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EXPERIMENTAL EVIDENCE THAT THE ACTIVE PRINCIPLE OF THE POISONOUS PLANT *THESIUM LINEATUM* L.f. (SANTALACEAE) IS A BUFADIENOLIDE

L. A. P. ANDERSON[1], J. P. J. JOUBERT[2], R. ANITRA SCHULTZ[1], T. S. KELLERMAN[1] and BARENDINA J. PIENAAR[3]

ABSTRACT

ANDERSON, L. A. P., JOUBERT, J. P. J., SCHULTZ, R. ANITRA, KELLERMAN, T. S. & PIENAAR, BARENDINA J., 1987. Experimental evidence that the active principle of the poisonous plant *Thesium lineatum* L.f. (Santalaceae) is a bufadienolide. *Onderstepoort Journal of Veterinary Research*, 54, 645–650 (1987).

The toxicity of *Thesium lineatum* is confirmed in sheep. A toxic crystalline bufadienolide, with the suggested trivial name thesiuside, has been isolated from it. This is the first cardiac glycoside to be isolated from a member of the Santalaceae. The toxin appears to have no cumulative effect and the symptoms of intoxication and pathology in sheep are similar to that expected from acute cardiac glycoside intoxication.

Thesium lineatum, a root parasite, is described and its distribution given.

INTRODUCTION

Thesium species are root parasites on plants such as *Felicia muricata, F. filifolia, Chrysocoma tenuifolia, Pteronia sordida, Melianthus comosus* and *Lycium* spp. (Steyn, 1949; Varhmeijer, 1981).

Although *Thesium* species have long been known to be poisonous, very little has been published on their toxicity. *T. namaquense* plants suspected of poisoning sheep in the Middelburg district (Cape Province) was toxic when dosed to rabbits and sheep, but similar trials with *T. triflorum* (Steyn, 1935; Steyn, 1936; Steyn, 1937) and *T. lineatum* (Van der Walt & Steyn, 1943) were negative.

Experimental evidence will be presented in this presentation that *T. lineatum* was responsible for losses of sheep in the Karoo and that the toxic principle is a cardiac glycoside.

DESCRIPTION, DISTRIBUTION AND ECOLOGY OF THE PLANT

Family: Santalaceae.

Name: Thesium lineatum L.f.

Common name: Witstorm, Vaalstorm.

Description: (Fig. 1). A woody bush up to *c.* 1 m high, densely branched, spreading. Stems spinous, prominently grooved, glabrous. *Leaves* mostly few, falling quickly; and inconspicuous, sometimes fairly numerous and conspicuous, linear, flattened and fleshy, but more often linear or occasionally linear-oblanceolate, subterete, subacute, 2–4 mm long, glabrous. *Bracts* small and scale-like, triangular-ovate or sublanceolate, glabrous. *Pedicel* up to 2 mm long with 2 very small bracteoles at the apex. *Flowers* solitary in bract axils and arranged in simple racemes, white; perianth *c.* 2,5 mm long, segments 1,5 mm, ovate-lanceolate, sub-acute, hooded, with slightly inflexed margins; anthers exserted, minute; filaments 0,25 mm; style 1,5 mm. *Fruits* woody, white when mature, oblong-ellipsoid, *c.* 6 mm long, 3,5 mm in diameter, with slightly impressed reticulation and some shallow longitudinal grooves, not ribbed. *Seed* solitary, basal, creamy white, with a bulky endosperm. *Flowering time* appears to be throughout the year, with a peak period in late summer to autumn.

Distribution: (Fig. 2) The plant inhabits the drier areas of South West Africa and the Cape Province.

In South West Africa/Namibia it has been recorded in the Etosha Pan area, Grootfontein, Gobabis, Okahandja, Kamanjab, Klein Karas, Grünau, Spitzkop, Swakopmund, Maltahöhe, Rehoboth, Aus, Lüderitz, Keetmanshoop, Klinghardt (from which the type specimen comes) and Oranjemund. In the Cape Province its distribution includes the following districts: Vioolsdrift, Springbok, Aggenys, Kamieskroon, Van Rhynsdorp, Calvinia, Willeston, Clanwilliam, Wuppertal, Zoutrivier, Ladismith, Carnarvon, Oudtshoorn, Graaff-Reinet, Somerset East, Cradock, Beaufort West, Sutherland, Loxton, Victoria West, Steynsburg, Prieska, Kenhardt and Hay.

