THE EFFICACY OF ACETAMIDE FOR THE TREATMENT OF EXPERIMENTAL
DICAPETALUM CYMOSUM (GIFBLAAR) POISONING IN SHEEP

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ABSTRACT


High mortality of livestock is caused annually by the plant, Dichapetalum cymosum (gifblaar), in the Northern Transvaal. So far no therapeutic measures have been developed for the prevention or treatment of this poisoning. In this presentation, the efficacy of acetamide as therapy for experimental gifblaar poisoning was tested in 18 sheep. When 2.5–5 g/kg of acetamide was dosed to sheep at various intervals before and sometimes after the administration of 5 g/kg of gifblaar, 1 out of 5 survived, compared with 0 out of 2 controls. Dosing of 2 g/kg of acetamide before and/or simultaneously with or after 1 g/kg gifblaar prevented mortality as 5 out of 5 treated sheep survived compared with none of the 5 controls.

The experiments indicate that acetamide has demonstrable therapeutic value as an antidote for the prevention of experimental gifblaar poisoning in sheep. Further investigations should determine the feasibility and applicability of these findings under field conditions in sheep and cattle.

INTRODUCTION

Dichapetalum cymosum (Hooker) Engler & Pranti is one of the most toxic indigenous plants of Africa. Its distribution covers the Transvaal province of the Republic of South Africa, South West Africa (Namibia), Zimbabwe and Botswana (Vickery & Vickery, 1973). The Afrikaans name of the plant (gifblaar = poison leaf) refers to its toxicity, known to the early settlers of Transvaal about 150 years ago. Grazing livestock, usually cattle, are often fatally poisoned by ingesting gifblaar leaves, and economic losses due to gifblaar are substantial.

Considerable experimental work has been done on the toxicity of gifblaar in domestic animals (Steyn, 1928) and antelopes (Basson, Norval, Hofmeyer, Ebedes & Schultz, 1982), and the toxic principle in the plant was identified as monofluoro-acetic acid (Marais, 1944).

The mode of action of monofluoroacetic acid (MFA), elucidated by Peters (1952), led to the discovery of a novel concept in toxicology, named “lethal synthesis”. This term indicates an increased toxicity as a result of in vivo metabolism of MFA to an extremely toxic metabolite, fluoroacetic acid. The latter is an irreversible inhibitor of the enzyme aconitase in the citric acid (Krebs)-cycle, causing an impairment of cellular respiration with all its consequences. There are more recent indications that MFA complexes with, and inactivates a carrier substance responsible for transferring acetate, an essential nutrient, into the mitochondria (Loomis, 1978). It is thus not surprising that compounds, regarded as “acetate donors”, may prevent experimental MFA and fluoroacetamide (an MFA derivative used in some countries as a rodenticide) poisoning in laboratory animals (Chenoweth, 1949; Gitter, Blank & Bergmann, 1953) and chickens (Egyed & Shlosberg, 1977).

These data have not been utilized for control of gifblaar poisoning in farm animals, as the course of the poisoning is invariably extremely short and clinical signs may remain unnoticed, especially under extensive agricultural conditions. Wide-scale eradication of the plant is very difficult and, at present, impractical. Activated charcoal has been efficacious in the treatment of plant-induced cardiac glycoside intoxications in farm animals in South Africa (Joubert & Schultz, 1982 a,b). However, this was not found to be the case in experimental gifblaar poisoning of guinea-pigs receiving 2–6 g/kg of gifblaar and 4–10 g/kg of charcoal orally (Egyed & Schultz, unpublished data, 1984) and in 1 sheep (Egyed & Kellerman, unpublished data, 1984) receiving 2.5 g/kg of gifblaar and treated with 5 g/kg of highly activated charcoal. These negative findings prompted an experiment with a specific antidote for the therapy of experimental gifblaar poisoning in sheep, and in this presentation the findings related to the efficacy of acetamide (CH₃CONH₂) are detailed.

