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PYRIDOXINE (A VITAMIN B6) AND ITS DERIVATIVE PYRIDOXAL AS TREATMENT FOR *ALBIZIA VERSICOLOR* POISONING IN GUINEA-PIGS

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ABSTRACT

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In the course of three experiments it was established that all the toxic effects of a lethal dose of *Albizia versicolor* pods (> 4,5 g/kg) in guinea-pigs could be countered by concurrent subcutaneous injection of pyridoxine (10 mg/kg). This treatment was also successful once severe symptoms had set in. Pyridoxal, on the other hand, was found to be ineffective as a therapeutic agent. The fact that pyridoxal does not counter the action of the toxin indicates an atypical site of action by the toxin as regards the normal pathways which require vitamin B6 as a co-factor.

INTRODUCTION

Vitamin B6 was unequivocally differentiated from other members of the vitamin B complex during the period 1934–1937 by Gyorgy, Edgar and Chick (Snell, 1958). By 1939, "pyridoxine" and "vitamin B6" had become synonymous terms (Snell, 1958). Further work demonstrated that oxidation of pyridoxine yielded an aldehyde, while amination yielded an amine, both of which were highly active for micro-organisms, and for which there were only six possible structures (Snell, 1944). The use of tissues from vitamin B6 deficient animals on one hand and vitamin B6 deficient micro-organisms on the other, permitted implication of pyridoxal phosphate as co-enzyme in many enzymatic reactions (Snell, 1958). It is thus currently accepted that the active form of vitamin B6 is pyridoxal phosphate.

With the elucidation of the structure of two neurotoxins from *Albizia tanganyicensis* by Steyn, Vleggaar & Anderson (1987), it was noted by them that the major toxin, 4-methoxy-pyridone (Fig. 1), may well be an antagonist to the vitamin pyridoxine (Fig. 1). This investigation was aimed at testing their hypothesis and establishing an effective treatment for *Albizia* toxicoses. Three experiments were carried out, designated Experiments 1, 2 and 3 according to the sequence in which they were done.

EXPERIMENT 1

Method: Seed pods of *Albizia versicolor* collected in the young semi-green state were air dried for 7 days before desiccation in an oven for 24 h at 37 °C. The dry pods were ground in a micro-hammer mill with a final pore size of 0,5 mm; the resultant powder was then mixed with methyl cellulose to facilitate oral administration and to obtain a uniform suspension.

Twenty male guinea-pigs were divided into 5 equal groups of 4. Each guinea-pig in Groups 1 to 4 received *c* 5 g/kg plant material per os. Simultaneous to the dosing of plant material, Group 2 was injected subcutaneously (S/C) with 0,1 mg pyridoxine and Groups 3 and 5 with 1 mg pyridoxine (S/C). Group 4 received 1 mg pyridoxine intra-muscularly (I/M) when severe symptoms began to be manifested. One animal in Group 4 died of natural causes before the experiment commenced. Group 5 acted as a control and received no plant material (Table 1).

Results: The toxic response indices (Table 1) were calculated as described in the discussion for Experiment 1. One out of 4 animals died in Group 1 (non-treated group), while the remaining three showed moderate symptoms before recovering (Table 1). Two out of 4 guinea-pigs died in the low treatment Group 2, while the remaining 2 showed moderate symptoms (Table 1). One of the two that died in this group was found to have a ruptured stomach at necropsy (Table 1). In the high treatment group, Group 3, only one guinea-pig showed very mild symptoms while the rest appeared not to be adversely effected (Table 1). All the guinea-pigs in Group 4 developed severe symptoms (Table 1) and were treated. One subsequently died shortly after treatment while the remaining two recovered fully within approximately 4 h. Group 5, which only received pyridoxine, showed no symptoms throughout the trial (Table 1).

Statistical methods

Bartlett's test for homogeneity of variances was utilised to determine if the assumptions for the one-way analysis of variance could be satisfied at $P = 0,01$, to compare the toxic response index values obtained for the different treatments. For experiments 1, 2 and 3, the variances were homogeneous. The toxic response index means were compared by Sheffe's multiple comparison test (Browne, 1985).

