Neurotoxicity in calves induced by the plant, *Nierembergia hippomanica* Miers var. *violacea* Millán in South Africa

C.J. BOTHA[1], R. ANITRA SCHULTZ[2], J.J. VAN DER LUGT[3], ELIZABETH RETIEF[4] and LEONIE LABUSCHAGNE[2]

ABSTRACT

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The plant *Nierembergia hippomanica* var. *violacea* has been incriminated in field outbreaks of neurotoxicity in calves in the Free State Province. Hepatotoxicity and electrocardiogram (ECG) deviations were induced in a sheep dosed with 5 g/kg dried plant material on four consecutive days. A calf dosed with 2,5 g/kg dried plant material, on two consecutive days, did not show overt clinical changes. Voluntary ingestion of approximately 30 g/kg fresh flowering plants by a second calf resulted in nervous signs characterized by chewing motions, protrusion of the tongue, dysphagia, hypermetria, ataxia, paresis and lateral recumbency. Salivation, dehydration and cardiac irregularities completed the clinical picture. Clinical chemistry changes revealed muscle damage and increased serum urea and creatinine concentrations indicative of kidney involvement. This is the first confirmed outbreak of *Nierembergia hippomanica* var. *violacea* intoxication of stock in South Africa.

Keywords: Calves, hepatotoxicity, neurotoxicity, *Nierembergia hippomanica* var. *violacea*, Solanaceae

INTRODUCTION

Intoxication of cattle, sheep, goats, horses and rabbits by *Nierembergia hippomanica* has been reported in Argentina and Uruguay (Buschi & Pomilio 1987; Riet-Correa, Rivero, Dutra, Timm & Méndez 1998). Mortalities, which may occur some hours after the plant has been ingested, are preceded by diarrhoea, mydriasis, locomotory ataxia, weakened heart action, dyspnoea, excitement and convulsions. Necropsies on acute cases reveal evidence of gastro-intestinal irritation and hyperaemia of the brain and meninges (Buschi & Pomilio 1987).

Riet-Correa *et al.* (1998) observed salivation, abdominal pain, diarrhoea, restlessness and periodic motion of the head and limbs in cattle during field outbreaks in which an estimated morbidity rate of up to 80 % in 3 to 4-year-old oxen or dairy cows occurred. There were no mortalities. Affected cattle recovered within one week after removal from infested pastures or stubble lands. Ondini, Rivero, Riet-Correa, Méndez & Giannechinni (1995, cited by Riet-Correa *et al.* 1998) reported that the minimum toxic dose of fresh plant material administered to cattle and sheep under experimental conditions was 10–15 g/kg, but concluded that the dried plant was not toxic to cattle and sheep. All the cattle and sheep in the dosing

[1] Department of Pharmacology and Toxicology, Faculty of Veterinary Science, University of Pretoria, Private Bag X4, Onderstepoort, 0110 South Africa

[2] Division of Toxicology, Onderstepoort Veterinary Institute, Private Bag X5, Onderstepoort, 0110 South Africa

[3] Department of Pathology, Faculty of Veterinary Science, University of Pretoria, Private Bag X4, Onderstepoort, 0110 South Africa

[4] National Botanical Institute, Private Bag X101, Pretoria, 0001 South Africa

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trials recovered in 1–8 d, except one calf that died after ingesting 50 g/kg green plant material. Necropsy revealed focal haemorrhages in the large intestine and enteritis of the small intestine.

A flavone glycoside (pinocembrin 7-neohesperidoside) and four acetylated forms of the parent flavone glycoside have been isolated from *N. hippomanica* (Pomilio & Gros 1979; González, Pomilio & Gros 1981; Ripperger 1981; González & Pomilio 1982). Buschi & Pomilio (1987) isolated pyrrole-3-carbamidine from *N. hippomanica* and considered this compound to be the lethal principle. Three cardenolides have been extracted from an Argentinian species, *N. aristata* (Gil, Lin, Chai, Pezzuto & Cordell 1995).

Nierembergia veitchii has been incriminated in enzootic calcinosis in sheep in southern Brazil. This species contains a 1,25-dihydroxycholecalciferol-like compound which on prolonged ingestion causes hypercalcaemia, hyperphosphataemia and soft tissue calcification (Riet-Correa, Schild, Méndez, Wasserman & Krook 1987; Riet-Correa, Méndez, Schild & Petiz 1993).

