

CARDIAC GLYCOSIDE POISONING IN SHEEP CAUSED BY *URGINEA PHYSODES* (JACQ.) BAK. AND THE ISOLATED PHYSODINE A

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ABSTRACT

NEL, P. W., SCHULTZ, R. ANITRA, JORDAAN, P., ANDERSON, L. A. P., KELLERMAN, T. S. & REID, CLARE, 1987. Cardiac glycoside poisoning in sheep caused by *Urginea physodes* (Jacq.) Bak. and the isolated physodine A. *Onderstepoort Journal of Veterinary Research*, 54, 641-644 (1987)

Urginea physodes (Jacq.) Bak., a species closely related to or possibly synonymous with *U. pusilla*, is described and its distribution given.

Four bufadienolides were isolated from *U. physodes* and the approximated LD₅₀ and cumulative effect of some of them determined in guinea pigs. The most toxic one proved to be mildly cumulative.

Typical signs of acute cardiac glycoside poisoning, involving the locomotory, gastro-intestinal, respiratory and cardiac systems, were seen in the field cases and/or were experimentally induced by the plant. Similar signs could also be induced by injecting the isolated bufadienolide, physodine A, to a sheep.

INTRODUCTION

The plant families Iridaceae, Liliaceae, Melianthaceae and Crassulaceae contain many species having bufadienolides (cardiac glycosides) as their toxic principles (Naudé, 1977). In the Liliaceae, bufadienolides were isolated from *Urginea rubella* (Louw, 1949), *U. burkei* (= *U. sanguinea*) (Louw, 1952), *U. depressa* (Rees, Schindler & Reichstein, 1959) and *U. altissima* (Lichti & Von Wartburg, 1960). As in the case of the other *Urginea* species, *U. physodes* is colloquially known as "slangkop" from the snake-like appearance of the flowers. These appear before the leaves in spring.

The toxicity of *U. physodes* and its bufadienolides were investigated after stock had died of suspected "slangkop" poisoning on veld where the plant was present.

DESCRIPTION, DISTRIBUTION AND ECOLOGY OF THE PLANT

Family: Liliaceae.

Name: *Urginea physodes* (Jacq.) Bak.

Common name: None specific, but most *Urginea* species are known as "slangkop".

Description: (Fig. 1 a & b) Bulb large, globose, c. 4-5 cm in diameter, prolonged into a short neck, inner tunics white, fleshy, outer tunics golden-brown, papery. Leaves 6-12, produced long after the flowers, lanceolate, c. 10-14 cm long, c. 15-25 mm wide, glabrous, darkgreen. Peduncle several per bulb, terete, 5-15 cm long, 1-2 mm in diameter, purplish. Raceme dense, many-flowered, 4-10 cm long, 4-5 cm in diameter, capitate when young. Pedicels subpatent, 12-18 mm long. Bracts small, ovate, not distinctly spurred, membranous. Perianth segments spreading, 6 mm long, white, purple-keeled. Stamens shorter than the perianth, filaments papillose basally. Capsule ovoid, 8-10 mm long, dark-green. Seeds oblong, c. 8 mm long, 5 mm wide, black, smooth (Baker, 1897).

Flowering time: December-February, also earlier or



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FIG. 1a & b *Urginea physodes* (Jacq.) Bak.

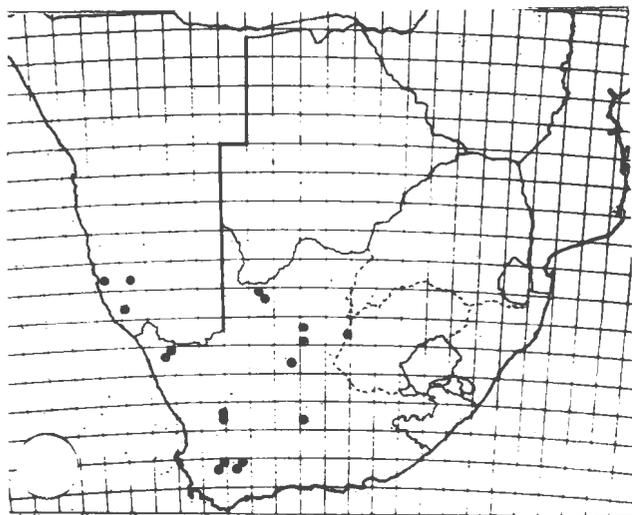


FIG. 2 Distribution of *U. physodes* (Jacq.) Bak. in southern Africa.

