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ACUTE TOXICITY OF ECHIMIDINE IN MALE WISTAR RATS

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Abstract- There is very little published information on the oral toxicity of the pyrrolizidine alkaloid echimidine. The acute oral toxicity of echimidine was investigated in male Wistar rats, using OECD Test Guideline 425 with minor modifications. The calculated single-dose 72-hour oral LD50 of echimidine in male Wistar rats is 518 (228.9-654.3) mg/kg bw. Echimidine did not cause pulmonary lesions consistent with acute pulmonary hypertension. Echimidine caused hepatic lesions of haemorrhage, necrosis and apoptosis but hepatic venous endothelial cells did not appear to be a target of echimidine toxicity.

Keywords- echimidine, pyrrolizidine alkaloid, oral toxicity, rat, hepatotoxicity

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Introduction

Pyrrolizidine alkaloids (PAs) are a class of naturally-occurring toxins produced by over 6000 plants around the world. More than 660 PAs and PA *N*-oxides have been identified. These compounds have caused numerous cases of human hepatotoxicity, including cases resulting from ingestion of herbal preparations, and some large-scale epidemics resulting from contamination of staple foods [1]. All reported cases of human toxicosis due to PAs have been due to ingestion or, in the case of neonatal toxicosis, by intrauterine exposure to PAs ingested by the mother. It is therefore surprising that for the vast majority of PAs, the oral toxicity has not been investigated in experimental animals. For most PAs for which any experimental animal toxicity data exist, only intraperitoneal toxicity data are available, which are not useful to assess the hazard of ingestion of PAs. Improved characterisation of the toxicological profile of individual PAs, administered by the route of exposure most relevant to humans, is an important contribution to understanding the risk of PA exposure to human health.

Literature data indicate that there are substantial differences in the toxicity of different PAs within a species, and of the same PA in different species. Acute toxicity data allow, however, for some comparison of toxicity between related compounds, such as different PAs. In addition, information from acute oral toxicity studies may be used to select doses for subsequent short-term and subchronic toxicity tests when no other toxicology information is available [2].

Echimidine is a PA synthesised by a number of plants in the genera *Echium* and *Symphytum*, and is the predominant PA in comfrey (various species in the genus *Symphytum*). Ingestion of comfrey as a vegetable or herbal tea has led to several cases of veno-occlusive disease (VOD) of the liver in human beings [3]. However, because exposure in these cases was chronic and the level of echimidine in the comfrey products was not determined, there is a lack of robust information on the actual toxic dose of echimidine to human beings.

There is no information in the available literature concerning the short-term oral toxicity of echimidine alone, without the concurrent presence of other PAs, in any experimental animal species. The purposes of the present study were to determine the median lethal dose of echimidine by the oral route in male Wistar rats, to identify target organs of toxicity, and to obtain dose-rangefinding information for subsequent studies from which to determine a No Observed Adverse Effect Level (NOAEL).

The design of this study was based on OECD Test Guideline 425 [4], with the following exceptions:

- The gender of the test rats. The guideline recommends the use of female animals because for most toxicants, females are more susceptible than males, but this is not generally the case for PAs. Male rats have been reported to be more susceptible than female rats to a number of PAs, including monocrotaline, riddelliine, senecionine, seneciphilline, and retrorsine [5], although female rats are more susceptible than males to lasiocarpine N-oxide toxicity [6]. Male rats are more susceptible than females to heliotrine toxicity by the intraperitoneal route [6].
- The time-point at which the animals were considered to have survived or died. The guideline specifies that the acute oral toxicity is determined at 24 hours post-dosing, but for this study the deadline was extended to 72 hours on the basis of a study in which liver lesions induced by a single oral dose of monocrotaline were not obvious by light microscopy at 48 hours post-dosing, but were severe at 72 hours post-dosing [7].

- The Guideline generally includes a post-dosing observation period of 14 days, although shorter times are allowed. In this study, scheduled termination was performed at 7 days, because the study was focussed on acute effects and intended to exclude consequences of dysrepair.