MATERIALS AND METHODS

Field outbreaks

Eighteen weaned calves on the farm Elyssum (27°05'S, 27°44'E) near Koppies, northern Free State, Republic of South Africa were placed in a camp where a flowering herb, unfamiliar to the farmer, grew in abundance. Shortly after ingesting large quantities of this plant, the calves exhibited nervous signs such as circling, paresis and paralysis. Six calves died. The attending veterinarian submitted specimens of the herb for botanical identification. Plant material (approximately 5 kg) was subsequently obtained from the farm. It was dried and used for dosing trials.

Another incident of poisoning due to this unknown poisonous plant was reported from Frankfort, northern Free State. Shorthorn calves showed nervous signs (slight head nodding, rotation of the eye balls, continuous licking motions, stiffness, falling, opisthotonus and leg paddling), nasal discharge and salivation. In this outbreak no mortalities ensued and the calves recovered within 3 d of removal from the infested camp. The private practitioner collected specimens from the incriminated plant for botanical identification.

Dosing trials

A 1-year-old Dorper ewe (animal 1) and a 4½-month-old Jersey cross calf (animal 2) were housed in individual pens with concrete floors at the Onderstepoort Veterinary Institute. The animals had free access to water and were fed milled lucerne hay and a maize-based concentrate. During adaptation, periodic clinical examinations and electrocardiogram (ECG) recordings, utilizing Lead 2 of the electrocardiograph (Lectromed, Medical Distributors), were carried out to establish baseline values. Dried, milled plant material collected on the farm at Koppies was administered by stomach tube according to the dosing regimen presented in Table 1. Clinical examinations and ECG recordings were performed daily throughout the experimental period, but the recordings were performed more frequently once cardiac arrhythmia was detected on auscultation. Before and during the dosing trials blood samples were collected from the jugular vein and submitted for determination of serum activities of aspartate transaminase (AST), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH) and creatine kinase (CK), and serum urea and creatinine concentrations. Serum inorganic phosphate and total calcium concentrations were also ascertained for animal 1. These parameters were determined with standard spectrophotometric methods by the Section of Clinical Pathology, Faculty of Veterinary Science, University of Pretoria using an automated chemical analyzer (Technicon RA-1000, Bayer Health Care Division, Isando, South Africa).

Feeding trial

A 5-month-old Afrikaner calf (animal 3) was housed and maintained under similar husbandry conditions as described above. Clinical examinations and ECG recordings were performed daily throughout the experimental period. Before and during the trial blood samples were collected from the jugular vein and submitted for determination of AST, GGT, LDH, CK and glutamate dehydrogenase (GLDH) activities, and serum urea and creatinine concentrations. Serum inorganic phosphate and total calcium were also determined. These serum chemistry parameters were determined by standard spectrophotometric methods. *Nierembergia* seedlings (botanically verified as *N. hippomanica*) were obtained from a local nursery, transplanted in containers and nurtured to maturity. The calf was deprived of fodder on the day preceding the feeding trial. Twenty-nine containers in each of which were three flowering plants were placed in the pen and offered to the calf (Table 1).

Pathology

Animals 1 and 3 were euthanazed by administering an overdose of pentobarbitone (Euthanaze, Bayer AH) intravenously on days 9 and 2, respectively. Necropsies were performed and the brain and spinal cord and tissue specimens of the myocardium and a range of other organs including lung, spleen, liver, kidney, adrenal glands, intestines and lymph nodes were collected in 10 % buffered formalin for histological examination. Following fixation, coronal sections were made of the brain and spinal cord of

TABLE 1 Dosing regimen, clinical signs and cardiac changes observed in a sheep and 2 calves dosed with *Nierembergia hippomanica*

Animal						Dosing regimen					
No	Species	Breed	Age (months)	Sex	Body mass (kg)	Plant material	Days	Dose (g/kg/d)	Total dose (g)	Clinical signs[a]	Cardiac changes[a]
1	Ovine	Dorper	12	Ewe	31,4	Dried, milled	0–3	5,0	628	Pollakiuria, soft faecal matter, uneasiness, panting, depression (day 0) Inappetence, tucked-in appearance, head held low (day 1) Double expiratory effort, weak rumenal movements, stiffness (day 2) Recumbency, tremors in the hindquarter (day 3) Laboured, jerky breathing (day 4)	Sinus arrhythmia, tachycardia (day 0) Suppression of ST-segment (day 1) Amplitude variation of T-wave (day 2) Coupled rhythm (day 3) Increased QRS-complex amplitude (day 8)
2	Bovine	Jersey	4½	Heifer	129,0	Dried, milled	0–1	2,5	645	Soft faeces (day 1)	Sinus arrhythmia (day 1) Coupled rhythm (day 2)
3	Bovine	Afrikaner	5	Heifer	109,5	Fresh, flowering	0	30,0[b]	3 285	Frothing at mouth, mouthing movements, protrusion of tongue, licking movements, agitation, front limb hypermetria, diarrhoea, posterior paresis, ataxia, wide-base stance (day 0) Bellowing, crossing of limbs, head tilt, dehydration, transient hindquarter tremor, recumbency, weak rumenal movements, salivation and inability to swallow water (= dysphagia), inappetence (day 1) Lateral recumbency, rumenal stasis, tympany, paresis, falling (day 2)	Sinus arrhythmia, increased QRS-complex and T-wave amplitude, single atrioventricular dissociation (day 1)