MATERIALS AND METHODS

Plant

D. cymosum plants were collected in February 1984 on a farm near Rust de Winter in the District of Cullinan. The plants were dried in the shade and stored at room temperature before being milled and dosed to animals.

Toxicity trials

Guinea-pigs. The toxicity of the milled (0.5 mm mesh) D. cymosum, suspended in 1.5 % cellofas (methyl cellulose), was assayed in young (c. 200 g) male albino guinea-pigs. Doses between 1 and 4 g/kg body mass were administered per polyethylene stomach tube (Table 1).

Sheep. Eight Merino sheep (milk tooth–full mouth) of both sexes, with live masses varying between 20 and 61 kg, were dosed, as set out in Table 2. The plants were coarsely-milled, suspended in c. 2 litres of water and dosed per stomach tube. One sheep (Sheep 2) received unmilled leaves via a ruminal fistula.

Antidotal therapy with acetamide (BDH, technical grade)

Guinea-pigs. Lethal doses of D. cymosum and various doses acetamide (50 % m/v solution) were dosed to young (c. 200 g) male albino guinea-pigs (Table 1).

Sheep. Ten Merino sheep (milk tooth–full mouth) of both sexes, with live masses varying between 16 and 41 kg, were dosed, as set out in Table 3. Aqueous solutions of acetamide were administered orally at various intervals relative to the time of administration of gifblaar and at doses of 2–5 g/kg body mass.

RESULTS

Guinea-pig experiments. Two and 4 g/kg of gifblaar leaves were found to be lethal in every instance. Dosages

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Received 26 August 1986—Editor
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Table 1: The administration of gifblaar and acetamide per os to guinea-pigs

<table>
<thead>
<tr>
<th>Control group</th>
<th>Treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of guinea-pigs</td>
<td>gifblaar g/kg</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

Table 2: Experimental gifblaar poisoning in sheep. Control experiments on the antidote effects show that a single dose of acetamide administered (2 g/kg, administered 1,5 h and 24 h before the administration of 5 g/kg gifblaar) was unable to prevent mortality. Identical (5 g/kg) but multiple doses of acetamide, given at various intervals before and 2,5 g/kg acetamide administered shortly after dosing with 5 g/kg gifblaar, saved 1 sheep of lethal poisoning (Table 3, Sheep 5). This animal had severe clinical signs for 4 days but recovered on the 5th day following treatment. A similar treatment was found to be ineffective in a sheep which was force-fed with gifblaar (Table 3, Sheep 4).

The 100% efficacy of acetamide as antidote in gifblaar poisoning was apparent when 2 g/kg was administered to sheep poisoned with the lethal dose of 1 g/kg gifblaar. Acetamide was administered either in multiple doses (before and simultaneously with gifblaar) (Table 3, Sheep 6–7) or in a single dose given simultaneously with gifblaar (Table 3, Sheep 8–10). A single dose of acetamide administered simultaneously with gifblaar prevented the appearance of clinical signs. The efficacy of acetamide is summarized in Table 3. Necropsy findings in sheep dying in these experiments were practically identical to those detailed by others (Steyn, 1928; Egued, 1973; Schultz, Coetzee, Kellerman & Naude, 1982; Newsholme & Coetzee, 1984) in gifblaar or MFA.

Table 3: Experimental gifblaar poisoning in sheep. Antidotal therapy with acetamide