FIG. 1a & b *T. lineatum* L.f.

[1] Veterinary Research Institute, Onderstepoort 0110

[2] Veterinary Regional Laboratory, Private Bag X528, Middelburg CP 5900

[3] Botanical Research Institute, Private Bag X101, Pretoria 0001

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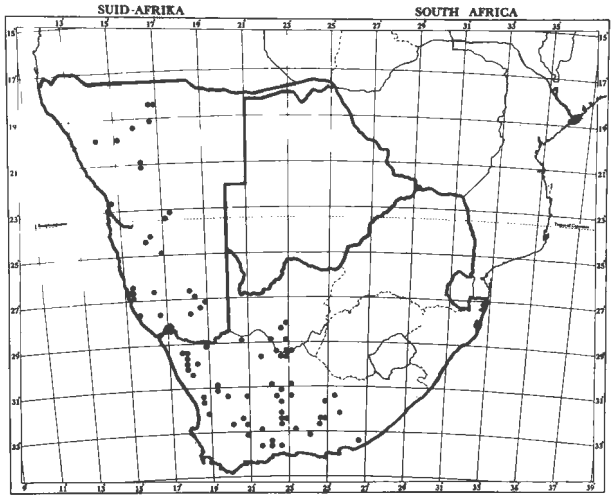

FIG. 2 Distribution of *T. lineatum* L.f.

One record comes from as far east as Lusikisiki in Transkei.

Ecology: T. lineatum prefers well-drained rocky soil of the broken Karroid veld type, listed by Acocks (1975) as type 33. It is often found on rocky hill slopes on all aspects in full sun, or in dry sandy riverbeds and banks or sand and clay or shales on edges of dams, pans, etc.

HISTORY

In a letter to the State Veterinarian, Middelburg (C.P.) T. F. Adelaar referred to dosing trials with *T. lineatum* in sheep during August, and in September 1964 at the Veterinary Research Institute (VRI), Onderstepoort (Table 1). The material, probably dried branches with flowers and fruits, was collected on the shady side of a hill in the Murraysburg area.

A field case of suspected *T. lineatum* poisoning was also reported in the Carnarvon district during October 1982, (P. Jordaan, S. V. Upington, personal communication, 1982). Grazed plants were collected from the 'toxic' camp on the south-eastern slopes (where the deaths occurred) and from the north-western slopes (where no deaths occurred). The 2 batches of chopped-up stems were dosed separately to 2 Merino wethers with a live mass of 25 and 32 kg respectively (Table 2).

Farmers in the Beaufort West, Murraysburg and Carnarvon districts aver that *T. lineatum* growing on the southern slopes (shady side) of the hills are more toxic to animals, especially from April to August. They also believe that the yellow flowers and white matured fruits are more toxic than the stems. Sheep, goats, and sometimes cattle, are affected, and young and newly introduced stock are more often intoxicated than those that are familiar with the plant (W. Crous, S. V. Beaufort West, and P. Jordaan, S. V. Upington, personal communication, 1983).

DOSING TRIALS AT MIDDELBURG

Materials and Methods

T. lineatum was collected on the southern slopes of hills and in the open veld of a known 'toxic' farm in the Beaufort West district during the winter of 1984.

The fairly dry plants were then chopped up, milled, and dosed to 3 4-tooth Merino wethers (per rumen fistula) and a milk tooth Dorper ram (Sheep 3, per stomach tube) with live masses varying between 40,2 and 49 kg (Table 3).

TABLE 1 Observations on sheep dosed per stomach with dried, milled *T. lineatum* collected in the Murraysburg area (T. F. Adelaar & J. D. Smit, VRI, Onderstepoort, unpublished data, 1964)

Sheep No.	Dosing regimen g/kg/d × n	Clinical signs	Fate	Histopathology
1	5 × 2	—	—	Congestion
2	5 × 3	—	Died on Day 2	Haemorrhages, congestion, oedema, light leucocyte infiltration
3	5 × 4	—	Died on Day 4	Lung emphysema and mild fatty degeneration of the liver
4	5 × 5	Dark coloured diarrhoea	Died on Day 12	Outspoken focal fibroses as seen in gousiekte
5	5 × 6	Dark coloured watery diarrhoea and faeces	Died on Day 23	Mild, small focal fibroses especially in the apex and severe lung oedema and congestion as seen in gousiekte

