Plant-based treatment of snakebites

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Snakebite is listed as a neglected tropical disease by the WHO. Up to 2.5 million people are bitten each year, resulting in up to 125,000 deaths, and 400,000 permanent disabilities due to tissue necrosis. We investigated 94 plant species from Africa, which are used in traditional medicine to treat snakebites, for inhibitory activity against the necrosis enzymes hyaluronidases, phospholipase A$_2$ and proteases. Six species, which were active against one or more of the necrosis enzymes after tannins were removed, were investigated for their ability to penetrate skin and for their wound healing activity in the scratch assay. The extracts could not pass the skin membrane and showed cytotoxicity in the cell scratch assay. Therefore, none of these extracts can be recommended for treatment of snakebites – which is in line with official guidelines on not using plant extracts on snakebites.

Keywords: Africa, Necrosis enzymes, Snakebites, Skin permeability, Tissue necrosis, Wound healing

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Snakes have been feared by humans of all cultures from before mythological times, and with good reason as many snakes are deadly venomous. The majority of the World’s dangerous snakes occur in developing countries, where access to treatment is limited. It is estimated that 1.8-2.5 million people are bitten annually, resulting in up to 125,000 deaths, and 400,000 permanent disabilities from severe complications, which may lead to amputation$^{1,2}$. The WHO has in 2009 declared snakebites ‘a neglected tropical disease’.

When bitten, the first choice of treatment should always be mono- or polyvalent antiserum. However, many of the incidences occur where antiserum therapy is not easily accessible$^3$ and antiserum does not provide protection against local tissue damage$^4,5,6$. Snake venoms contain compounds with specific effects on the nervous system, coagulation of the blood or on the cardiac muscle, but also enzymes causing necrosis of the tissue, which causes the complications leading to amputation.

The inaccessibility of hospital care results in about 80% of snakebite victims first being seen by a traditional practitioner, and only subsequently treated with anti-venom therapy$^3$. The traditional treatment is most often plant-based, and the traditional practitioners have extensive knowledge of which plant species are useful against snakebites$^7,8$. We have complied a database with over 1500 plants reported to be used traditionally against snakebites worldwide, based on available literature from PubMed, Web of Sciences and Science Direct$^9$.

Results and discussion

Sub-Saharan Africa is one of the regions of the world that is affected by snakebites, which prompted us to investigate the medicinal plants used for snakebites. Africa is a large continent with varying habitats. In order to try and cover several geographical areas, we investigated snakebite-plants from West Africa (Mali), Central Africa (DR Congo) and Southern Africa (South Africa).

As anti-serum should always be the first-choice treatment for the fatal effects of envenomation, we deliberately did not look for plants to counter these effects, but instead we focused on a possible anti-necrosis activity of the plant extracts. The tissue necrosis is caused by enzymes from the classes hyaluronidase, phospholipase A$_2$ and protease.

Water and ethanol extracts, in total 226 extracts, from 94 plant species collected in Mali, DR Congo or South Africa were tested in phospholipase A$_2$-, protease- and hyaluronidase enzyme assays using
venom from *Bitis arietans* and *Naja nigricollis* as enzyme source. *B. arietans* and *N. Nigricollis* (Fig. 1) are the two snakes that cause most of the envenomation in Sub-Saharan Africa.

Forty of the tested species exhibited more than 90% inhibition in one or more assay. The IC$_{50}$ value were determined for these extracts, and those extracts that had IC$_{50}$-values below 100 µg/ml were selected for HPLC fingerprinting. The chromatography was done on an analytical C18 column with a gradient of 5-95 % MeOH over 30 min. The fingerprint revealed the presence of tannins in many of the samples, as a hump eluting with a retention time of 5-20 min. Tannins are a problem, as they unspecifically bind to proteins, and thereby may show non-specific (false-positive) activity in enzyme assays. The tannins were therefore removed by passing the extracts through Oasis HLB 3 cc (60 mg) cartridges (Waters). It was then investigated whether the extracts had retained their activity.

Water extracts of aerial parts of *Pupalia lappacea* Juss. (Amaranthaceae), leaves of *Combretum molle* R.Br. ex G.Don (Combretaceae) and *Strrychnos innocua* Delile (Loganiaceae), bark of *Grewia mollis* Juss. (Malvaceae) and roots of *Bauhinia thomningii* Schum. (Fabaceae) and the ethanol extract of bark of *Lannea acida* A.Rich. (Anacardiaceae) were active in either the hyaluronidase or protease bioassays after removal of tannins, indicating that they besides tannins also contained specific enzyme inhibitors.

These six extracts were selected for further investigations. They were tested for their ability to heal a wound in the so-called scratch assay, where a cell mono-layer of mouse fibroblasts are scratched to produce an artificial wound, and the closing of the wound is then monitored. The extracts were tested at two concentrations, 10 and 100µg/ml. At the highest concentration, the extracts of *C. molle*, *G. mollis* and *S. innocua* were cytotoxic. All extracts had a negative effect on closing of the scratch, except the extracts of *L. acida* and *S. innocua*, which at 10µg/ml had a slight, though not significant, positive effect. The plants are traditionally applied as poultices, which would result in a relatively high concentration of ‘plant extract’ at the bite site, so it is not very positive to see that the extracts were cytotoxic, as that would have a detrimental effect on the necrotic wound.

Right after a bite, the skin is still intact except for the two puncture marks from the fangs. Therefore, active compounds from the poultice would have to be able to pass the skin barrier to have an effect. The permeability of the six extracts across the skin barrier was tested in the Franz cell set up. It was tested by HPLC whether any metabolites passed through the skin, and it was also tested in the enzyme bioassays if any active compounds had passed. No active compounds from any of the extracts were able to permeate the skin. The *G. mollis* extract was also investigated in an *ex vivo* model testing venom neutralization in porcine skin biopsies. In this study, we opted to inject the plant extract into the tissue, to overcome the inability of the active compounds to pass the skin barrier. The extract was not able to prevent the tissue damage caused by the injected venom.

Thus, these extracts would not be of much use immediately after the bite, as the active compounds cannot pass through the skin. If used when a necrotic wound forms, they might cause more damage, as they show cytotoxicity. Overall, none of these extracts can be recommended for treatment of snakebites – which is in line with official guidelines on not using plant extracts on snakebites.

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