<table>
<thead>
<tr>
<th>Sheep No.</th>
<th>Dosing regimen g/kg × n</th>
<th>Clinical signs Latency Time</th>
<th>Clinical signs</th>
<th>Latency Time</th>
<th>Fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5 × 1</td>
<td>Tremor, polypnoea</td>
<td>Abdominal breathing, grinding of teeth</td>
<td>5 h 30 min</td>
<td>Died after 5 h</td>
</tr>
<tr>
<td>2</td>
<td>5 × 1</td>
<td>Tremor, depression</td>
<td></td>
<td>4 h 15 min</td>
<td>Died after 4 h 35 min</td>
</tr>
<tr>
<td>3</td>
<td>2.5 × 1</td>
<td>Depression, head shaking, polypnoea</td>
<td></td>
<td>2 h</td>
<td>Died after 6 h</td>
</tr>
<tr>
<td>4</td>
<td>1 × 1</td>
<td>Mild hyperecstibitity</td>
<td></td>
<td>7 h</td>
<td>Died after 6 h</td>
</tr>
<tr>
<td>5</td>
<td>1 × 1</td>
<td>Hyperecstibitity, staggering</td>
<td></td>
<td>4 h</td>
<td>As Sheep 4</td>
</tr>
<tr>
<td>6</td>
<td>1 × 1</td>
<td>Polypnoea, grinding of teeth</td>
<td></td>
<td>7 h</td>
<td>As Sheep 4</td>
</tr>
<tr>
<td>7</td>
<td>1 × 1</td>
<td>Depression, abdominal breathing</td>
<td></td>
<td>7 h</td>
<td>Found dead after 12 h</td>
</tr>
<tr>
<td>8</td>
<td>1 × 1</td>
<td>Depression, abdominal breathing</td>
<td></td>
<td>7 h</td>
<td>Found dead after 12 h</td>
</tr>
</tbody>
</table>

* Given unmilled leaves through rumen fistula
** Given unmilled leaves through rumen fistula

Sheep experiments. The results of the toxicity experiments in sheep (Table 2) indicate that 5, 2.5 and 1 g/kg body mass of gifblaar leaves were 100% lethal to sheep. Two sheep dosed with 5 g/kg of leaves died within 5 h, 1 sheep receiving 2.5 g/kg of leaves died within 6 h and 5 sheep receiving 1 g/kg of leaves, died overnight (probably between 12–20 h after the administration of gifblaar). In the antidote experiments (Table 3), 3 of the 5 sheep receiving 5 g/kg gifblaar and acetamide died overnight (probably after 15–20 h), 1 died after 4.5 h and another recovered clinically after 5 days. Five sheep, receiving 1 g/kg of gifblaar and treated with acetamide (2 g/kg) at various times (Table 3), remained either clinically unaffected (3 sheep) or recovered within 24 h (2 sheep), after showing mild clinical signs. The results of the antidote experiments show that a single dose of 5 g/kg acetamide, administered 1.5 h and 24 h before the administration of 5 g/kg gifblaar (Table 3, Sheep 1–3), was unable to prevent mortality. Identical (5 g/kg) but
poisoning. There appeared to be no difference in the toxicity of gitblaar administered orally or through ruminal fistula. The overall experimental results are summarized in Table 4.

**DISCUSSION**

MFA (FCH\_2\_COOH) is structurally closely related to acetic acid (CH\_3\_COOH). It is apparent that they can compete with each other to combine with coenzyme-A to form fluoroacetyl coenzyme-A (with FMA) or acetyl coenzyme-A (with acetic acid). The formation of fluoroacetate, the toxic metabolite of MFA, is the condensation product of fluoroacetyl coenzyme-A with oxaloacetic acid. The concept of rational (specific) therapy of MFA poisoning is dependent on the use of an antidote able to prevent fluoroacetate formation. In this respect, therapeutic successes have been achieved with compounds regarded as "acetate donors". It was found that sodium acetate and ethanol (a source of acetate formation in vivo) had some protective effect in experimental MFA poisoning in mice, and the combination of the 2 had a synergistic effect (Tourtelotte & Coon, 1949). It is very interesting to note that farmers in South Africa, without this knowledge, used equal parts of vinegar (acetic acid) and sorghum beer (ethanol) as a folk medicine for the treatment of gitblaar poisoning in farm animals (Steyn, 1934). Striking therapeutic successes were noted with glycerol monoacetate in rabbits, dogs and monkeys (Chenoweth, Kandel, Johnson & Bennett, 1951) and acetamide in rats (Gitter et al., 1953). Glycerol monoacetate (monacetin) given i.m., and acetamide, given by oral administration or by s.c. injection, were equally efficacious. For rats the oral LD\_50 of acetamide is 30 g/kg, and the s.c. LD\_50 of monacetin is 6.6 g/kg (Anon., 1968).