TABLE 2 Observations on sheep intoxicated with *T. lineatum* collected in the Carnarvon district (P. Jordaan, S. V. Upington, personal communication, 1982)

Sheep No.	Site of collection	Dosing regimen			Fate	Histopathology
		g/kg/d × n	Day dosed	Total dose g/kg		
1	South-eastern slope	*c.* 31 × 1	0	31,25	Died after 8h 45 min	General congestion, lung oedema, haemopericardium, haemorrhages in diaphragma, rumen, intestine and kidneys. The intestine was filled with gas
2	North-western slope	40 × 3 10 × 5 20 × 3	0–2 3–21 31–38	230	Died on Day 38	Mild general congestion, hepatosis and nephrosis

TABLE 3 Observations on sheep intoxicated with *T. lineatum* collected in the Beaufort-West district

Sheep No.	Site of collection	Dosing regimen g/kg × n	Clinical signs	Fate	Pathology	Histopathology
1	Southern slopes	6,2 × 1	Dyspnoea, cyanosis	Died after 4,5 h	Severe generalized congestion, varying size subcutaneous & visceral haemorrhages, congestion, oedema and emphysema of the lungs, hydropericardium & myocardial haemorrhages especially subepicardially	Multifocal haemorrhages and hyaline degeneration and necrosis of myocardial fibres, mild infiltration of lymphocytes and macrophages around blood vessels, acute nephrosis and cloudy swelling of hepatocytes
2	Southern slopes	2 × 1	Uneasiness, frequent urination, grunt on expiration	Died after 5 h	As in Sheep 1	As in Sheep 1
3	Southern slopes	0,5 × 4 (Day 0–7)	Anorexia, watery to haemorrhagic diarrhoea, dehydration	Died on Day 7	Dehydration, moderate submandibular oedema, ascites, petechiae on mucosal surface of abomasum and small intestine, watery, greyish-yellow to haemorrhagic contents in small intestine, mild fatty degeneration of liver.	Multifocal hyaline necrosis of myocardial fibres, focal disseminated interstitial lymphocytic myocarditis and fibrosis, centrilobular cloudy swelling of hepatocytes, multifocal necrosis of proximal convoluted tubules in kidneys
4	Open veld	6 × 1	n/u	Discharged	—	—

Results

The observations on sheep intoxicated with plants from the Beaufort West district are given in Table 3.

EXPERIMENTS AT THE VETERINARY RESEARCH INSTITUTE, ONDERSTEPOORT

MATERIALS AND METHODS

Plant material

T. lineatum plants collected in winter on the southern slopes of hills in the Beaufort West district, were dried in the shade and stored at room temperature.

Isolation of the toxic principle

The finely ground plant material (57 kg) was extracted 3 times with hot methyl alcohol. The combined extracts were evaporated under reduced pressure on a water-bath. The resultant syrup was partitioned between 1:1 ethyl acetate and water (5 ℓ). Evaporation of the ethyl acetate extracts yielded a non-toxic residue. Extraction of the water phase with water saturated normal butyl alcohol (3 × 1 ℓ) resulted in a toxic butyl alcohol extract and a non-toxic water extract. The butyl alcohol extract was evaporated on a water-bath under reduced pressure to yield a syrup (530 g).

The syrup was chromatographed on a silica gel column (4,5 kg). Elution with chloroform-acetone-methanol-water (20:20:60:2, v/v/v/v) yielded a number of toxic fractions, while elution with methanol yielded non-toxic material only. Fractions were assayed for toxicity in guinea pigs, *vide infra*.

The combined toxic fractions on evaporation to dryness yielded an amorphous residue (275 g).

Repeated chromatography of the toxic residue on silica gel, using ethyl acetate-methanol-water (90:10:2, v/v/v), yielded a crystalline toxic compound (3,56 g). The trivial name of thesiuside is suggested for the toxin.