[a] Clinical signs and cardiac changes in order of appearance
[b] Estimated voluntary consumption

animal 3. Tissue blocks from the organ specimens were embedded in paraffin wax, and sections 4–5 µm in thickness were cut and stained with haematoxylin and eosin (HE).

RESULTS

Plant identification

The plant implicated in both field outbreaks was identified as *Nierembergia hippomanica* Miers var. *violacea* Millán (Fig. 1 and 2) by the National Botanical Institute, Pretoria where voucher specimens are lodged.

Common names

Purple Robe (Brickell 1994), Nierembergia.

Description

Perennial herb, a rounded bush up to 0,2 m in height and spread; plant hairy—hairs short and rigid, sessile and stipitate glands present.

Stems	erect or decumbent, diffusely branched, hairy.
Leaves	linear, 8–12 x 0,5–1,5 mm, alternate, simple, entire, hairy.
Flowers	solitary opposite a leaf.
Calyx	campanulate, deeply 5-lobed; tube 5–6 mm long, strongly veined, lobes 3–6 mm long, narrowly lanceolate; hairy.
Corolla	dark bluish-purple or violet, cup-shaped, limb 5-lobed, 15–20 mm in diameter; glandular hairy (Fig. 3).
Stamens	borne on corolla, fertile stamens 4, in pairs, staminode 1; stipitate glands present on filaments.
Fruit	a two-valved capsule with many seeds (Brickell 1994; Mabberley 1997).

Flowering time

Spring and summer.

Habitat

A garden escapee occurring on abandoned land, in waste places and along roadsides.

Distribution

Nierembergia hippomanica var. *violacea* is native to Argentina. It is often grown for its attractive flowers and is moderately fast growing (Brickell 1994; Mabberley 1997). The species has become naturalized in the Free State and Eastern Cape Provinces (Fig. 4).

Clinical signs

Signs of toxicity observed during the trials are summarized in the order of appearance in Table 1. The most prominent clinical signs encountered in animal 1 were depression, inappetence, weak rumenal movements, dyspnoea, a tucked-in appearance when standing, stiff gait and recumbency.

FIG. 1 *Nierembergia hippomanica* Miers var. *violacea* Millán

FIG. 2 Bluish-purple flowers of *N. hippomanica* var. *violacea*

FIG. 3 Scanning electron micrograph of the glands on the corolla of *N. hippomanica* var. *violacea* (6,5 mm = 15,7 µm)

