Towards a yellow tulp vaccine: preliminary studies exploiting the potential for cross-reactivity with related bufadienolides

Isa H. I.[1,3], *Ferreira, A.*[1], *Crafford J.E.*[2], *Botha C.J.*[1]

[1] Department of Paraclinical Sciences, University of Pretoria, South Africa
[2] Department of Veterinary Tropical Diseases, University of Pretoria, South Africa
[3] Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria, Nigeria

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Veterinary Science

Introduction

Cardiac glycoside poisoning

Poisoning by *Moraea pallida* Bak. (yellow tulp) (Fig 1) is the most important of all cardiac glycoside induced toxicoses which collectively account for 33% and 10% deaths in large and small stock due to plant poisoning, respectively, in the Republic of South Africa (Kellerman *et al*., 1996). Yellow tulp contains epoxyscillirosidine (Fig. 2), a bufadienolide type of cardiac glycoside (Enslin *et al*., 1966)

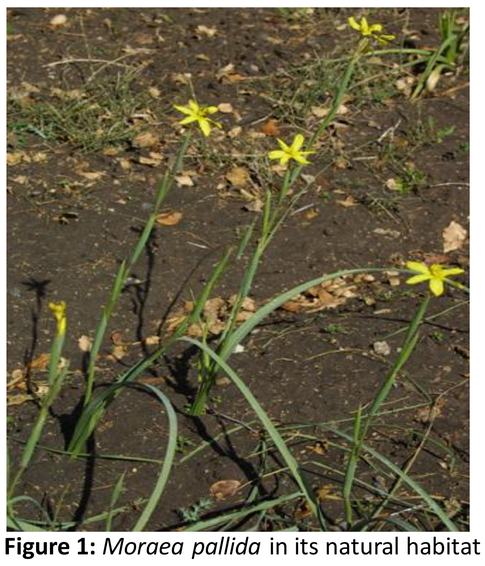

Figure 1: *Moraea pallida* in its natural habitat

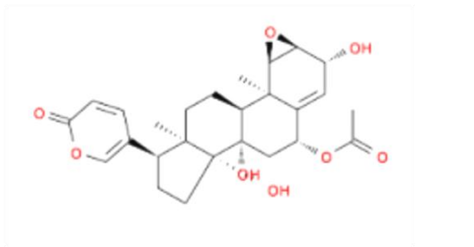

Figure 2. Structure of epoxyscillirosidine

Management of poisoning

- Treatment
  Oral administration of activated charcoal is the most effective
- Prevention
  - Eradication of yellow tulp is difficult, costly, involve changing the environment (e.g. herbicides, mechanical).
  - Conditioned feed aversion has been found to be a natural mechanism for protecting stock against poisoning.
  - Potential alternative: immunoprophylaxis with vaccines.

Aim of the study

- Ascertain if antibodies raised against related bufadienolides will cross-react with epoxyscillirosidine.

Objectives

To:

- synthesize epoxyscillirosidine, bufalin and proscillaridin conjugates, to immunize animals.
- evaluate efficacy of the synthetized conjugates in inducing an immunological response.
- determine the degree of cross-reactivity between the different generated antibodies.

Methods and Materials

- Epoxyscillirosidine was extracted, isolated, purified and confirmed using ^{13}C NMR spectroscopy.
- Bufalin and proscillaridin were purchased and together with epoxyscillirosidine were coupled to bovine serum albumin (BSA), ovalbumin (OVA) and keyhole limpet haemocyanin (KLH) to render them immunogenic (Fig. 3).

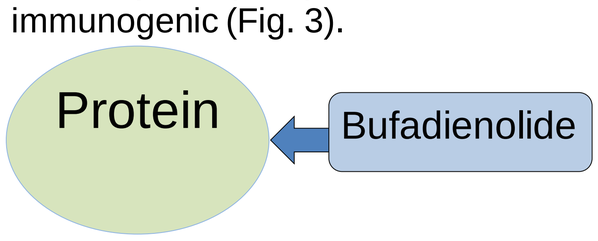

Figure 3: Synthesis of immunogens

- The immunogens (4 mg/ml) were emulsified with an equal volume of Montanide ISA, as an adjuvant (Fig. 4).
- Adult male New Zealand White rabbits (n=15) were randomly assigned to 5 equal groups. Rabbits in groups I, II, III and IV were vaccinated with proscillaridin-BSA, bufalin-BSA, epoxyscillirosidine-KLH and epoxyscillirosidine-BSA conjugates, respectively. Group V served as control where animals were administered BSA only (Fig. 5).

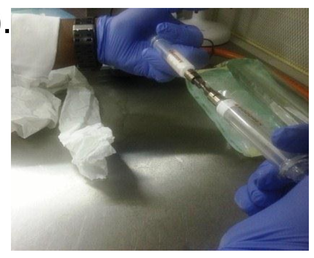

Figure 4: Preparation of vaccine

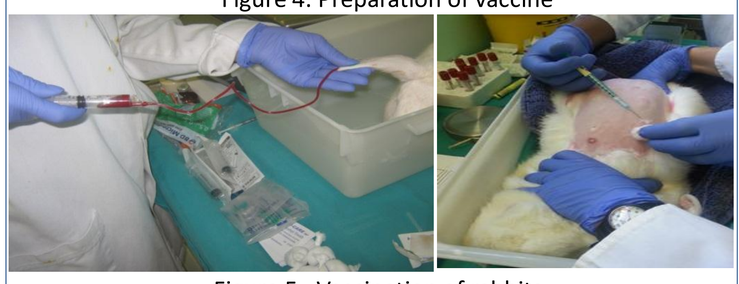

Figure 5 : Vaccination of rabbits

- The rabbits were vaccinated on Days 0, 21 and 42 by intradermal injection of 0.1ml of the vaccine at four sites on the dorsum.
- Blood was collected prior to each vaccination and on Day 67.
- An ELISA was performed to determine antibody response.