Materials and Methods

Test Article Preparation

Echimidine was obtained from Planta Analytica, Connecticut, USA and formulated into stock suspensions by Grayson Wagner Co. Ltd., Penrose, Auckland, New Zealand. Echimidine was dissolved in dichloromethane and dispersed onto fumed silica, after which the methylene chloride was removed by evaporation. The solid echimidine on fumed silica was ground in a food-grade low-foaming phosphate-based diluent with a working pH of 4.0 to 5.0. For purposes of diluting the stock suspension to make dosing formulations, stock suspension was diluted with a similar diluent with pH 6.12. Composition of each diluent, as % w/w, is shown in [Table-1].

Table 1- Composition of diluents for echimidine dosing formulation, as % w/w

Ingredient	Grinding diluent	Gavaging diluent
Water	97.4, distilled water	97.43, tap water
Tetra potassium pyrophosphate	0.2	0.2
Phosphoric acid 68%	0.1 of 85%	0.07
Methyl paraben	0.1	0.1
Xanthan gum	0.2	0.2
Tergitol L 64	2	2
Total	100	100

Animal System

The study was conducted at Estendart Ltd., Palmerston North, New Zealand. The study plan complied with the New Zealand Animal Welfare Act 1999 and was approved (Approval number AEC 010/13) by the Kaiawhina Animal Ethics Committee. Male Wistar rats obtained from Otago University, New Zealand were approximately 9 weeks old at the time of receipt. Prior to the start of the study, rats were acclimated for at least 7 days to the study conditions, which included individual housing in polycarbonate cages, environmental temperature of 22(±3)°C, mean relative humidity of 45.1% (sd ± 8%; range 26.3-60.8%), and artificial lighting set to 12 hours light:12 hours dark. Water and a balanced rat chow, Massey University Rat Diet, were provided *ad libitum* with the exception of 4.7 to 5.9 hours prior to test article administration, when food was withheld to ensure that the test article was administered on an empty stomach. Food and water consumption and body weight were determined daily. Clinical observations were made at least once daily prior to dosing and at least twice daily after dosing. Each rat was weighed daily for at least four days prior to dosing, on the day of dosing and on days subsequent to dosing.

Echimidine suspension (10 mL/kg body weight) was administered using a flexible 18 gauge gavage tube. The first rat was dosed with 50 mg echimidine/kg body weight (bw). Subsequent rats were dosed based on the outcome for the previous rat as determined at 72 hours after dosing, using the dose calculation method specified by OECD Test Guideline 425 [4], with the exception that after three rats had been dosed from the first vial of stock suspension, Grayson Wagner Co. Ltd advised that the concentration of that vial was 206.00 mg/mL rather than 204.02 mg/mL as had been originally reported to Estendart. Some slight adjustments in dose level were made to maintain consistency as a result of this change. All rats were dosed with echimidine, because OECD Test Guideline 425 [4] does not include the use of control animals.

All rats were humanely terminated using carbon dioxide, either on Day 7 or earlier if they reached predetermined endpoints, which included any of the following clinical observations: overt icterus, severe ascites, severe dehydration, dyspnoea, reluctance or inability to move, coldness when handled, loss of righting reflex, or loss of either 10% of bodyweight in 24 hours, or 20% of bodyweight since dosing. All rats were subject to gross necropsy. Liver was collected from all rats, and liver weight was determined for rats that were terminated prior to scheduled necropsy on Day 7. The right lobe of each liver was preserved in saline-buffered formalin for subsequent histopathological examination. Lung, kidney, heart and the gastrointestinal tract were also collected from rats terminated prior to scheduled necropsy on Day 7. Tissues were processed to slides, and stained with haematoxylin and eosin.

Results

Mortality and Median Lethal Dose

Doses and 72-hour outcomes are shown in [Table-2].

