The Good and the Bad of Poisonous Plants: an Introduction to the USDA-ARS Poisonous Plant Research Laboratory

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Published online: 25 February 2012

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Abstract This article provides an overview of the Poisonous Plant Research Laboratory (PPRL), about the unique services and activities of the PPRL and the potential assistance that they can provide to plant poisoning incidences. The PPRL is a federal research laboratory. It is part of the Agricultural Research Service, the in-house research arm of the U.S. Department of Agriculture. The mission of the PPRL is to identify toxic plants and their toxic compounds, determine how the plants poison animals, and develop diagnostic and prognostic procedures for poisoned animals. Furthermore, the PPRL's mission is to identify the conditions under which poisoning occurs and develop management strategies and treatments to reduce losses. Information obtained through research efforts at the PPRL is mostly used by the livestock industry, natural resource managers, veterinarians, chemists, plant and animal scientists, extension personnel, and other state and federal agencies. PPRL currently has 9 scientists and 17 support staff, representing various disciplines consisting of toxicology, reproductive toxicology, veterinary medicine, chemistry, animal science, range science, and plant physiology. This team of scientists provides an interdisciplinary approach to applied and basic research to develop solutions to plant intoxications. While the mission of the PPRL primarily impacts the livestock industry, spinoff benefits such as development of animal models, isolation and characterization of novel compounds, elucidation of biological and molecular mechanisms of action, national and international collaborations, and outreach efforts are significant to biomedical researchers. The staff at the PPRL has extensive knowledge regarding a number of

poisonous plants. Although the focus of their knowledge is on plants that affect livestock, oftentimes, these plants are also poisonous to humans, and thus, similar principles could apply for cases of human poisonings. Consequently, the information provided herein could be of benefit to healthcare providers for human cases as well.

Keywords Poisonous plants · Chemical analysis · Plant toxins · Toxicology · Plant identification

Introduction

In the late 1800s, settlers migrated west with their livestock, and large losses began to occur as their animals encountered unfamiliar range plants. As a result of these losses, stockmen pleaded for help from the federal government. Dedicated research efforts were established by federal mandate around 1894. Early researchers traveled west each spring and summer to temporary field stations established in locations where the largest losses of livestock occurred. A permanent research location was created for the Poisonous Plant Research Laboratory (PPRL) in 1955 on the campus of Utah State University, Logan, UT, USA. In 2004, a new research laboratory was dedicated providing additional space and resources. Scientists at the PPRL provide information to and have active collaborations with scientists around the USA and all over the world including China, Brazil, South Africa, Croatia, and Australia.

The mission of the PPRL is to identify toxic plants and their toxic compounds, determine how the plants poison animals, and develop diagnostic and prognostic procedures for poisoned animals. Furthermore, PPRL's mission is to identify conditions under which poisoning occurs and develop management strategies and treatments to reduce

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losses. The PPRL currently has 9 scientists and 17 support staff, representing various disciplines consisting of toxicology, reproductive toxicology, veterinary medicine, chemistry, animal science, range science, and plant physiology. This team of scientists provides an interdisciplinary approach to applied and basic research to develop solutions to intoxication. Major projects that are currently under investigation at PPRL encompass a variety of plants and can be reviewed on the PPRL website (<http://www.pprl.ars.usda.gov>). Criteria for selecting research problems vary but typically include scope of the problem (e.g., local, regional, or national importance), severity of the losses, in both animal numbers and financial terms, current focus of the research team, and uniqueness of the poisoning problem. Table 1 lists many of the plants that PPRL scientists have researched in the past or are currently studying. Table 2 is a list of the plant toxins for which PPRL scientists actively perform analyses.