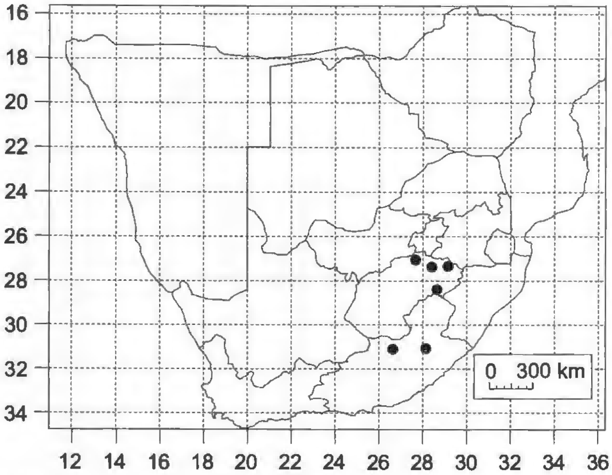

FIG. 4 Distribution of *N. hippomanica* var. *violacea*

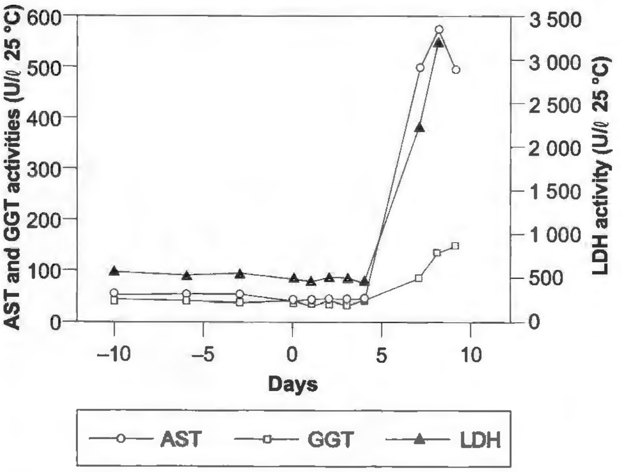

FIG. 5 Serum enzyme activities of AST, GGT and LDH determined in animal 1 during the trial

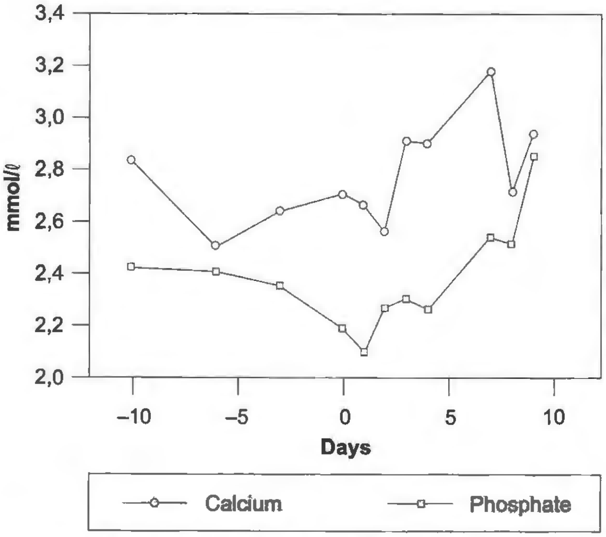

FIG. 6 Total calcium and serum inorganic phosphate concentrations in animal 1

The clinical signs slowly improved from day 4 until euthanasia on day 9. Tachycardia (170 beats/min) was only detected on day 0 and sinus arrhythmia was recorded on days 0–4. Suppression of the ST-segment occurred on days 1, 2, 4, 7, 8 and 9 and variations in the T-wave amplitude were present on days 2–4. Clinical chemistry revealed increased AST, GGT and LDH activities from day 7 (Fig. 5). Total serum calcium and inorganic phosphate increased from days 3 and 7, respectively (Fig. 6). No increase in CK activity and urea and creatinine concentrations occurred throughout the dosing period.

No clinical abnormalities were observed in animal 2, except softening of the faecal matter. Sinus arrhythmia and coupled rhythm were detected on days 1 and 2, respectively. Clinical chemistry examination revealed no aberrations and the calf was discharged from the experiment on day 4.

The most apparent clinical abnormalities observed in animal 3 were chewing movements with protrusion of the tongue (Fig. 7), bellowing, hypermetria of the front limbs, posterior paresis progressing to lateral recumbency (Fig. 8) and rumenal stasis with free-gas bloat.

ECG interpretation on day 1 revealed sinus arrhythmia with a single atrioventricular dissociation. Salivation and inability to swallow water were interpreted as dysphagia and contributed to the dehydration. On day 1 the clinical signs were more pronounced in the morning and the calf was perceived to have made an apparent recovery during the day, but relapsed during the night and was euthanased *in extremis* on day 2. Clinical chemistry revealed increased AST, LDH and CK activities and increased serum urea and creatinine concentrations from day 1 (Fig. 9 and 10). The serum GGT and GLDH activities determined after ingestion of the plant material fluctuated within or near the pre-dosing ranges and the total calcium and inorganic phosphate levels decreased.

Pathology

Gross lesions noted in animal 1 comprised mild brain oedema, mild interstitial pneumonia, slight liver swelling with more distinct lobulation, and congestion of the wall of the gall bladder and mucosa of the small and large intestines. Microscopical lesions in the liver included degeneration of hepatocytes, scattered necrotic hepatocytes and small accumulations of neutrophils in some of the lobules. Significant macroscopical lesions were not present in animal 3. Histologically, mild cerebral oedema of the white matter in periventricular areas was evident.