Table 2- 72-hour single-dose acute oral toxicity determination of echimidine by up-and-down procedure adapted from OECD Test Guideline 425.

Subject	Dose (mg/kg)	Outcome at 72 hrs.	Termination
1	50.5[a]	Alive	Scheduled termination on Day 7
2	161.8[a]	Alive	Scheduled termination on Day 7
3	518[a]	Alive	Scheduled termination on Day 7
4	1650	Dead	Humane euthanasia on Day 3
5	518	Dead	Humane euthanasia on Day 3
6	160	Alive	Scheduled termination on Day 7
7	518	Dead	Humane euthanasia on Day 4
8	160	Alive	Scheduled termination on Day 7
9	518	Dead	Humane euthanasia on Day 2

[a]Intended doses were 50 mg/kg, 160 mg/kg and 510 mg/kg respectively, but higher doses were given because the concentration of the stock suspension was initially reported incorrectly.

The day of dose administration is counted as Day 1. For the purposes of this report the rat that received 161.8 mg/kg is regarded as having received 160 mg/kg, because the difference between intended and actual dose is considered to be without toxicological significance.

The median lethal dose (LD50) of echimidine was 518 mg/kg bw, with lower and upper bounds of 228.9 mg/kg bw and 654.3 mg/kg bw, respectively, although three of four rats given 518 mg/kg bw required humane euthanasia within 72 hours after dosing. The dose averaging estimate was 389.5 mg/kg bw.

All rats treated with echimidine doses ≤ 160 mg/kg bw survived to scheduled termination on Day 7.

Clinical Observations

Clinical signs were mild or absent at echimidine doses ≤ 160 mg/kg bw. Marked clinical signs, described below, were observed in most rats dosed with ≥ 518 mg/kg bw, with the exception of the first rat administered that dose. Clinical signs were nonspecific rather than indicating an adverse effect specific to any target organ or system.

Clinical observations in the rat administered 50.5 mg/kg bw were limited to a positive skin fold test, indicating dehydration, on Day 3 and reduced activity on Day 4.

No clinical abnormalities were observed in Subject 2, which was administered 161.8 mg/kg bw, whereas Subject 6, dosed with 160 mg/kg, exhibited mild porphyrin staining around the nares on Day 1 and pink staining of the head and neck from Day 1 to Day 7. The same clinical signs were observed in the third rat, Subject 8, dosed with 160 mg/kg bw. Subject 8 was also noted to have porphyrin staining at the nares on the day of dosing, and pink staining of the fur from Day 1 to Day 7, and the coat of this rat was noted to be rough on Days 6 and 7.

Chromodacryorrhea on the day of dosing, with pink staining of the fur until Day 7, was observed for the first rat dosed with 518 mg/kg bw, Subject 3. This rat was also noted to have a pale pinna on Day 1 and reduced activity on Day 3. The second rat dosed with 518 mg/kg bw exhibited rough coat, pink staining of the fur and a positive skin fold test on Day 2. These clinical observations persisted on Day 3 and the rat developed pallor of the mucous membranes, reduced activity, hunched posture and dyspnoea, and was terminated for humane reasons. The next rat dosed with 518 mg/kg bw, Subject 7, followed a similar clinical course with rough coat, chromodacryorrhea, mucous membrane pallor and reduced activity on Day 2. These clinical signs persisted until termination on Day 4. On Day 3 this rat also developed dehydration and hunched posture. On Day 4, Subject 7 was cold to touch and exhibited dyspnoea, and was terminated for humane reasons. The final rat dosed with 518 mg/kg bw was terminated on Day 2 due to clinical signs including dehydration, rough coat, chromodacryorrhea, mucous membrane pallor, reduced activity, dyspnoea and hunched posture.

The rat dosed with 1650 mg/kg bw, Subject 4, exhibited reduced activity, hunched posture, rough coat, dyspnoea and a positive skin fold test on Day 2. These clinical signs persisted on Day 3 and the rat also developed mucous membrane pallor and loss of righting reflex, and felt cold to touch. The rat was terminated for humane reasons on Day 3.