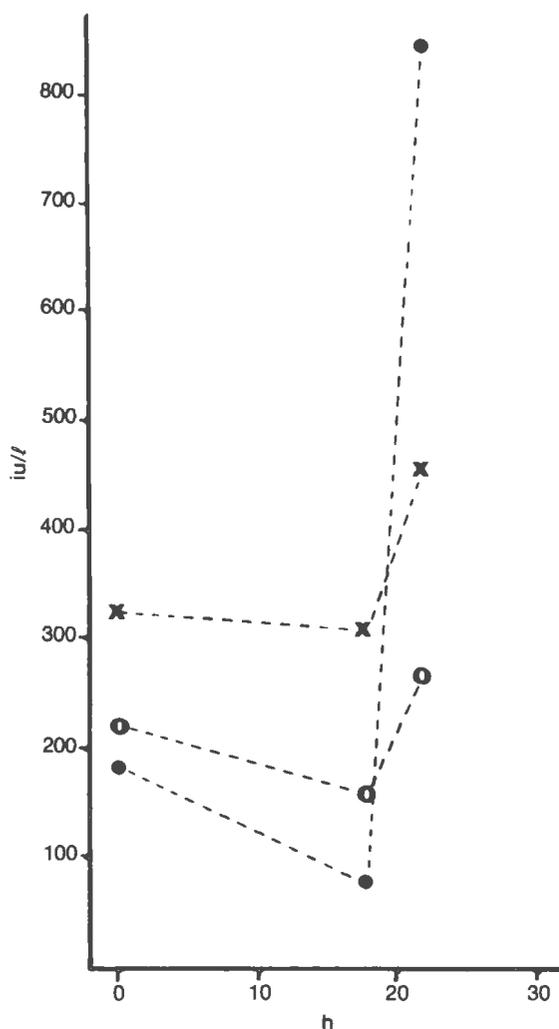


FIG. 3 Increased LD (x-x), HBDH (o-o) and CK (e-e) activity in Sheep 3 after being dosed with *U. physodes* bulbs.

later in summer. Flowers open in the late afternoon, about 15h00, and close in the evening, about 20h00, lasting only one day. Since of the numerous flowers in the inflorescence only about five open each day, the flowering period is fairly prolonged.

Note: The genus is at present under revision. *U. physodes* is currently regarded as being closely related to *U.*

pusilla (Jacq.) Bak. and it is possible that in future the 2 species will be regarded as synonymous.

Ecology: Alluvial washes at mountain bases, or compacted gravel on pan margins.

Distribution: The specific localities are cited (Fig. 2).

HISTORY

In a letter to the State Veterinarian, Bloemfontein, in 1979, J. P. J. Joubert (State Veterinarian, Middelburg, C.P.) mentioned that a farmer in the Petrusburg area had lost 3 cattle after they had been on the farm for 5 days. The cattle had suffered from a haemorrhagic diarrhoea and a plant, suspected of being responsible for their deaths, was identified as *U. physodes*.

One of the authors (P. Jordaan) also reported a field case of suspected *U. physodes* poisoning during June 1984 in the Upington district. Twenty-eight Dorper sheep died within a week after *U. physodes*, which was the only greenery in the camp, had been heavily grazed. The clinical signs were anorexia, apathy, arrhythmia, weakness in the hindquarters, paralysis, semi-coma and death. At necropsy, subendocardial haemorrhages in the left ventricle were seen.

MATERIALS AND METHODS

Plant material

Fresh *U. physodes* bulbs from the 'toxic' farm in the Upington area were dried in the shade and stored at room temperature.

Isolation of the toxic principles

The bulbs (26 kg) were crushed and extracted 3 times with ethyl acetate in a Waring blender. The solvent was evaporated under reduced pressure on a water-bath to yield a syrup (179 g). Treatment of the syrup with petroleum ether removed 50 g of non-toxic material. The residue was dissolved in acetone (1ℓ) and left overnight at room temperature. Crystalline material (15 g), non-toxic to guinea pigs (see Dosing trials), was filtered off. The filtrate was evaporated down on a water-bath under reduced pressure to yield a syrup which was chromatographed on a silica gel column (1 kg). Further sequential elution with chloroform yielded non-toxic material. Elution with chloroform-methanol (95:5, v/v) and chloroform-methanol (90:10, v/v) yielded toxic material (48 g). The final elution with methanol yielded non-toxic material.