DISCUSSION

These are the first reported outbreaks of *N. hippomanica* var. *violacea* poisoning of stock in South

FIG. 7 Chewing, protrusion of the tongue and salivation displayed by animal 3

FIG. 8 Animal 3 exhibiting posterior paresis

Africa. The genus *Nierembergia* was named after Juan Eusebio Nieremberg (1595–1658), a Spanish Jesuit and first professor of natural history at the University of Madrid (Mabberley 1997). According to Mabberley (1997), the genus comprises 23 species occurring from Mexico to Chile and Argentina. They are found as decumbent to erect, diffusely branched, usually glabrous perennial herbs and subshrubs. The open bell-shaped flowers are pale violet to blue or white.

Nierembergia belongs to the Solanaceae, a sub-cosmopolitan family, known for various edibles such as peppers, tomatoes and potatoes. Solanaceae can be divided into two subfamilies, namely Solanoideae and Cestroideae. Genera such as *Capsicum*, *Datura* and *Solanum* of the Solanoideae have an accrescent calyx and the fruit is generally a berry. Members of the Cestroideae are characterized by a non-accrescent calyx and usually a capsular fruit. *Nierembergia*, *Cestrum*, *Nicotiana* and *Petunia* are well-known

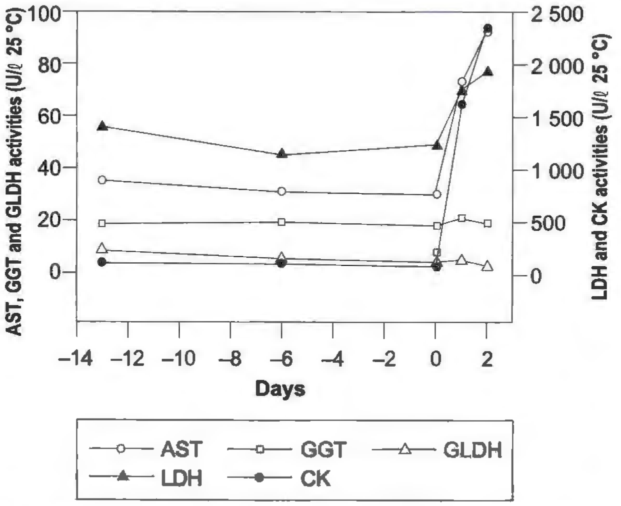

FIG. 9 Serum enzyme activities of AST, GGT, GLDH, LDH and CK determined in animal 3 during the trial

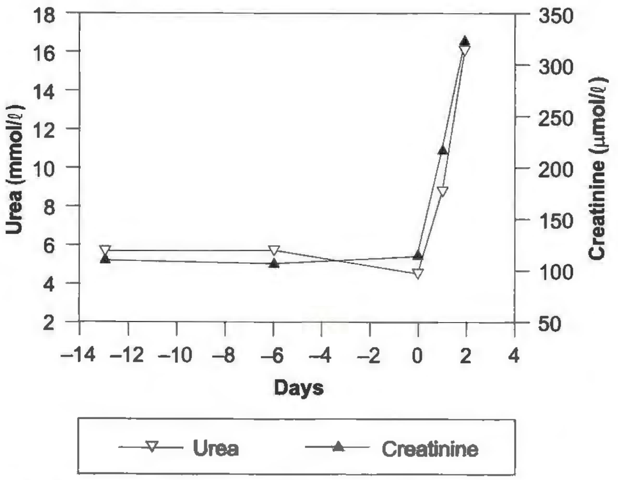

FIG. 10 Serum urea and creatinine concentrations in animal 3

genera of this subfamily. The colour of the corolla distinguishes *Nierembergia hippomanica* var. *violacea* from other southern African species of the genus. The only other species known to be commonly cultivated in southern Africa, *Nierembergia repens* Ruiz & Pav., has white, yellow-centred flowers which are occasionally flushed pink with age. *Nierembergia repens* is a mat-forming, perennial herb with ovate leaves. These characters distinguish it from those of *N. hippomanica* var. *violacea* with a bluish-purple corolla and narrowly ovate leaves (Brickell 1994; Mabberley 1997).

Nierembergia hippomanica is also cultivated in South African gardens. This popular flowering plant adapts well to local climatic conditions and has escaped from gardens to become naturalized in the camps where the field outbreaks occurred.

