Toxicity of Extract from Three Larkspur Species (*Delphinium barbeyi, D. glaucescens, D. occidentale*) Measured by Rat Bioassay

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Highlight: Differences in toxicity among species of larkspur have been suspected but until now no standard method of comparison has been made. A rat bioassay has recently been developed and was used to estimate the toxicity of an extract from three larkspur (*Delphinium barbeyi, D. glaucescens,* and *D. occidentale*). *Delphinium barbeyi* was the most toxic and *D. occidentale* the least, as measured by this bioassay.

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Larkspur poisoning of cattle in North America was reported as early as 1760 (Miller). About 1900, the United States Department of Agriculture and Agricultural Experiment Stations began to investigate and report extensive death losses of cattle and sheep due to larkspur poisoning in the western United States (Chestnut and Wilcox 1901; Wilcox 1897; Wilcox 1899). There was some confusion at that time concerning the relative toxicity of larkspur species. Irish (1889) fed the tops of 24 plants of *Delphinium trolliifolium* to one steer and the roots to another and observed no signs of toxicity. He also fed 30 plants of "white larkspur" and observed no signs of toxicity. Wilcox (1897) reported that *D. menziesi* was poisonous to sheep, but Nelson (1906) concluded that *D. menziesi* was not poisonous to sheep. Glover (1906) reported that at least 18 *Delphinium* species had been found in Colorado and that the four most abundant species were poisonous to livestock. Experimental feeding of larkspur to cattle confirmed field observations of symptoms caused by larkspur poisoning (Chestnut and Wilcox 1901; Marsh et al. 1916) and the report that toxicity of the plant seemed to decrease during the growing season (Marsh et al. 1916).

Toxicity studies with guinea pigs and rabbits have been limited (Beath 1919; Crawford 1907) and no standard method for

Table 1. Median lethal dose (LD_{50}) for rats of an alcohol extract from *Delphinium barbeyi, D. glaucescens,* and *D. occidentale*.

Plant sample	Mean body weight of rats[1] (g)	No. of rats injected	Dose[2] (mg/g)	Mortality rate[3]	LD_{50}[4] (mg/g)	Confidence interval[5]
D. barbeyi	297	6	2.93	0	4.5	4.0 – 5.1
	297	6	3.64	0		
	304	6	4.51	4		
	291	6	5.59	5		
D. glaucescens	255	5	12.33	2	16.2	12.3–21.4
	226	5	14.43	1		
	247	5	16.88	3		
	228	5	19.75	4		
D. occidentale	245	5	37.06	1	40.4	37.6–43.5
	250	5	39.29	2		
	257	5	41.65	3		
	251	5	44.14	4		

[1] After 24-hour fast.
[2] Dose levels used to determine LD_{50} expressed as milligrams of dried plant material equivalent to extract injected subcutaneously, per gram of body weight.
[3] Number of rats that died within 24 hours after injection.
[4] Calculated by method of Weil expressed as milligrams of dried plant material equivalent to extract subcutaneously injected, per gram of body weight.
[5] Estimation of a confidence interval that will encompass the LD_{50} 95 times in 100 tests.

measuring toxicity of larkspur has been established. A rat bioassay to estimate the toxicity of an alcohol extract from larkspur has recently been developed (Olsen 1977).

This report describes the toxicity to rats of extracts from *D. barbeyi, D. glaucescens,* and *D. occidentale*. The purpose of the study was to investigate whether differences in toxicity between larkspur species from different locales could be measured.

Methods

The above-ground parts of the three species of larkspur were collected in 1973 during the early flower bud stage, i.e., small, unopened buds were visible on some plants. *Delphinium barbeyi* was collected at an elevation of 3,109 m at the head of Manti Canyon in central Utah; *Delphinium claucescens* was collected at an elevation of 2,438 m on the border of Idaho and Montana north of Dubois, Ida.; and *Delphinium occidentale* was collected at an elevation of 1,829 m east of Ashton, Ida. Extracts of air-dried, ground plant material were tested for toxicity to rats as previously described (Olsen 1977). An alcohol extract was made, evaporated to dryness, and reconstituted in saline solution. Rats of either sex were individually identified, weighed, and caged in groups of six. The undiluted saline extract was subcutaneously injected. At each site, 1 ml or less was injected. The dose for each rat was calculated on the basis of the amount of saline extract injected (dose expressed as mg of dry plant extracted) per gram of body weight required to cause toxicosis. Four dose levels, increasing in a geometric progression, were chosen after preliminary tests. The median lethal dose (LD_{50}) was calculated from the mortality rate at 24 hours after injection; the method of Weil was used (Weil 1952).

Results and Discussion

Clinical signs of toxicosis could be observed 1.5 hours after injection, and most animals died between 1.5 and 18 hours after injection.

The LD_{50} for extracts from each larkspur species has been listed in Table 1. The LD_{50} was significantly different for each species. *D. barbeyi* was the most toxic and *D. occidentale* the least. The confidence interval was much narrower for *D. barbeyi* than for the others. This narrow confidence interval was a result, in part, of the fact that the death loss at the intermediate doses (mortality rate in Table 1) was sharply different for *D. barbeyi*.

The difference in toxicity between species, as measured, could result from several factors.

A number of potentially toxic alkaloids have been isolated from *Delphinium* sp. (Pelletier and Keith 1970). The type of major alkaloid, or the chemical form in which it exists, may vary among these species. Also, the absolute concentration of alkaloid present in these *Delphinium* species may be different, or the concentration ratio of the various alkaloids present may vary.

Another factor is the stage of growth of the plant. Even though the plant material tested for each species was generally in the same stage of growth, the change in toxic alkaloids throughout the growing season may not be the same for each species.

The geographical site of growth and the seasonal weather conditions may also be a potential factor contributing to a difference in toxicity between species.

The method of extraction used in this study was relatively simple and yielded suitably reproducible results for an individual sample of plant material; however, extraction of toxic alkaloidal material may not have been exactly the same for different species. More detailed chemical determinations will be required to clarify this point.

We will continue to use the bioassay method for more detailed study of larkspur toxicity. Potential differences between years of growth, within a growing season and among species and the effect of environmental factors are being studied.

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