Body Weights, Body Weight Changes, Food Consumption and Water Consumption

Data on body weight changes, food consumption and water consumption are presented in [Table-3].

The rat dosed with 50.5 mg echimidine/kg bw gained weight each day while all rats dosed with 160 mg/kg lost weight in some 24-hour periods, and two of them had a lower body weight at termination than at the day of dosing [Table-4]. Daily food consumption did not appear to be affected in the animals treated with 160 mg/kg bw.

The only rat dosed with 518 mg echimidine/kg bw to survive to scheduled termination, Subject 3, lost body weight from Day 2 through to Day 5 and then began to gain weight again. This rat had markedly decreased food consumption until Day 6. All other rats dosed with ≥ 518 mg echimidine /kg bw showed marked weight loss and decreased food consumption. Water consumption was also decreased in Subjects 7, 9 and 4.

Gross Pathology and Histopathology

Necropsy findings, including terminal bodyweight change relative to dosing bodyweight, and liver weight as a percentage of terminal bodyweight, are presented in [Table-4].

Table 3- Body weight changes, food consumption and water intake of rats dosed with echimidine

Subject	Day[a]	Body weight change (g)	Feed consumed(g)	Water consumed (g)
50mg/kg				
1	2	5.5	29.1	44.4
	3	3.1	32.2	30.8
	4	3.9	26.4	41.6
	5	4.8	25.4	57.1
	6	1.8	26.2	56.4
	7	3.2	25	48.2
160 mg/kg				
2	2	6.9	20.4	105.6[b]
	3	-3.5	28.6	42.5
	4	1.7	31.5	30.9
	5	0.7	25.6	40.3
	6	3.1	22.5	35
	7	0.4	23.9	52.5
6	2	1.9	21.4	24.7
	3	-6.1	26.4	32.3
	4	-0.8	22	26.9
	5	-3.7	22.2	28.7
	6	-0.5	22.1	22.7
	7	-0.8	18.7	24.6
8	2	3.7	29.4	37
	3	-0.8	25.3	36.9
	4	-5.3	27.6	40.7
	5	3.1	33.4	52.2
	6	1.3	27.6	62.6
	7	-1.6	26.1	53.9
518 mg/kg				
3	2	-4.6	11.3	67.2[b]
	3	-9.6	12.9	70.7[b]
	4	-2.5	12.9	72.4[b]
	5	-8.4	14.9	87.1[b]
	6	2.6	16.6	63.4
	7	2	20.6	50.7
5	2	-10.8	13.7	48
	3	-22.7	2.8	31.3
7	2	-9.1	13.2	9.9
	3	-27.4	4.5	18.9
	4	-13.1	3.4	1.1
9	2	-24.9	16.5	19.6
1650 mg/kg				
4	2	-10.1	16.4	27.5
	3	-13.9	2.3	13.5

[a]Day of dosing was Day 1 for each rat
[b]known or suspected water bottle leakage

No gross abnormalities were discovered on necropsy of rats administered echimidine at doses ≤ 160 mg/kg bw. In contrast, only the first rat dosed with 518 mg/kg bw did not have gross abnormalities on necropsy, as specified in [Table-4].

No significant abnormalities were discovered on microscopic examination of the livers of rats administered echimidine at doses ≤ 160 mg/kg bw, or in the liver of Subject 3, the first rat dosed with 518 mg/kg bw.

In contrast to the liver of Subject 3, marked changes were found in the liver tissue of the other three rats dosed with 518 mg/kg bw. Their livers showed extensive coalescing zones of acute necrosis and haemorrhage, with mild to moderate infiltration by polymorph neutrophils. Apoptotic figures were frequently observed. The distri-

bution of necrosis was primarily centrilobular although some central veins were spared. Marginating macrophages and polymorph neutrophils were common in central veins but the endothelial cells of the central veins were generally intact [Fig-1].