Repeated chromatography of the toxic concentrate on silica gel, using chloroform-methanol (90:10, v/v) and chloroform-acetone-methanol-water (70:30:10:2, v/v/v/v) eventually yielded 4 colourless crystalline compounds. The trivial names of physodine A, B, C and D are suggested for these compounds.

Reagents and apparatus

Melting points were done on a Büchi melting-point apparatus. Ultra-violet absorption refers to methanol, nuclear magnetic resonance (NMR) to CDCl_3 solutions and infra red (IR) to KBr discs. UV absorptions were measured on a Shimadzu UV-260 spectrophotometer and IR spectra on a Perkin Elmer 257 spectrophotometer. ^1H NMR spectra were recorded at the National Chemical Research Laboratory of the CSIR on a Bruker WM-500 (500 MHz) spectrometer. Merck silica gel (0,063–0,200 mm) was used for column chromatography. Pre-coated silica gel 60 F₂₅₄ TLC aluminium sheets were used for thin layer chromatography. The plates were developed with chloroform-methanol (90:10, v/v) or chloroform-acetone-methanol-water (70:30:10:2, v/v/v/v) and the spots were visualized by spraying with 85% sulphuric acid and heating for 1 min. at 120 °C.

TABLE 1 Observations on guinea pigs intoxicated by subcutaneous injection of bufadienolides isolated from *U. physodes*

Bufadienolide	24 h LD ₅₀ mg/kg	Cumulative effect	
		25 % LD ₅₀	50 % LD ₅₀
Physodine A	c. 0,22	Mild changes occurred after 3 doses	Progressive clinical signs were seen from Day 1 onwards. One died after 4 doses
Physodine B	c. 1,0	Not tested because of relatively low toxicity	
Physodine C	> 100	ditto	
Physodine D	> 100	ditto	

TABLE 2 Intoxication of sheep with dried *U. physodes* bulbs

Sheep No.	Dosing regimen	Clinical signs	ECG changes	Fate	Pathology
	g/kg × n				
1	5 × 1	Not observed	—	Died overnight (Day 0)	—
2	2 × 1	Forced abdominal respiration, inappetence, ruminal stasis and watery diarrhoea (Day 1). Increased serum urea (Day 2)	—	Treated with 5 g/kg of activated charcoal on Day 2 and discharged on Day 5	—
3	3 × 1	Forced respiration, foaming at mouth, inappetence, ruminal stasis, diarrhoea (17 h), followed by bloating and cyanosis (19 h). Shallow and deep irregular respiratory movements (20 h—death). Increased serum urea (17 h) and HBD, LD and CK (20,5 h)	Tachycardia (19 h), AV-block with intermittent coupled rhythm (20 h—death) transient ventricular tachycardia and atrial flutter	Died after 22 h	Mild congestion, oedema and diffuse emphysema of the lungs. Severe bloat

TABLE 3 Observations on a sheep intoxicated with the bufadienolide physodine A by intravenous injection

Experimental time	Dose (g/kg × h)	Clinical signs	ECG changes	Fate
0	0,09 × 1	Ruminal stasis, grinding of teeth, forced respiration	Tachycardia	
14 min				
32 min				
1 h 49	0,18 × 1		After 2nd dose firing of ectopic foci, followed by AV dissociation and ventricular tachycardia	
1 h 54				

Dosing Trials

Oral administrations were carried out by dissolving the toxins in 0,1–0,2 ml of propylene glycol and adding 5 ml of water. Subcutaneous administrations were carried out by dissolving the toxins in 1–5 ml of ethyl alcohol and diluting it with saline.

Guinea pigs. The toxicity of the plant extracts and fractions was monitored by dosing them to weaned guinea pigs, while the acute and cumulative effects of the 4 physodines (Table 1) were assayed in young (c. 200 g) male albino guinea pigs. The approximate subcutaneous 24 h LD₅₀ of each bufadienolide was determined (2 animals/dose) and the cumulative effect by the subcutaneous injection of 25 % and 50 % of the LD₅₀/day for 4 days (4 animals/dose).

Sheep. Three Merino wethers and 1 ewe (milk-tooth) with live masses varying between 15 and 22 kg were dosed per stomach-tube with minced bulbs (Table 2) or injected intravenously with the isolated toxins (Table 3). The sheep were examined regularly, and electrocardiographic (ECG) changes were recorded. The following standard chemical pathological determinations were done periodically on the blood: erythrocyte sedimenta-

tion rate, haematocrit, haemoglobin and serum glucose, urea nitrogen, aspartate transaminase (AST), γ -glutamyltransferase (GGT), calcium, sodium, potassium, magnesium, lactate dehydrogenase (LD), α -hydroxybutyrate dehydrogenase (HBD) and creatine kinase (CK).

