

## Raising Antibodies against Epoxyscillirosidine, the Toxic Principle Contained in *Moraea pallida* Bak. in Rabbits

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**Abstract :** *Moraea pallida* Bak. (yellow tulip) poisoning is the most important plant-induced cardiac glycoside toxicosis in South Africa. Cardiac glycoside poisonings collectively account for about 33 and 10 % mortalities due to plants, in large and small stock respectively, in South Africa. The toxic principle is 1 $\alpha$ , 2 $\alpha$ -epoxyscillirosidine, a bufadienolide. The aim of the study was to investigate the potential to develop a vaccine against epoxyscillirosidine. Epoxyscillirosidine and the related bufadienolides proscillaridin and bufalin, which are commercially available, were conjugated to the carrier proteins [Hen ovalbumin (OVA), bovine serum albumin (BSA) and keyhole limpet haemocyanin (KLH)], rendering them immunogenic. Adult male New Zealand White rabbits were immunized. In Trials 1 and 2, rabbits (n=6) were, each assigned to two groups. Experimental animals (n=3; n=4) were vaccinated with epoxyscillirosidine-OVA conjugate, while the control (n=3; n=2) were vaccinated with OVA, using Freund's complete and incomplete and Montanide adjuvants, for Trials 1 and 2, respectively. In Trial 3, rabbits (n=15), randomly allocated to 5 equal groups (I, II, III, IV and V), were vaccinated with proscillaridin-BSA, bufalin-BSA, epoxyscillirosidine-KLH, epoxyscillirosidine-BSA conjugates, and BSA respectively, using Montanide as adjuvant. Vaccination was on Days 0, 21 and 42. Additional vaccinations were done on Day 56 and 63 for Trial 1. Vaccination was by intradermal injection of 0.4 ml of the immunogen (4 mg/ml [Trial 1] and 8 mg/ml for Trials 2 and Trial 3, respectively). Blood was collected pre-vaccination and at 3 week intervals following each vaccination. Antibody response was determined using an indirect ELISA. There was poor immune response associated with the dose (0.4 mg per rabbit) and adjuvant used in Trial 1. Antibodies were synthesized against the conjugate administered in Trial 2. For Trail 3, antibodies against the immunogens were successfully raised in rabbits with epoxyscillirosidine-KLH inducing the highest immune response. The antibodies raised against proscillaridin and bufalin cross-reacted with epoxyscillirosidine when used as antigen in the ELISA. The study successfully demonstrated the synthesis of antibodies against the bufadienolide conjugates administered. The cross-reactivity of proscillaridin and bufalin with epoxyscillirosidine could potentially be utilized as alternative to epoxyscillirosidine in future studies to prevent yellow tulp poisoning by vaccination.

**Keywords :** antibodies , bufadienolides, cross-reactivity, epoxyscillirosidine, *Moraea pallida*, poisoning

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