Statistical results

The analysis of variance on the response indices given in Table 1, resulted in a significant F-value (3,10) of 2,7 ($P<0,01$). Comparison of treatment means was as follows:

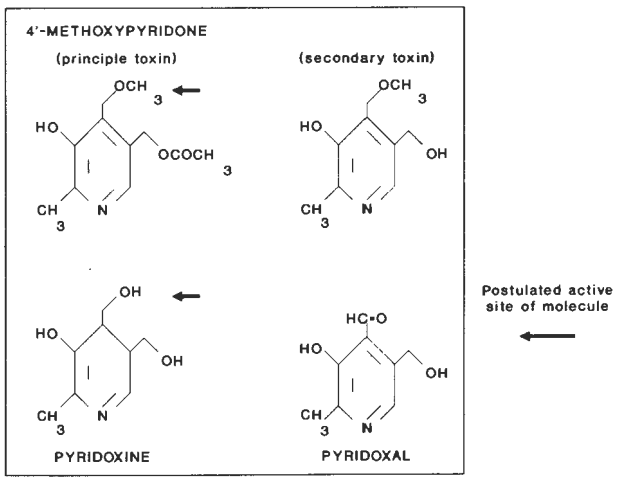

FIG. 1 Chemical structures of *Albizia* toxins and vitamin B6 derivatives

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TABLE 1 Summary results of Experiment 1

Group No. Animal No.	Dose of Pyridoxine mg/kg	Plant dose received g/kg	Time to most severe symptoms min	Grade of symptoms	Toxic response index
1/1	0	4,2	230	2	7,77
1/2	0	5,6	330	5	12,92
1/3	0	3,9	230	2	8,37
1/4	0	3,8	230	2	8,59
2/1	0,53	4,5	180	2	7,87
2/2	0,52	4,4	330	5	16,44
2/3	0,48	4,0	180	2	8,85
2/4	0,59	5,0	135*	5	—
3/1	4,6	3,9	—	0	0
3/2	5,0	4,2	180	1	4,22
3/3	4,27	3,6	—	0	0
3/4	4,6	3,9	—	0	0
4/1	4,76	4,0	75	3	17,78
4/2	6,3	5,1	90	5	21,88
4/3	4,25	3,6	75	3	19,76
5/1;2;3;4	—	0	—	0	—

* Died of a ruptured stomach

Group	3	1	2	4
Pyridoxine	1 mg S/C	none	0,1 mg S/C	1 mg I/M
Mean response index	1,05	9,41	11,05	19,81

The underlined treatment means do not differ significantly at 1 %.

Discussion. Experiment 1 showed that plant material in excess of 4,5 g/kg was probably fatal to guinea-pigs, while lower doses produced nervous symptoms similar to those of cattle and sheep (Kellerman, Coetzer & Naudé, 1988). An examination of the occurrence of symptoms in guinea-pigs revealed that the time lapse between dosing and manifestation of clinical signs should be taken into account in evaluating the toxic response. In addition, since each animal received slightly different doses of plant material, it was also necessary to reflect these differences in the overall assessment of the response of the animals to the toxin. Hence a toxic response index was devised to incorporate the grade of symptoms, the time to most severe symptoms and the toxic dose received. The grade of symptoms were classified in retrospect once the symptoms in Experiment 1 had been noted. It was possible to grade the symptoms in order of severity from 0 to 5 as follows:

0= no symptoms
1= mild symptoms —shivering and ruffled appearance
2= moderate symptoms —shivering, muscle spasms and convulsions when excited
3= severe symptoms —muscle tremors, incoordination, hypersensitivity, twisting of body, non-stimulated convulsions
4= very severe symptoms —listlessness, hypersensitivity, convulsions and lateral recumbency
5= death

The grade of the most severe symptom noted for each guinea-pig was then used directly in the calculation of the toxic response index for that animal.

The time taken for the most severe symptoms to develop was difficult to incorporate into the index. While acknowledging that the time factor did play a role, there was insufficient evidence to support a linear relationship between time and toxic plant dose. It could be assumed, however, that an animal that took longer to die at a particular toxic dose had a less severe toxic response than one which died rapidly; hence an inverse relationship between time and the ultimate toxic response would give a better interpretation of the data. As the grade of symptoms was a more important indicator of response than the time taken for the symptoms to occur, it was felt that time could not be given the same weight in the index as the grade of symptoms. Hence, if the cube root of time was used, this would have the combined effect of reducing the weight of the time factor in the index and also of normalising the results within a group. Thus any individual variation between animals, (that may influence the time taken for the most severe symptoms to develop) would then, to some extent, be eliminated.

Since the relationship between plant dose and grade of symptoms was an inverse relationship, probably equal in importance to the grade of symptoms, this was multiplied directly with the cube root of the time to most severe symptoms to give the denominator of the toxic response index.

Hence:

$$\text{Toxic response index} = \left\{ \frac{\text{grade of most severe symptom noted}}{\sqrt[3]{\text{time to most severe symptom} \times \frac{\text{oral}}{\text{plant dose}}}} \right\} \times 100$$

It was felt that this index would give the best reflection of how each animal ultimately responded to the toxin.