PPRL scientists are currently pursuing five areas of investigation. These areas include (1) developing science-based guidelines for grazing livestock on rangelands infested with toxic plants; (2) reducing the risks of livestock losses due to variations in quantitative and qualitative differences in toxin accumulation over time and in plant species by quantifying the influence of endophytes, climate changes, and genotype on plant toxin accumulation; (3) enhancing feed and food safety by improving risk assessment and diagnosis of plant-induced poisoning to livestock by improving analytical methods for analyzing plant and animal tissues, measuring toxicokinetics, assessing carcinogenic and genotoxic potential, and identifying toxin metabolites and biomarkers of toxicoses; (4) developing improved procedures and guidelines for diagnostic and prognostic

evaluation to reduce negative impacts of poisonous plants on livestock reproduction and embryo/fetal growth; and (5) developing guidelines to aid producers and land managers in making genetic-based herd management decisions to improve livestock performance and animal safety on rangelands infested with poisonous plants.

Methods to Identify Plant Poisonings in Livestock

The PPRL provides information and support to livestock owners, managers, veterinarians, and extension agents to help them determine if an animal was poisoned by a plant. Despite the high frequency of occurrence, arriving at a positive diagnosis of plant poisoning is challenging. To help make an accurate diagnosis, a history of the poisoning incident is obtained, which includes understanding the animal involved, the location, plant communities involved, and management strategies being used (Table 3). Compiling animal information such as type of animal, overall condition, age, sex, vaccination histories, and nutritional status as well as identifying clinical signs, lesions, number of affected animals, and progression of disease are the first steps in identifying a cause. Once this information is gathered, a list of potential causes is developed. This list is used to develop an investigative plan that focuses on confirming the right diagnosis and excluding all other possibilities. Making a complete and thorough list requires engagement of appropriate experts when needed (Table 4). Many infectious, degenerative, and immunologic diseases produce clinical signs, biochemical changes, and lesions identical to those caused by poisonous plants and thus must be ruled out. An extensive differential diagnosis is created so that a plan can be formulated to confirm or rule out possible causes. The plan includes field investigations, physical examinations, biochemical or serologic evaluations of the blood, possible postmortem evaluations, and chemical or microscopic evaluations of plants and/or animal tissues (Tables 3 and 5). Rapid institution of this process oftentimes allows for an accurate diagnosis, practical solutions, and aids in avoidance of catastrophic animal losses.

Scientists at the PPRL help with plant poisoning incidences by identifying poisonous plants and analyzing plant and animal tissues for plant toxins. Plants that are included in the list of differential diagnoses or unidentified plants are collected for positive identification. The entire plant is collected if possible or a high-quality photograph if it is the only option. Flowering plants are most easily identified. Most state land grant (agricultural) colleges have herbaria with experts able to identify most plants. However, if local options are not readily available, scientists at the PPRL can help. Additionally, due to the fact that the toxicity of a plant is often variable, additional plant samples are oftentimes needed for chemical evaluation

Table 1 Poisonous plants for which the PPRL has information/recommendations for livestock poisonings

Arrowgrass	Horsebrush	Ponderosa pine
Western bracken fern	Horsetail	Rayless goldenrod
Bitter rubberweed	Jimsonweed	Selenium-accumulating plants
Broom snakeweed	Larkspur	Sneezeweed
Chokecherry	Locoweed	Spring parsley
Cocklebur	Lupine	St. Johnswort
Copperweed	Milkvetch	Tansy mustard
Death camas	Milkweed	Tansy ragwort
Desert bailey	Nitrate-accumulating plants	Riddell's groundsels
Drymary or inkweed	Oak	Water hemlock
False hellebore	Colorado rubberweed	Yellow star thistle
Halogeton	Poison hemlock	Yew
Hemp dogbane		