Reagents and apparatus

The melting point is uncorrected and was done on a Büchi melting point apparatus. Ultraviolet (UV) absorption refers to methanol and infra-red (IR) to KBr discs. UV absorptions were measured on a Beckman DK-2A spectrophotometer and IR spectra on a Perkin Elmer 257 spectrophotometer and NMR spectra on a Varian XL100 spectrometer. Merck silica gel (0,063–0,200 mm) was used for column chromatography. Pre-coated silica gel 60 F_{254} TLC aluminium sheets were used for thin layer chromatography. The plates were developed with ethyl acetate-methanol-water (90:10:5, v/v/v) and the spots were visualized by spraying with 85 % sulphuric acid and heating for 1 min at 120 °C.

Toxicity assay

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 toxin was assayed in young male albino guinea pigs, (*c*. 200 g) (Table 4). The approximate subcutaneous LD_{50} of the toxin was determined (3 animals/dose) and the cumulative effect by the daily subcutaneous injection of 25 % and 50 % of the estimated LD_{50}/day for 5 days (4 animals).

Dosing trials

Five Merino wethers (milk-tooth) with live masses varying between 17 and 26 kg were dosed with the isolated toxin per stomach tube as set out in Table 5. The sheep were examined daily and electrocardiograms were recorded periodically.

Pathology

One guinea pig was destroyed for necropsy by intraperitoneal administration of pentobarbitone sodium[1] (Table 4).

TABLE 4 Observations on guinea pigs intoxicated by subcutaneous injection of the bufadienolide isolated from *T. lineatum*

Guinea pig No.	Dose mg/kg × n	Clinical signs and/or fate	Pathology
1	0,5 × 1	Died after 25 min	—
2	0,3 × 1	Died after 40 min	—
3	0,14 × 1	Died after 48 h	—
4	0,14 × 1	Died after 48 h	—
5	0,14 × 1	Neck paresis and severe tremors Destroyed at 53 h	Mild pulmonary congestion
6	0,07 × 5	Mass loss, general weakness, tremors Destroyed	—
7	0,035 × 5	Mass loss, general weakness, mild tremors Destroyed	—

All the sheep that died were necropsied (Table 5).

RESULTS

Toxic principle

Thesiuside (3,56 g) was obtained as white crystals from methanol, m.p. 172–175 °C; λ_{max} 299 nm; υ_{max} (KBr) 3400, 2920, 1705, 1695, 1685, 1630, 1540, 1450, 1370, 1265 and 840 cm^{-1}. R_f = 0,31 (ethyl acetate-methanol-water 90:10:5, v/v/v). It gave a light powder-blue Lieberman colour reaction changing to a deeper blue after 1 min.

The toxin was identified as a bufadienolide by virtue of its positive Lieberman colour reaction, characteristic IR absorptions at *c.* 1705, 1630, 1540, 1265 and 840 cm^{-1}, ultraviolet absorption at 299 nm and signals in the ^1H NMR spectrum characteristic of the 3 protons of the α-pirone ring (Anderson & Koekemoer, 1968).

Clinical signs and pathology

Guinea pigs. The clinical signs of thesiuside poisoning were neck paresis and tremors. This bufadienolide is not cumulative and the clinical signs seen after repeated administrations (5 × 50 % LD_{50} and 5 × 25 % LD_{50}, Table 4) were merely general weakness. The approximate subcutaneous LD_{50} of thesiuside is 0,14 mg/kg (Table 4).

Sheep. The observations on sheep intoxicated with the toxin isolated from *T. lineatum* (thesiuside) are summarized in Table 5. The electrocardiographic changes and clinical signs were consistent with those of cardiac glycoside poisoning.

DISCUSSION

Many plants in South Africa are known to contain cardiac glycosides. Some of these plants, despite being highly toxic, are relatively unimportant, while others cause heavy stock losses. Generally speaking those that contain cardenolides are of minor consequence, e.g., *Nerium* spp., *Strophanthus* spp., *Acocanthera* spp., *Asclepia* spp. and *Adenium* spp. All the important cardiac glycoside-containing plants, on the other hand, have bufadienolides as their active principles. Some of them count amongst the most destructive poisonous plants of the subcontinent, e.g., tulp, slangkop and plakkies (Naudé, 1977). *T. lineatum* can now be classified with this growing list of bufadienolide-containing plants that cause significant losses of stock in the field.