From the comparison of the means of the toxic response indexes of Experiment 1, it was apparent that the toxic response of Group 4 was significantly greater than that of the untreated control Group 1. Normally, it would be expected that the toxic response of Group 4 would be almost as severe as that of Group 1; as treatment in Group 4 should be withheld until just prior to death (the maximum toxic response) and all animals in the control Group 1 can be expected always, to exhibit this maximum toxic response. In this experiment, however, since the lethal dose for guinea-pigs was unknown, the control Group 1 was in fact underdosed and in 3 out of the 4 cases failed to show the maximum toxic response. Why Group 4 should still show a more violent reaction to these sub-lethal doses of toxin is not known,

TABLE 2 Summary results of Experiment 2

Group No. Animal No.	Dose of Pyridoxine mg/kg	Plant dose received g/kg	Time to most severe symptoms min	Grade of symptoms	Toxic response index
1/1	0	4,87	53	5	27,33
1/2	0	5,10	51	5	26,44
1/3	0	4,43	125	5	22,57
1/4	0	4,90	108	5	21,14
2/1	9,61	4,81	—	0	0
2/2	8,93	4,46	—	0	0
2/3	7,94	3,96	—	0	0
2/4	9,92	4,96	—	0	0
3/1	4,63	4,63	105	5	22,89
3/2	4,72	4,72	100	5	22,82
3/3	4,35	4,35	100	4	19,81
3/4	5,00	5,00	100	5	21,54
4/1	8,99	4,50	120	4	18,02
4/2	11,90	5,95	75	3	11,96
4/3	8,93	4,46	90	2	10,01
4/4	8,33	4,70	75	2	10,09
5/1;2;3;4	$\bar{x} = 9,3$	0	—	0	—

although it is suspected that the discrepancy lies more in Group 1 than Group 4 as the magnitude of the toxic response of the latter is of the same magnitude as those obtained for Experiment 2. Some individual variation between guinea-pigs can be expected and a greater subjectivity in distinguishing between the grades of milder symptoms may also have played a part, however these explanations still do not entirely explain the apparent discrepancy between Groups 1 and 4. More in line with expectations is the fact that Group 3, which received higher treatment doses (1 mg) of pyridoxine at the onset of the experiment, had a significantly less toxic response than Group 4. This indicates that the pyridoxine treatment afforded some protection but, at these drug doses, apparently not sufficient to distinguish Group 3 from the control Group 1. The high dose treatment Group 3 also had a significantly less toxic response than the low dose treatment (0,1 mg) Group 2, which in turn did not differ significantly in its toxic response from that of the control (Group 1) and late treatment Group 4. This implied that the low dose of pyridoxine did not protect the guinea-pigs from toxicity and that in future higher doses would be necessary.

Thus Experiment 1 indicated that pyridoxine did have some effect in the treatment of *Albizia* toxicoses but insufficient data was available to confirm this. It also established that higher doses of plant material would be necessary to evoke a maximum toxic response in guinea-pigs and that this response would probably need higher doses of pyridoxine to counteract it. With the lessons learned from Experiment 1, Experiment 2 was desinged to test the efficacy of pyridoxine as a treatment more conclusively.

EXPERIMENT 2

Method: Five groups of 5 guinea-pigs were used. Groups 1 to 4 were dosed with *c.* 5 g/kg body mass (bm) plant material (Table 2) and Group 1 received no treatment. Together with the plant material, Groups 2 and 5 received *c.* 10 mg/kg pyridoxine S/C and Group 3 received *c.* 5 mg/kg. Group 4 was given *c.* 10 mg/kg pyridoxine I/M when severe symptoms were first observed (Table 2).

Results: As a result of the higher dose of plant material administered per os in this experiment, all the guinea-pigs in Group 1 (which received no treatment) died (Table 2). In contrast, all the guinea-pigs in Group 2, which received a high dose of pyridoxine prior to the dosing of plant material, showed no symptoms (Table 2). Three out of the 4 guinea-pigs that received a low dose of pyridoxine together with the plant material died. The lone survivor of this group showed very severe symptoms (Table 2) but recovered completely after about 4 h.

The guinea-pigs in Group 4 developed moderate to very severe symptoms before being treated with approximately 9,5 mg/kg pyridoxine (Table 2); but all four later recovered completely. Group 5's guinea-pigs, which only received pyridoxine, manifested no symptoms throughout the experiment.