Autopsies were performed as soon as possible after death.

RESULTS

Chemistry of toxic principles

Since no sharp melting-points could be obtained, melting-points were not recorded.

Physodine A: This component (1,1 g) was obtained as white crystals from methanol-ether, λ_{\max} (MeOH) 299 nm; ν_{\max} (KBr) 3450, 2940, 1705, 1630, 1535, 1245 and 835 cm⁻¹. $R_f = 0,154$ (chloroform-acetone-methanol-water, 70:30:10:2, v/v/v/v). It gave a pink Liebermann colour reaction, changing to light-blue in 5 s and blue after 1 min.

Physodine B: The component (1,43 g) was obtained as white crystals from methanol-ether, λ_{\max} MeOH 299 nm; ν_{\max} (KBr) 3500, 3490, 2940, 1715 (br), 1630,

1535, 1240 and 835 cm^{-1} . $R_F = 0,118$ (chloroform-acetone-methanol-water, 70:30:10:2, v/v/v/v). It gave a powder-blue Lieberman colour reaction changing through sea-green to blue within 1 min.

Physodine C: The component (0,25 g) was obtained as white crystals from methanol-ether, λ_{\max} MeOH 299 nm; ν_{\max} 3400, 2925, 1720, 1700, 1630, 1535, 1240, 1250, 835 cm^{-1} . $R_F = 0,09$ (chloroform-acetone-methanol-water, 70:30:10:2, v/v/v/v). No Lieberman colour reaction was obtained.

Physodine D: This component (0,46 g) was obtained as white crystals from methanol-ether, λ_{\max} (MeOH) 299 nm; ν_{\max} 3420, 2925, 1715, 1630, 1530, 1240 and 830 cm^{-1} . $R_F = 0,068$ (chloroform-acetone-methanol-water, 70:30:10:2, v/v/v/v). No Lieberman colour reaction was obtained.

Spectral data confirmed these compounds to be bufadienolides, but their structures are still being investigated.

Clinical signs

Plant material. The findings are summarized in Table 2. Amongst the first clinical signs seen were those affecting the gastro-intestinal and respiratory systems, followed by cardiac changes (Fig. 3 & Table 2). All the changes persisted until death.

Bufadienolides. The results are summarized in Tables 1 & 3.

Guinea pigs. The approximate LD_{50} of physodine A was 0,22 mg/kg and it was mildly cumulative. The clinical signs noted were weakness, muscular tremors, neck paresis and mass loss. Physodine B had a lower toxicity of c. 1 mg/kg. Two bufadienolides, physodine C and D, were found to be non-toxic at doses of 100 mg/kg administered subcutaneously (s.c.).

Sheep. Only physodine A was administered. The gastro-intestinal and respiratory systems were the first to be affected, accompanied, after the 2nd dose, by conspicuous cardiac conduction changes.

DISCUSSION

As a result of this investigation, *U. physodes* can be added to other species of this genus, such as *U. sanguinea*, *U. rubella* and *U. altissima*, which have been shown to contain cardio-active bufadienolides.

An unusual finding was the isolation of bufadienolides (C and D) that were non-toxic for guinea pigs at doses of 100 mg/kg s.c. Considerably lower yields of non-toxic

than toxic bufadienolides (0,5 g cf. 1 g) were obtained, probably because the non-toxic fractions were disposed of during the purification process. Being harmless to guinea pigs at 100 mg/kg s.c., some fractions containing C and D were almost certainly discarded before it was realized that they had contained bufadienolides.

Typical signs of cardiac glycoside poisoning, affecting the gastro-intestinal, respiratory and cardiac systems, were noted in sheep by drenching the milled bulbs or injecting intravenously an isolated bufadienolide. In addition, nervous signs consistent with locomotory depression, such as weakness of the hindquarters and paralysis, were reported in the field cases near Upington. Like *U. sanguinea* plants, which reportedly have some cumulative effect in rabbits (Steyn, 1935), physodine A was found to be mildly cumulative in guinea pigs. This mild cumulativity is interesting in the light of the paralysis reported in the sheep poisoned by *U. physodes* near Upington and the rare instances of krimpsiekte-like signs recorded in stock poisoned by *U. sanguinea* in the field (Steyn, 1945).

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