Table 2 Plants and plant toxins commonly analyzed at PPRL

Plant scientific names	Plant common names	Class of toxin	Examples of toxin
<i>Delphinium</i> spp.	Larkspurs	Norditerpenoid alkaloids	Methyllycaconitine, deltaline
<i>Astragalus</i> and <i>Oxytropis</i> spp.	Locoweeds	Indolizidine alkaloids	Swainsonine
<i>Astragalus</i> spp.	Vetches	Nitrotoxins	3-Nitropropanol
<i>Astragalus</i> spp.	Selenium accumulator plants	mineral	Selenium
<i>Lupinus</i> spp.	Lupines	Quinolizidine and piperidine alkaloids	Anagyrine, ammodendrine
<i>Senecio</i> spp., <i>Crotalaria</i>	Tansy ragwort, Riddell's groundsel	Pyrrolizidine alkaloids	Riddelliine
<i>Zigadenus</i> spp.	Death camas	Steroidal alkaloids	Zygacine
<i>Veratrum</i> spp.	Skunk cabbage, corn Lily, false hellebore	Steroidal alkaloids	Cyclopamine, veratramine
<i>Pinus</i> and <i>Juniperus</i> spp.	Ponderosa pine, juniper trees	Labdane acids	Isocupressic acid
<i>Conium maculatum</i>	Poison hemlock	Pyridine alkaloids	Coniine
<i>Cicuta maculata</i>	Water hemlock	Acetylenic alcohols	Cicutoxin

of potential toxins. It is best to contact the appropriate laboratory prior to submission to ensure the sample is properly prepared and that an adequate sample size is being sent.

Biomedical Applications of Poisonous Plant Research

While the mission of the PPRL primarily impacts the livestock industry, spinoff benefits such as development of animal models, isolation and characterization of novel compounds,

elucidation of biological and molecular mechanisms of action, national and international collaborations, and outreach efforts are significant to biomedical researchers. Traditionally, searches for new drugs in human medicine investigate plants that have an ethnopharmacological basis for their use, whereas plants that are commonly poisonous to livestock have been less well studied. However, numerous advances in understanding and potentially treating human diseases have resulted from investigations of the toxic principles derived from poisonous plants that are commonly responsible for livestock

Table 3 Components essential for an accurate diagnosis in suspected plant poisoning episodes

History of the poisoning incident: (this includes all information and analyses already performed)

Pertinent facts: breed, sex, age, number, condition, vaccination status, mineral supplements, feeding or pasture changes, and other treatments

Clinical disease: number affected, signs, clinical course and progression, lesions, and mortality

Clinical tests: blood tests to evaluate inflammation and organ function and to evaluate immunologic responses to infectious agents (blood cell counts and serum element, metabolite and biochemical analyses, and serologic tests)

Field studies: (this includes additional evaluations and analyses to be performed)

Animals: general condition of affected animals, unusual behaviors, clinical signs, and lesions. Additional clinical tests may be indicated to analyze samples for plant toxins or metabolites in the tissues, blood, urine, or feces

Pasture:

1. Determine forage availability, plant species composition, and evidence of grazing patterns
2. Collection of potential problem plants or unidentified plants (dried samples for identification, frozen samples for chemical analysis)
3. Note physical location, weather conditions at the time of the incident (for example, snow depth) and their effect on forage and forage availability

Prepared feeds such as hay, silage, or concentrate feed samples (frozen for analysis)

Water, salt, and mineral supplements including location and use (samples frozen for analysis)

Check for other potential hazards, such as old batteries and pesticide-laced feed

Postmortem examination:

Animal condition and lesions

Rumen and gastrointestinal tissues and contents

Tissues for histologic studies fixed in 10% neutral buffered formalin (brain, lung, heart, liver, spleen, gastrointestinal tract, kidney, skeletal muscle, and any gross lesions)

Tissues for chemical or microscopic studies stored in plastic bags and frozen (rumen and gastrointestinal contents, complete eye, liver, kidney, serum, whole blood, body fat, bone, urine, and milk if lactating)

Table 4 Resource list

USDA/ARS Poisonous Plant Research Laboratory

Tel: +1-435-7522941

<http://www.pprl.ars.usda.gov>

State agricultural extension service

<http://www.csrees.usda.gov/Extension>

State animal disease diagnostic laboratory

[http://www.aavld.org/index.php?](http://www.aavld.org/index.php?option=com_content&view=article&id=18)option=com_content&view=article&id=18. Id=aavld
(laboratories accredited by American Association of
Veterinary Diagnostic Laboratories)

Local herbarium

<http://herba.msu.ru/mirrors/www.helsinki.fi/kmus/botmus.html>
(lists of public herbaria throughout the world)

Microscopic analysis of feces and ingesta

Texas Veterinary Medical Diagnostic Laboratories

Tel: +1-888-6465623

<http://tvmdl.tamu.edu>

poisoning. The following are a few examples of how information gained through livestock-based research efforts have also resulted in human biomedical applications.