Table 4- Body weight and gross liver findings on gross necropsy of rats dosed with echimidine on Day 1

Subject	Day and nature of death[1]	Bodyweight change between Day 1 and termination (g)	Findings recorded at gross necropsy	Liver weight as % of body weight
		50 mg/kg		
1	7,T	16.3	No abnormalities discovered	5.14
		160 mg/kg		
2	7,T	3	No abnormalities discovered	5.07
6	7,T	-14.7	No abnormalities discovered	4.13
8	7,T	-3.2	No abnormalities discovered	4.35
		518 mg/kg		
3	7,T	-25.8	No abnormalities discovered	4.62
5	3, M	-36.6	Stomach dilated with 15 g ingesta. Very little ingesta in intestines. Liver enlarged and pale. Multifocal reddening of lungs.	4.11
7	4, M	-50.6	Stomach distended with 18 g ingesta. No ingesta in small intestine and first 13 cm of small intestine reddened. Few fecal pellets in large intestine. Liver enlarged and pale.	4.18
9	2, M	-25.1	Stomach distended with ingesta. Very little ingesta in intestines, and few fecal pellets in colon. Liver dark and mottled. Lungs dark and mottled.	3.96
		1650 mg/kg		
4	3, M	-24	Stomach distended with 21 g of ingesta Liver mildly enlarged and pale	4.6

[1]Relative to dosing on Day 1. T = Scheduled termination, M = moribund termination

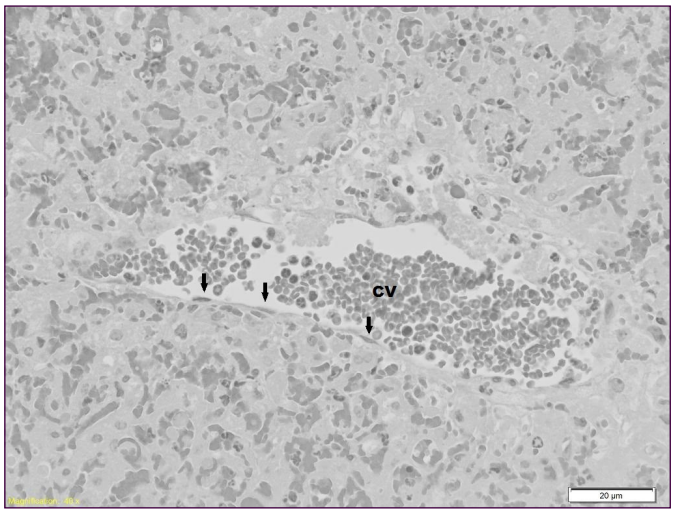

Fig. 1- Liver of Subject 7. Although the surrounding liver tissue is completely necrotic, the endothelial cells (arrows) of this central vein (CV) appear to be intact and normal in morphology. HE 40x

The liver of the rat dosed with 1650 mg/kg bw showed extensive haemorrhage, cellular degeneration, necrosis and apoptosis, and loss of sinusoidal architecture. Degenerative changes were severe and generalised, but less severe in islands of cells around some portal triads. There was mild infiltration of polymorph neutrophils. Endothelial cells of central veins appeared to be normal.

Lung tissue from rats with acute liver necrosis (Subjects 4, 5, 7 and 9) showed severe congestion with moderate thickening of alveolar septa.

No significant abnormalities were observed on microscopic examination of kidney, heart or gastrointestinal tract of any rats.

Discussion

According to the OECD-specified approach [4] the LD50 of echimidine in male Wistar rats was calculated to be 518 mg/kg bw. This dose resulted in severe liver damage.

