bioactive molecules, including phospholipases A2, metalloproteinases, and serine proteases, many of which display therapeutic potential. Here, we investigated SVs fractions as a source of antimalarial leads by screening crude SVs and their fractions for (i) antiplasmodial activity against erythrocytic stages of *P. falciparum* and (ii) cytotoxicity and hemolysis.

Two sample sets were analyzed. Set 1 comprised a panel of *Bothrops jararaca* fractions, including crude venom and a pooled fraction, and was evaluated for hemolysis and cytotoxicity in THP-1 monocytes and V79 fibroblasts. Set 2 included the remaining *B. jararaca* fractions and the *Bothrops atrox* panel and was assessed for cytotoxicity in THP-1 and U937 cell lines; hemolysis was not assessed for Set 2. All samples were screened against the *P. falciparum* 3D7HT-GFP clone. Fractions showing >70% inhibition were selected for further determination of IC_{50}.

Across both sets, only fraction P1 and the Pool inhibited parasite growth by >70% and their IC_{50} profiles suggest an effect on egress and/or reinvasion. In Set 1, no fraction caused significant hemolysis (<10%). P1 and the Pool exhibited low cytotoxicity in V79 cells, whereas only the crude venom was highly toxic to THP-1 cells. Set 2 presented consistently higher cytotoxicity in U937 cells than in THP-1 cells.

These findings identify a SVs fraction with selective antiplasmodial activity, supporting further investigation as a lead for antimalarial development. Although most fractions did not present inhibitory activity against *P.* falciparum, cytotoxicity profiling provides an essential safety baseline and will support prioritization of fractions for follow-up antiparasitic screens. Ongoing work will expand fraction testing and identify active components.