Bufadienolides, to the best of our knowledge, have never been isolated from a member of the Santalaceae. The families of plants in South Africa known to contain cardiac glycosides are the Apocynaceae, Asclepiadaceae, Iridaceae, Liliaceae, Melianthaceae and Crassulaceae. A cumulative neurotoxic effect (krimpsiekte) has been demonstrated in certain members of the Crassulaceae and some of their purified bufadienolides (Terblanche & Adelaar, 1965; Naudé & Schultz, 1982; Anderson, Joubert, Prozesky, Kellerman, Schultz, Procos & Olivier, 1983). Clear evidence of cumulativity has not been found in *Thesium;* in fact, field intoxication induced by this plant seems to be of a more subacute type. This opens up the possibility that, as in other acute cardiac glycoside intoxications, activated charcoal (2 g/kg) will be an appropriate therapy (Joubert & Schultz, 1982).

The toxic *Thesium* plants used in this experiment did not grow in close proximity to known cardiac glycoside-containing host plants. The as yet unidentified bufadienolides of *T. lineatum* were therefore probably produced by the plants itself rather than by the species on which it parasitized. This is in marked contrast to the parasitic *Tapinanthus* sp. (= *Loranthus* sp.) that become vicariously toxic by taking up bufadienolides from the host plant (*Melianthus* spp.) on which they grow (Marloth, 1913; T. F. Adelaar & M. Terblanche, VRI, Onderstepoort, unpublished data, 1964).

The post-mortem changes in the sheep poisoned by *T. lineatum* generally fit in with those expected in acute heart failure and cardiac glycoside intoxication in general, but on their own are not sufficiently specific to support a diagnosis.

In the animal that died peracutely after only 3,5 h, no microscopical changes were observed in the heart. The lesions in the heart muscle of the 3 less acutely affected sheep are representative of the early changes seen in acute degeneration and they appear to develop shortly before death, as there is no associated cell reaction. Similar changes are observed from time to time in heart muscle in deaths unassociated with known heart affectation, presumably due to terminal anoxia. Because of this similarity, the changes observed in the hearts of *Thesium* intoxicated animals should be regarded as non-specific and, therefore, not a direct primary effect of the toxin.

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[1] Sagatal V, Maybaker

TABLE 5 Observations on sheep intoxicated with the toxin isolated from *T. lineatum*

Sheep No.	Dosing regimen mg/kg/d × n	Clinical signs	ECG changes	Fate	Pathology	Histopathology
1	0,55 × 1	Transient bloating, decreased ruminal movements, polypnoea, forced respiratory movements and elevated SUN values (Day 0–2)	Tachycardia caused by struggling followed by sinus arrythmia (Day 1)	Discharged on Day 3	—	—
2	0,75 × 1	Forced respiratory movements (Day 0)	N/u	Died overnight on Day 0	Mucosal pallor and slight cyanosis; mild, patchy pulmonary emphysema	Myocardium: Single to small group of fibres stain darker, more pronounced eosinophilia of cytoplasms, loss of cross striations and sacroplasm may appear clumped or have small vacuoles. Nuclei also stain darker, with almost pycnotic appearance
3	0,75 × 1	N/u	N/u	Died overnight on Day 0	Extensive pulmonary emphysema and mild oedema. Scattered ecchymoses under parietal pleura. Subepicardial petechiae (3) in left ventricle	Milder than in Sheep 2
4	0,75 × 1	Progressively decreasing ruminal movements; forced, shallow, irregular respiratory movements; apathy, reluctance to stand	Transient tachycardia (Day 1). Double P waves, ST depression, firing of ectopic foci and runs of complete AV block (Day 2)	Died overnight on Day 2	Upper respiratory cyanosis, aspiration of rumen contents, mild right ventricular dilatation	As in Sheep 2
5	1,5 × 1	Polypnoea, forced, irregular respiratory movements (2 h 30 min). Terminally erratic breathing, muscular tremors and uneasiness	Progressive tachycardia (2 h 3 min), ST elevation, complete AV dissociation, intermittent firing of ectopic foci but fairly constant rhythm	Died after 3,5 h	Mild cyanosis, few petechiae in epicardium of ventricles, moderate serious pericardial effusion	N/u

N/u = nothing unusual

FIG. 1a & b *T. lineatum* L.f.

FIG. 2 Distribution of *T. lineatum* L.f.

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