The difference in toxicity between the first rat dosed at 518 mg/kg bw and subsequent rats is similar to findings in previous up-and-down toxicity studies conducted with other pyrrolizidine alkaloids [8,9]. For example, the first rat gavaged with 80 mg riddelliine /kg bw showed only mild congestion of the liver whereas three other rats subsequently administered the same dose of riddelliine developed severe haemorrhagic necrosis of the liver [8]. In a separate study, all four rats treated with 510 mg heliotrine /kg bw developed liver lesions, but the severity appeared to increase over the course of the up-and-down toxicity study. The greater susceptibility of male rats to PAs has been attributed to gender-specific differences in expression of cytochrome P450 enzymes, particularly those in the CYP3A subfamily [5]. In an up-and-down toxicity study rats are dosed sequentially, and therefore the rats increase in age at dosing over the course of the study. It is possible that the first rat dosed with 518 mg echimidine /kg bw in the current study, which was 8 weeks old, did not have the same CYP3A enzyme activities as the other three rats administered the same dose of echimidine, which ranged from 10 to 12 weeks old. The literature concerning the age-related expression of CYP3A2 is conflicting [10], probably because CYP3A2 shares a 97% homology with CYP3A9, leading to antibody cross-reaction [11]. However, CYP3A9 mRNA expression first appears at puberty in Sprague Dawley rats, and expression levels increases between 51 days and 91 days. CYP3A18 also increases after puberty in male Sprague Dawley rats, whereas CYP3A2 expression declines between days 51 and 91 [10]. There is a lack of comparable information for Wistar rats. Age-related changes in expression of enzymes responsible for metabolic activation may

also account for the increasing severity of liver histopathology in the up-and-down toxicity studies of riddelliine and of heliotrine [8,9].

Liver lesions were generally similar to those observed in previous up-and-down toxicity studies with other PAs, with the exception of the normal appearance of endothelial cells in central veins. Necrosis and disappearance of these cells was commonly observed in affected livers of rats dosed with ≥510 mg heliotrine/kg bw [9] or ≥80 mg riddelliine/kg bw [8], and retrospective examination of slides from the monocrotaline study showed that these endothelial cells were commonly degenerating or absent in affected livers. The relative resistance of these endothelial cells to echimidine may be important for human health risk assessment. The characteristic human response to excessive PA ingestion is hepatic VOD and the primary lesion is necrosis/apoptosis and dehiscence of the endothelial cells lining the hepatic sinusoids and central veins [7]. If echimidine does not exert this toxic effect in human hepatic endothelial cells, this may greatly reduce its toxicity to human beings.

In contrast to findings of up-and-down toxicity studies of monocrotaline [8] and heliotrine [9], echimidine did not induce pulmonary changes consistent with acute pulmonary hypertension. Although they have similar chemical structures, different PAs have different effects on the liver and respiratory system.

The distension of the stomach with ingesta was also observed in previous up-and-down toxicity studies of PAs [8,9] and was attributed to inhibition of stomach emptying. This effect was previously observed in male Wistar rats following oral gavage with monocrotaline (≥510 mg/kg bw), riddelliine (255 mg/kg bw) and heliotrine (1600 mg/kg bw).

Table 5- Comparison of results from up-and-down studies of four pyrrolizidine alkaloids in male Wistar rats

PA	LD50 (mg/kg) Dose averaging estimate	LD50 (mg/kg) Calculated estimate	Lower to upper bounds of calculated estimate (mg/kg)	Histopathological No Observed Effect Level (NOEL) (mg/kg)
Monocrotaline	508	510	297.3 - 963.4	Not identified (<160)
Riddelliine	98.1	80	55.0 - 175.0	25 (n=3)
Heliotrine	677.4	510	405.2 - 1142.0	160 (n=1)
Echimidine	385.9	518	228.9 - 654.3	160 (n=3)

Table 6- Comparison of clinical and histopathological findings from up-and-down studies of four pyrrolizidine alkaloids