As with Experiment 1, the effect of the toxin in each group was assessed by means of a toxic response index (Table 2). The mean toxic response index for each group was then compared using Scheffe's method of multiple comparison (Browne, 1985). The analysis of variance on the toxic response indices given in Table 2 excluding Group 2, resulted in a significant F-value (2,9) of 8,02 ($P < 0,01$). Comparison of treatment means was as follows:

Group	1	3	4	2
Pyridoxine	none	5 mg/kg S/C	10 mg/kg I/M at simp.	10 mg/kg S/C at start
Mean response index	24,37	21,76	12,52	0,00

The underlined treatment means do not differ significantly at 1 %.

Discussion: The results of Exp. 2 confirmed the postulation that a dose of 4,5 g/kg or greater of fresh pod material was lethal to guinea-pigs. As expected, Group 1 which received no treatment, but a lethal dose of plant material, showed the greatest degree of intoxication, while Group 2, which received approximately 9 mg/kg pyridoxine at the same time as a lethal dose of plant material, showed no symptoms at all, giving a zero toxic response index. According to Scheffe's method of multiple comparison there was a significant difference between Groups 1 and 2 at a 99 % level of confidence in terms of their response to the toxin. Hence it can be accepted that, at a dose of 9 mg/kg or greater, pyridoxine completely countered the effects of a lethal dose (> 4,5 g/kg) of *Albizia versicolor* pods. However, since in nature it

TABLE 3 Summary results of Experiment 3

A.

Exp 3.1. Control group; no treatment given

Group No. Animal No.	Plant dose received g/kg	Time to most severe symptoms min	Grade of symptoms	Toxic response index	Group toxic response index mean
1/1	2,5	330	4	23,15	27,49 ± 6,9
1/2		300	4	23,90	
1/3		180	5	35,42	
2/1	3,0	165	5	30,39	24,82 ± 9,64
2/2		390	3	13,69	
2/3		165	5	30,39	
3/1	3,5	300	5	21,34	27,39 ± 11
3/2		45	5	40,16	
3/3		330	5	20,67	
4/1	4,0	60	5	31,93	23,48 ± 7,67
4/2		195	5	21,55	
4/3		400	5	16,96	
5/1	4,5	90	5	24,79	19,89 ± 4,3
5/2		225	5	18,27	
5/3		300	5	16,60	

B.

Exp 3.2 10 mg/kg pyridoxine treatment was given to each guinea-pig

Group No. Animal No.	Plant dose received g/kg	Time to most severe symptoms min	Grade of symptoms	Toxic response index	Group toxic response index mean
1/1	5,0	—	0	0	0
1/2		—	0	0	
1/3		—	0	0	
2/1	5,5	—	0	0	0
2/2		—	0	0	
2/3		—	0	0	
3/1	6,0	180	1	2,95	4,78 ± 1,59
3/2		180	2	5,90	
3/3		225	2	5,48	
4/1	6,5	135	1	3,00	5,50 ± 2,70
4/2		758	5	8,44	
4/3		225	2	5,06	
5/1	7,0	120	3	8,69	5,39 ± 2,99
5/2		75	1	3,39	
5/3		60	1	3,65	

C.

Exp. 3.3 10 mg/kg pyridoxal treatment was given to each guinea-pig

Group No. Animal No.	Plant dose received g/kg	Time to most severe symptoms min	Grade of symptoms	Toxic response index	Group toxic response index mean
1/1	5,0	75	5	23,71	19,38 ± 4,4
1/2		135	5	19,49	
1/3		300	5	14,94	
2/1	5,5	195	5	15,68	18,62 ± 6,0
2/2		240	5	14,63	
2/3		45	5	25,56	
3/1	6,0	90	5	18,59	19,07 ± 4,1
3/2		45	5	23,43	
3/3		165	5	15,19	
4/1	6,5	105	5	16,30	16,15 ± 1,0
4/2		135	5	14,99	
4/3		90	5	15,19	
5/1	7,0	90	5	15,94	14,00 ± 2,0
5/2		225	5	11,74	
5/3		120	5	14,48	

is rarely possible to treat animals upon first exposure to a toxin, it was considered necessary to test the treatment in guinea-pigs showing severe symptoms, as was done with Group 4. An I/M injection of approximately 9 mg/kg pyridoxine in prostrate guinea-pigs resulted in full recovery despite them having received lethal doses of pod material. Recovery was most dramatic, with animals standing up within 5 to 10 min after receiving the pyridoxine.

From these experiments it was apparent that pyridoxine could be used both prophylactically as well as therapeutically for the treatment of *Albizia versicolor* toxicity in guinea-pigs. It must be noted, however, that prophylactic treatment of guinea-pigs with pyridoxine 12 h before exposure to plant material failed to protect them (Anderson L.A.P., 1989, personal communication) and that only concurrent administration of pyridoxine and plant material had the desired results. This finding is probably related to the half-life of pyridoxine in guinea-pigs.