Results

- Experimental animals developed varying titres of antibodies against proscillaridin, bufalin and epoxyscillirosidine.
- Furthermore, proscillaridin and bufalin antibodies cross-reacted with epoxyscillirosidine (Fig. 6).
- Toxic signs were similarly absent in all the rabbits. However, all rabbits developed reactions at the injection sites, characterized by reddening and slight oedema a day after vaccine administration
- No rabbit died following the administration of immunogens.

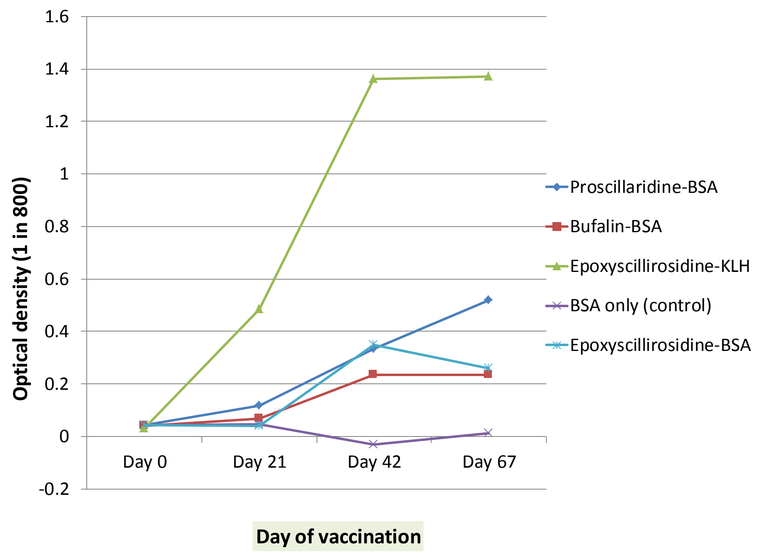

Figure 6: Optical density values showing degree of reactivity between proscillaridin, bufalin and epoxyscillirosidine antibodies and epoxyscillirosidine in an ELISA

Discussion

Antibodies could be raised in rabbits against bufadienolide conjugates evaluated herein. Antibodies were synthesized against proscillaridin-BSA, bufalin-BSA, epoxyscillirosidine-BSA and epoxyscillirosidine-KLH conjugates. The antibody response was however variable. The KLH conjugate of epoxyscillirosidine induced the highest response. This is likely because KLH is a better carrier protein due to its larger molecular mass, complexity and foreignness which are all properties that determine the degree of immunogenicity of a compound (Kuby, 1994) Antibodies against specific haptens were raised in earlier studies (Landsteiner, 1945). Butler and Chen (1967) first described the production of antibodies against cardiac glycosides. They raised and investigated antibodies specific for digoxin for possible use in immunotherapy (Digibind®). Cross-reactivity among cardiac glycosides has also been reported. Belz *et al*., (1973) demonstrated cross-reactivity between oubain, digoxin and digitoxin with proscillaridin. Antibodies against proscillaridin bound oubain, digoxin and digitoxin, albeit with low binding activity.

Conclusions

- Antibodies were successfully raised against proscillaridin, bufalin and epoxyscillirosidine.
- Furthermore, antibodies against proscillaridin and bufalin cross-reacted with epoxyscillirosidine. However, the degree of cross-reactivity was low.
- The immunogenicity of the bufadienolides may be enhanced by optimizing the vaccines to induce a stronger response.
- The antibodies will be evaluated to determine their neutralization and cross-neutralization efficacy against epoxyscillirosidine *in vitro*

Contact

Hamza Ibrahim Isa
Email:ihamzaisa@yahoo.com

References

1. Enslin, P.R., Naudé, T.W., Potgieter, D.J.J. and Van Wyk, A.J. (1966) '1α, 2α-Epoxyscillirosidine, the main toxic principle of *Homeria glauca* (Wood and Evans) N.E. Br', *Tetrahedron* 22: 3213–3220.
2. Kellerman, T.S., Naudé, T.W. and Fourie, N. (1996). The distribution, diagnoses and estimated economic impact of plant poisonings and mycotoxicoses in South Africa. *Onderstepoort Journal of Veterinary Research* 63: 65–90
3. Belz, G. G., Brech, W. J., Kleeberg, U. R., Rudofsky, G and Belz, G. (1973).Characterization and specificity of proscillaridine antibodies. *Archives of Pharmacology*, 279: 105- 114
4. Butler, V. P. and Chen, J. P. (1967). Digoxin-specific antibodies. *Proceedings of the National Academy of Sciences of the United States of America*, 57(1): 71-78
5. Kuby, J. (1994). Immunology. W. H. Freeman and Company, New York
6. Landsteiner, K.. (1945). The Specificity of Serological Reactions. Cambridge, Massachusetts: Harvard University Press