Experimentally, *N. hippomanica* induces clinical signs in sheep similar to those of naturally poisoned cattle in Northwest Uruguay (Riet-Correa *et al.* 1998). The symptomatology and pathological findings in animal 1 in the present dosing trial, however, did not conform to the nervous signs observed in calves during the field outbreaks. Depressed habitus, a tucked-in appearance, anorexia and weak rumenal movements could intimate a hepatotoxicosis. Liver damage, evinced by the increase in AST, GGT and LDH activities in the serum, manifested as a mild non-specific hepatosis with few scattered necrotic hepatocytes.

Contrary to the clinical chemistry findings in the sheep, no hepatic damage was noticed in the calves as GLDH and GGT fluctuated near or within the normal ranges. With the limited number of animals used in the trials it is difficult to explain this discrepancy, but it can possibly be attributed to species differences. In animal 1 the enzyme activities were only elevated after a week and a much more rapid clinical course was evoked in animal 3.

In South Africa, poisoning of cattle with *Cestrum laevigatum*, another South American member of the Solanaceae family, causes a hepatotoxicosis. Nervous signs (incoordination, tremor and aggression) in this intoxication are ascribed to hepatic encephalopathy (Kellerman, Coetzer & Naudé 1988; Kellerman, Naudé & Fourie 1996). *Cestrum laevigatum* flourishes along the banks of the Vaal River and is a burgeoning problem in the northern Free State (Kellerman *et al.* 1996).

Animal 3 exhibited severe neurological signs after voluntary consumption of approximately 30 g/kg of fresh flowering plant material. After drying fresh plant material in an oven at 55 °C, a 22 % dry mass was calculated which equates to ingestion of roughly 6,6 g/kg dry plant material. The clinical signs, such as chewing movements, protrusion of the tongue, salivation, ataxia, paresis and recumbency, observed in animal 3 correspond to the nervous signs described in the recent field outbreaks.

The increase in AST, LDH and CK activities in animal 3 indicate muscle damage. The increase in serum urea and creatinine concentrations can be attributed to reduced renal clearance of these endogenous substances due to dehydration. The negative result obtained after dosing the dry plant material (2,5 g/kg on two consecutive days) to animal 2 can be ascribed to an insufficient toxic dose or diminished toxicity of dry plant material (Riet-Correa *et al.* 1998).

Cardiac arrhythmia was detected in both species, but save for the single atrioventricular dissociation detected on day 1 in animal 3, the irregularities were not considered life threatening. Cardiac glycosides, such as the cardenolides isolated from *N. aristata*, can induce similar cardiac irregularities; but the flavone glycoside, its acetylated forms and pyrrole-3-carbamidine isolated from *N. hippomanica*, not having been demonstrated in other South African poisonous plants, are obscure entities.

Field cases of *Nierembergia* poisoning in cattle are reported from the northern Free State. Although it is considered that this poisoning will only be encountered infrequently in this region, it should nevertheless be differentiated from other important intoxications such as diplodiosis and tulp poisoning.

Diplodiosis, caused by ingestion of maize contaminated with the common cob-rot fungus, *Diplodia maydis*, occurs in winter when ruminants are kept on harvested maize lands and is characterized by ataxia (stiff-legged, high-stepping gait), paresis and paralysis (Kellerman *et al.* 1996). Tulp poisoning, caused by ingestion of *Homeria* spp. and *Moraea* spp., is characterized by cardiac arrhythmia, atrioventricular dissociation (heart-block), laboured breathing, rumenal stasis, diarrhoea and posterior paresis and should also be distinguished from *Nierembergia* poisoning (Kellerman *et al.* 1996). The salivation, dysphagia and bellowing noticed during *Nierembergia* poisoning must not be confused with rabies (Swanepoel 1994).

Riet-Correa *et al.* (1993) described hypercalcaemia and hyperphosphataemia in sheep fed *N. veitchii* within 48 h and 5 d, respectively. In the present investigation an increase in total serum calcium and inorganic phosphate was demonstrated in animal 1 (Fig. 5), whereas a decrease in total serum calcium and inorganic phosphate was detected in animal 3.

Other members of the Solanaceae, which may induce nervous signs in cattle in South Africa, are *Solanum kwebense* and *Nicotiana glauca*. Ingestion of large quantities of *S. kwebense* precipitates epileptiform seizures in cattle in the Northern Province. In this neurotoxicity, cerebellar atrophy is microscopically associated with degeneration and loss of the Purkinje cell layer (Kellerman *et al.* 1988; Kellerman *et al.* 1996). *Nicotiana glauca* poisoning is characterized by salivation, irregular gait, tremors, convulsions and dyspnoea, but intoxication is extremely rare and of no practical significance (Kellerman *et al.* 1988).

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