As no significant difference in response to the toxin could be observed between Group 1 and Group 3 it appears as if lower doses of pyridoxine (5 mg/kg) failed to protect the guinea-pigs from the effects of the toxin. This suggested a possible relationship between plant toxin and therapeutic dose of pyridoxine.

EXPERIMENT 3

Method: After having established that the lethal dose for guinea-pigs was *c.* 4,5 g plant material per kg body mass and the therapeutic dose of pyridoxine required to block this dose was *c.* 10 mg/kg bm, the following sub-experiments were designed. In all instances the effect of the plant material and drug was measured by means of the toxic response index.

Exp. 3.1: Five groups of 3 guinea-pigs were dosed orally with plant material: Group 1 received 2,5 g/kg bm of plant material; Group 2, 3 g/kg; Group 3, 3,5 g/kg; Group 4, 4 g/kg and Group 5, 4,5 g/kg. No treatment was given to any of the animals.

Exp. 3.2: Five groups of 3 guinea-pigs each received 10 mg/kg pyridoxine S/C at the time of dosing of plant material per os. Group 1 received 5 g/kg plant material/kg body mass; Group 2, 5,5 g/kg; Group 3, 6 g/kg; Group 4, 6,5 g/kg and Group 5, 7 g/kg.

Exp. 3.3: This was a repetition of Experiment 3.2, but here pyridoxal replaced pyridoxine as treatment. It was anticipated prior to the experiment that pyridoxal would be at least as effective as pyridoxine in the treatment of *Albizia* toxicoses.

Results: The toxic response index for each group within each of the three sub-experiments are given in Table 3. The group toxic response index means for sub-experiment 3.1 ranged from 19,89 to 27,49 (Table 3 A) giving an average toxic response for the control Exp. 3.1 of 24,6. The group toxic response index means for the pyridoxine treated sub-experiment 3.2 ranged from 0 to 5,5 (Table 3 B), giving an average toxic response index for Exp. 3.2 of 3,1. The group toxic response index means for the pyridoxal treated sub-experiment 3.3 ranged from 14 to 19,38 (Table 3 C), giving an average toxic response index for Exp. 3.3 of 17,4. The mean toxic response indexes for each sub-experiment were then statistically compared as described under Experiment 2. The analysis of variance on the toxic response indices resulted in a significant F-value (2,12) of 6,93 ($P < 0,01$). Comparison of the means was as follows:

Sub-experiment:	3.1 (control)	3.3 (pyridoxal)	3.2 (pyridoxine)
Mean toxic response index:	24,6	17,4	3,1

The underlined means do not differ significantly at 1 %.

Discussion: The objective of this experiment was originally to generate a dose-response curve from which the type of antagonism between pyridoxine and the *Albizia* toxins could be established. However, due to the lack of precision in the measuring of the response it was decided that the results would not give the degree of accuracy required for the interpretation of dose response relationships and this aspect of the experiment was not pursued further. The experiment did nevertheless reveal another interesting finding.

As stated in the introduction, pyridoxal phosphate is generally regarded as the active and intracellular form of Vit. B6, while pyridoxine merely acts as a precursor to this form of the vitamin. Pyridoxal, the intermediate between pyridoxine and pyridoxal phosphate, can therefore be expected to act more rapidly and possibly more efficiently than pyridoxine. Because of this reasoning, the use of pyridoxal was explored as an alternative treatment for *Albizia* toxicoses. However, contrary to expectations, pyridoxal, unlike pyridoxine, did not prevent *Albizia* toxicoses. This finding is born out by the results which show no significant difference in the toxic response indexes for Experiment 3.1 (the untreated control group) and Experiment 3.3 which received pyridoxal at the same dose as the pyridoxine administered in Experiment 3.2. On the other hand, as expected, a significant difference in toxic responce was recorded between the controls (Exp. 3.1) and those receiving 10 mg/kg pyridoxine treatment (Exp. 3.2). There was also a significant difference between the toxic response indexes of the pyridoxine and pyridoxal treated groups, further supporting evidence that pyridoxal was not working as a treatment. No firm explanation can be offered for this finding, other than to suggest that symptoms of toxicity may not be related to blockage of any one of the pathways where pyridoxal is thought to play a role as co-factor, thus implying some other mechanism of action is in operation here. The active portion of the toxin/s may be cited at C4 of that molecule, since this is the only area where both toxins and pyridoxal differ from pyridoxine (Fig. 1). Further work is now being done to shed more light on these findings.

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