Cancer Therapy

During the mid-20th century, up to 25% of pregnant ewes that grazed in the mountains of central Idaho gave birth to lambs suffering from serious craniofacial defects [1]. These anomalies varied from the extreme malformation, cyclopia, to mildly deformed upper jaws. Ingestion of *Veratrum californicum* by sheep on day 14 of gestation was shown to induce severe birth defects in offspring [2]. Although *Veratrum* is relatively unimportant as a plant poisonous to adult sheep, it is extremely toxic to a developing embryo. Experiments also showed that the damage to the developing embryo can be severe enough to cause embryonic death [3]. Scientists at the PPRL determined that the primary alkaloid responsible for *Veratrum*-induced terata was 11-deoxojervine, which was named cyclopamine. In addition to sheep, cyclopamine induced craniofacial defects in rabbits and chick embryos [4]. Recent advances in molecular biology and genetics have demonstrated that the molecular mechanisms underlying the induction of terata by cyclopamine are due to the inhibition of the sonic hedgehog signaling pathway. Studies using a variety of sonic hedgehog-dependent cell types have shown that virtually all aspects of sonic hedgehog signaling are interrupted upon exposure to cyclopamine [5, 6]. These studies concluded that the cyclopamine exerts its primary teratogenic

Table 5 Partial list of tests, samples, sample size, and preservation for investigation of potential poisonous plant poisoning

Test	Sample	Size	Shipping
Blood counts	Whole blood	3–5 mL	Chilled on ice
Serum biochemistries	Serum	5–10 mL	Chilled on ice or if frozen, serum should be separated from the cells
Microscopic evaluation of tissues	Various tissues	1 × 1 × 2-in pieces	Fixed formalin
Postmortem or necropsy	Dead or moribund animal	Whole animal	Fresh
Chemical evaluation of serum, blood, urine, or milk	Serum, whole blood, urine, or milk	20 mL	Stored in plastic tubes on ice or frozen
Chemical evaluation of tissue	Various tissues	2 × 2 × 4-in pieces	Stored in plastic bags and frozen
Chemical evaluation of feces or gastrointestinal contents	Feces or ingesta	1–2 lbs (about a sandwich bag full)	Stored in plastic bags and frozen
Plant identification	Whole plant	Whole plant including flowers, pods, leaves, stems, and roots	Fresh if delivered that day, dried if hand-delivered later, pressed and dried if shipped
Plant chemical analysis	Whole plants	5 or 6 whole plants	Fresh if delivered that day, dried if shipped, or frozen if they can be maintained frozen during shipping
Hay for weed contamination and weed identification	Stored baled hay	5 or 6 bales	Dry
Hay for nitrate analysis	Hay	Several representative samples (these can be core samples), 1–2 lbs	Dry
Prepared feeds	Feeds	Representative feed samples such as cubed feed, 1–2 lbs	Dry
Silage or green-chopped feed	Feeds	Representative feed samples, 5–10 lbs	Frozen

Be sure to check with the laboratory because they often require specific sampling, sample preparation, and shipping instructions. Label all materials with ink, provide date, owner, location and contact information

effect on developing embryos by selectively blocking sonic hedgehog signal transduction [5, 6]. Consequently, cyclopamine has become a valuable research tool to study the sonic hedgehog signaling pathway and its biological effects. The sonic hedgehog signaling pathway is not only essential for proper embryonic development but it also plays a role in the division of adult cells. Thus, this signaling pathway has been shown to be involved in the formation of several cancers [7, 8]. Consequently, several pharmaceutical companies are currently testing derivatives of cyclopamine as potential therapeutics [9].