Echimidine		Heliotrine		Monocrotaline		Riddelliine	
Dose (mg/kg BW)	Comments	Dose (mg/kg BW)	Comments	Dose (mg/kg BW)	Comments	Dose (mg/kg BW)	Comments
-	-	-	-	-	-	25 (n = 1)	0 mortality Negl. clinical signs Negl. to + weight loss/day 0 liver histopathology
~50 (n = 1)	0 mortality Negl. clinical signs 0 weight loss/d 0 liver histopathology	50	0 mortality 0 clinical signs Negl. weight loss/d 0 liver histopathology	-	-	-	-
-	-	-	-	-	-	80	1 of 4 mortality + to ++ clinical signs 0 to ++ weight loss/day Liver histopathology from 0 (1 rat) to +++ (3 rats)
~160	0 mortality Negl. clinical signs 0 to + weight loss/d 0 liver histopathology	160	0 mortality Negl. clinical signs 0 weight loss 0 liver histopathology	160	1 of 3 mortality + clinical signs + to +++ weight loss/d 0 to ++ liver histopathology	-	-
-	-	-	-	-	-	255	2 of 2 mortality Marked clinical signs Severe weight loss/day +++ liver histopathology in both rats
518	3 of 4 mortality + (1 rat) to +++ (3 rats) clinical signs + (1 rat) to +++ (3 rats) weight loss/d 0 (1 rat) to +++ (3 rats) liver histopathology	510	1 of 4 mortality ++ clinical signs ++ weight loss/d ++ (2 rats) to +++ (2 rats) liver histopathology	510	2 of 4 mortality ++ to +++ clinical signs ++ to +++ weight loss/day ++ (1 rat) to +++ (3 rats) liver histopathology	-	-
1650	1 of 1 mortality +++clinical signs +++ weight loss/d +++ liver histopathology	1600	3 of 3 mortality +++ clinical signs ++ to +++ weight loss/d +++ liver histopathology	1600	2 of 2 mortality +++ clinical signs +++ weight loss/day[a] +++ liver histopathology[a]	-	-

Scale: + = mild, ++ = moderate, +++ = severe

[a]Weight loss and liver histopathology data do not include findings from the peracute death of one rat.

A comparison of oral toxicity data obtained by the authors for the four PAs is presented in [Table-5].

The calculation method specified in OECD Test Guideline 425 [4] lacks sensitivity when there are outcomes in which, at a given dose, some animals live and others die. In these situations the method will identify that dose as the LD50 regardless of the proportion of animals that live and animals that die. Thus, although three of four rats dosed with 510 mg heliotrine /kg bw survived, that dose was identified as the LD50 for heliotrine. In contrast, three of four rats dosed with echimidine at 518 mg/kg bw died, but that dose was identified as the LD50 for echimidine. The upper and lower bounds of the LD50 estimate [Table-5] are skewed, suggesting that the true LD50 for heliotrine is higher than calculated and the true LD50 for echimidine is lower.

The method also calculates a dose averaging estimate of the LD50, which when considered together with the clinical signs and histopathology [Table-6], provides additional information on the relative toxicities of the PAs studied. Using the dose averaging estimate, the relative toxicities would be ranked as follows: riddelliine>echimidine>monocrotaline>heliotrine. On the other hand, comparison of the clinical and histopathological findings [Table-5] and in [Table-6] suggest that at the dose of 160 mg/kg bw monocrotaline is more toxic than echimidine, although a firm conclusion cannot be drawn because of the small numbers of subjects.

In conclusion, the calculated single-dose 72-hour oral LD50 of echimidine in male Wistar rats is 518 (228.9-654.3) mg/kg bw. Echimidine does not cause pulmonary lesions consistent with acute pulmonary hypertension but causes hepatic lesions of haemorrhage, necrosis and apoptosis. Hepatic venous endothelial cells do not appear to be a target of echimidine toxicity.

Abbreviations

PA: pyrrolizidine alkaloid

LD50: median lethal dose

NOAEL: No Observed Adverse Effects Level

OECD: Organisation for Economic and Cooperative Development

VOD: veno-occlusive disease

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Conflicts of Interest: None declared.

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