The efficacy of acetamide is associated with its ability to penetrate the double barrier of the cell wall and of the mitochondria (Peiers, 1957). An important prerequisite for the antidotal effect of acetamide in laboratory animals is that it should be administered prior to MFA or simultaneously with it, but not later than a few minutes after the MFA administration (Gitter et al., 1953), i.e. well before the appearance of clinical signs.

Regarding the mechanism of action of acetate donors, we speak only about preventive therapy. Unfortunately, there are no antidotes available which are able to convert fluoroacetic acid back to MFA, although over 400 compounds have been tested without practical results (Pattison & Peters, 1966). No recommended dose of acetamide has been established for treatment of MFA (or fluoroacetamide) poisoning in livestock. It is postulated that its dose should be relatively high in order to compete successfully for the active site in the Krebs-cycle. Doses as low as 0.2 g/kg had no beneficial effect on the course of lethal fluoroacetamide poisoning in sheep (Egyed, 1971). Comparative toxicological data indicate that one of the most susceptible species is the sheep (Meldrum & Bignell, 1957). This is unexpected, since in ruminants acetate is a major product of fermentation in the rumen and the acetate concentration in blood in these animals is 3–10 times higher than that of non-ruminants (Annison, Hill, Lindsay & Peters, 1960).

The efficacy of acetamide in the treatment of sheep experimentally poisoned with gitblaar was demonstrated by the number of surviving animals in the treated group, in which recovery was noted in 6 out of the 10 sheep (60 %) that received acetamide after lethal doses of gitblaar, whereas in the control (untreated) group none of the 8 sheep survived. It should be noted that the acetamide-treated sheep that died did so later (Table 3, Sheep 1–3) than the untreated controls (Table 2). This delayed death is apparently associated with the incomplete protective effect of acetamide. Furthermore, even with the highest (apparently supralethal) dosage of gitblaar (5 g/kg) used in these experiments, 1 sheep treated with multiple doses of acetamide (Table 3, Sheep 5) recovered. Only 1 sheep in the group treated with acetamide (Table 3, Sheep 4) died as early as the untreated ones. However, this sheep was force-fed, and this additional stress apparently precipitated the cardio-toxic effects of gitblaar.

The efficacy of acetamide is dependent on factors, such as the toxicity of gitblaar, and on the dosage and timing of the application of the antidote. Further investigations must now be carried out in order to establish the therapeutic doses of acetamide for livestock poisoned by gitblaar in the field.

**ACKNOWLEDGEMENTS**

The encouragement and interest of Dr R. D. Bigalke in this research work during my sabbatical leave at Onderstepoort is highly appreciated. Special thanks are due to Prof T. W. Naudé and Dr T. S. Kellerman for valuable comments and discussions. I am also grateful to Dr Kellerman for his hospitality in the Toxicology Section and for his generosity in supplying me with experimental animals. I also appreciate the co-operation of the Staff of Toxicology and Pathology sections and the devoted and excellent technical assistant of Mr B. P. Maartens.

**REFERENCES**


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**TABLE 4 Summary of the results of the toxicity of gitblaar leaves and the efficacy of acetamide as therapeutic agent in experimental gitblaar poisoning in sheep**

<table>
<thead>
<tr>
<th>Gitblaar (g/kg)</th>
<th>Control group No. of sheep</th>
<th>Results</th>
<th>Gitblaar (g/kg)</th>
<th>Treated group No. of sheep</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>2</td>
<td>Lethal</td>
<td>5</td>
<td></td>
<td>Died: 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Delayed death: 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Recovery: 1</td>
</tr>
<tr>
<td>2.5</td>
<td>1</td>
<td>Lethal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>Lethal</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Rapid clinical recovery: 2

Clinically unaffected: 3

Delayed death: 3

Recovery: 1

Clinically unaffected: 3

Rapid clinical recovery: 2

*Died: 1
Delayed death: 3
Recovery: 1
Clinically unaffected: 3
Rapid clinical recovery: 2*
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