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

M. N. EGYED[1] and R. ANITRA SCHULTZ, Veterinary Research Institute, Onderstepoort 0110

ABSTRACT

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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 & Prantl 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, fluorocitric 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, unpulished 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 (CH_3CONH_2) 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 ℓ 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

[1] Permanent address: Kimron Veterinary Institute, P.O. Box 12, Bet-Dagan 50250, Israel

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TABLE 1 The administration of gifblaar and acetamide per os to guinea-pigs

Control group			Treated group			
No. of guinea-pigs	Gifblaar g/kg	Survival rate	No. of guinea-pigs	Gifblaar g/kg	Acetamide g/kg	Survival rate
2	1	2/2	—	—	—	—
2	2	0/2	4	2	2,5	4/4
10	4	0/10	8	4	5	8/8

TABLE 2 Experimental gifblaar poisoning in sheep. Control experiments on the toxicity of gifblaar

Sheep No.	Dosing regimen g/kg × n	Clinical signs	Latency	Fate
1	5 × 1	Tremor, polypnoea	4 h 30 min	Died after 5 h
2	5 × 1*	Tremor, depression	4 h 15 min	Died after 4 h 35 min
3	2,5 × 1	Depression, head shaking, polypnoea	2 h	Died after 6 h
4	1 × 1	Mild hyperexcitability	7 h	Died after *c.* 15–20 h
5	1 × 1	Hyperexcitability, staggering	4 h	As Sheep 4
6	1 × 1	Polypnoea, grinding of teeth	7 h	As Sheep 4
7	1 × 1	Depression, abdominal breathing	7 h	As Sheep 4
8	1 × 1	Depression, abdominal breathing	7 h	Found dead after 12 h

* Given unmilled leaves through rumen fistula

TABLE 3 Experimental gifblaar poisoning in sheep. Antidotal therapy with acetamide

Sheep No.	Dosing regimen		Time	Clinical signs	Latency	Fate
	Gifblaar g/kg × n	Acetamide g/kg × n				
1	5 × 1	5 × 1	1,5 h before gifblaar	Abdominal breathing, grinding of teeth	5 h 30 min	Died overnight
2	5 × 1	5 × 1	24 h before gifblaar	Inappetence	7 h 30 min	Died overnight
3	5 × 1	5 × 1	24 h before gifblaar	Abdominal breathing	7 h 30 min	Died overnight
4*	5 × 1	5 × 2	18,5 h before and 1 h after gifblaar	Tremor, recumbency, abdominal respiration	3 h	Died after 4 h 35 min
5**	5 × 1	5 × 3 2,5 × 1	24, 19 and 1 h before gifblaar 10 min after gifblaar	Depression, abdominal breathing, fasciculation, tachycardia	3 h	Recovery after 5 days
6	1 × 1	2 × 3	18 h and 1 h before gifblaar and simultaneously with gifblaar	Decreased appetite	7 h	Recovery within 24 h
7	1 × 1	2 × 2	19 h before gifblaar and simultaneously with gifblaar	Rapid, shallow respiration	12 h	Recovery within 24 h
8	1 × 1	2 × 1	Simultaneously with gifblaar	Clinically normal		Discharged
9	1 × 1	2 × 1	Simultaneously with gifblaar	Clinically normal		Discharged
10	1 × 1	2 × 1	Simultaneously with gifblaar	Clinically normal		Discharged

* Force-fed (by opening the mouth and inserting coarsely-milled gifblaar. By closing the mouth, swallowing was induced)
** Given unmilled leaves through rumen fistula

of 2,5 and 5,0 g/kg of acetamide, administered orally and simultaneously with 2–4 g/kg gifblaar leaves, gave full protection against lethal poisoning (Table 1).

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 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; Egyed, 1973; Schultz, Coetzer, Kellerman & Naudé, 1982; Newsholme & Coetzer, 1984) in gifblaar or MFA

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

Gifblaar (g/kg)	Control group No. of sheep	Results	Gifblaar (g/kg)	Treated group No. of sheep	Results
5	2	Lethal	5	5	Died: 1 Delayed death: 3 Recovery: 1
2,5	1	Lethal			
1	5	Lethal	1	5	Clinically unaffected: 3 Rapid clinical recovery: 2

poisoning. There appeared to be no difference in the toxicity of gifblaar administered orally or through ruminal fistula. The overall experimental results are summarized in Table 4.

DISCUSSION

MFA (FCH_2COOH) is structurally closely related to acetic acid (CH_3COOH). 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 fluorocitric acid, 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 fluorocitric acid 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 gifblaar 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 (Peters, 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 fluorocitric 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 gifblaar 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 gifblaar, 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 gifblaar (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 gifblaar.

The efficacy of acetamide is dependent on factors, such as the toxicity of gifblaar, 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 gifblaar in the field.

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