A preliminary study of chemistry and toxicology of a natural antiprotozoal agent.

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Introduction

- **Recently, there has been an increased interest in plant secondary metabolites for use as possible defaunating agents.**
- **Foliage from a variety of different leguminous trees have been tested *in vitro* and *in vivo* for antiprotozoal activity and it was suggested that saponins in the foliage were the antiprotozoal agent (Leng *et al*., 1992).**
- **Saponins are secondary compounds that function in plant defence against herbivores.**
- **The major objectives of these preliminary studies were to understand the chemistry and toxicology of the natural antiprotozoal agents present in *Enterolobium cyclocarpum* Griseb focusing on the isolation of the active agent, the membrane lytic effects of agent on sheep red blood cells and more particularly rumen protozoa and the proposed structural elucidation**

Methods

- The active agents were isolated by maceration of the water-soluble compounds from the dried leaves of *E. cyclocarpum,* followed by partitioning between water and n-butanol layer.
- A further fractionation process of the leaf extracts was guided by two bioassays, haemolytic assay and antiprotozoal assay *in vitro*.
- A series of haemolysis assays was undertaken to assay the lytic power of a fraction by determining the quantity required to lyse 50% of the red blood cells. The extent of lysis was determined spectrophotometrically.
- Antiprotozoal activity was assessed by: (1) measurement of decreased motility by subjective appraisal under the microscope and, (2) measurement of the degree of disintegration of protozoa by microscopic examination.
- The molar concentration of the active agents was approached by determination the type and quantity of the sugar contents by GLC based on the assumption that the analysed fractions contained only saponins with the aglycone machaeranic acid lactone. GLC analysis of TMS derivatives of monosaccharides liberated by acid hydrolysis was employed to determine the structure of the active agents.

Results

- It appears that the saponin extract exhibited strong haemolytic activity, however, the haemolytic activity may not reflect a parallel ability to lyse rumen protozoa (Table 1).
- The butanol extract of *E. cyclocarpum* showed relatively high ability to immobilise and lyse the protozoa cells (Table 2). Their activities were not affected after fractionation.
- It appears that the saponin molecule is unlikely substituted with more than one sugar.

Table1. The haemolytic activity of butanol extract (Extract) relative to white saponin and Alkanate 3SL3

Amount (μg)	% Lysis		
	Extract	White saponin	Alkanate 3SL3
100	19.0	85.2	1.0
250	57.1	91.5	32.9
500	90.1	92.1	91.2
750	93.5	103.8	94.4

Table 2. The lytic activity of butanol extract (Extract) on rumen protozoa compared to Alkanate 3SL3

Lytic agent	Conc. (mg/ml)	Motility*	Protozoa Number (10^4 ml^{-1})	% Lysis
Extract	1	+ + + +	120	57.1
	2	+ + + +	72	74.3
	5	+ + + +	32	88.6
Alkanate 3SL3	1	+ + + +	224	0.2
	2	+ + + +	152	45.7
	5	+ + +	71	74.6
Control		0	280	0

* The control appeared completely normal, the more (+) signs, the greater the effect on motility

Conclusions

- **Saponin fractions in *E. cyclocarpum* showed strong haemolytic and antiprotozoal activity.**
- **It appears that the structures of the active agents is unlikely to be substituted with more than one sugar. Glucose seems to be predominant follow by rhamnose, arabinose and xylose, while galactose is not present in all fractions.**
- **Further work aims to evaluate the relationship between structure of individual, purified saponins and activity against rumen protozoa.**

Reference

Leng, R.A., Bird, S.H., Klieve, A., Choo, B.S., Ball, F.M., Asefa, G., Brumby, P., Mudgal, V.D., Chaudhary, U.B., Haryono, S.U., Hendratno, N., 1992. FAO Animal Production Health Paper 102, pp. 177-191