Skeletal Malformations

Research at the PPRL on plant-induced skeletal malformations and cleft palate began in the late 1950s when musculo-skeletal defects in newborn calves known as “crooked calf disease” were attributed to maternal ingestion of *Lupinus* spp. [10, 11]. A high incidence of cleft palate was also associated with lupine-induced crooked calf disease [12, 13]. In the late 1960s and early 1970s in the Midwestern and Southern states, epidemic proportions of skeletal malformations in pigs were recorded. It was determined that poison hemlock (*Conium maculatum*) and burley tobacco (*Nicotiana tabacum*) were responsible. The induced malformations in newborn pigs appeared similar to those in lupine-induced crooked calf disease.

The proposed mechanism of action for *Lupinus*-induced malformations and cleft palate has been attributed to reduction in fetal movement [14]. The mechanism of action of the teratogenic effects of *Lupinus*, *Conium*, and *Nicotiana* spp. is believed to be identical [15]. Experiments using ultrasound demonstrated a direct relationship between reduced fetal activity and severity of contracture-type skeletal defects and cleft palate in sheep and goats. Further research suggested that this inhibition of fetal movement must be over a protracted period of time during specific stages of gestation. The skeletal malformations are theoretically caused by abnormal alignment, bowing, or twisting as a result of the in utero positioning and/or abnormal tendon and ligament development resulting from the lack of movement [14]. The cleft palate is believed to result from mechanical interference by the tongue between the palatal shelves at the programmed time of closure [16, 17].

Although the goat model was primarily developed to study the mechanism of action of crooked calf syndrome in cattle, this model has become an important tool in the study of the mechanism of cleft palate induction in humans and fetal biomedical research [18, 19]. Currently research is focused on the privileged period of fetal scarless healing and development of in utero surgical procedures to repair human cleft palates early in gestation. The methodology and techniques used to successfully repair congenital cleft palates in utero with successful scarless palatal healing and development after repair have been demonstrated [19]. This model

is also useful for histological comparison of the prenatal and postnatal-repaired cleft palate and for comparison of craniofacial growth and development. Therefore, the goat model provides an ideal congenital model to study the etiology of cleft palate in humans, develop fetal surgical techniques in utero, and compare palate histology after prenatal or postnatal repair.

Even though research at the PPRL has been limited to the three genera mentioned above, there are many other plant species that contain structurally similar alkaloids. These include species of the genera *Genista*, *Prosopis*, *Lobelia*, *Cytisus*, *Sophora*, *Pinus*, *Punica*, *Duboisia*, *Sedum*, *Withania*, *Carica*, *Hydrangea*, *Dichroa*, *Cassia*, *Ammonden-dron*, *Liparia*, *Colidium*, and others. Many of these plants may be included in human diets; however, toxicity and teratogenicity are a matter of dose, rate of ingestion, time of insult, and alkaloid concentration and composition in the plant, and thus toxicity/teratogenicity may not always be readily correlated with ingestion. Of note, there is circumstantial evidence that the alkaloids in *Lathyrus latifolius* may have been the cause of arm and hand deformities in a baby boy, via maternal ingestion of goat's milk [20].

Glycoprotein Processing

Some of the earliest research undertaken by the U.S. Department of Agriculture (USDA) (PPRL) to prevent widespread livestock losses was focused specifically on locoweeds. Consumption of locoweeds by animals produces a multitude of syndromes, including neurological problems, depression, emaciation, reproductive disturbances, birth defects, and abortion. Specific problems observed are a consequence of numerous factors, including animal species, nutritional status, prior exposure to the plant, pregnancy, and environmental conditions [21]. For example, cattle grazing the plants at high altitudes exhibits congestive right-heart enlargement and edema of the brisket [22, 23], with nursing calves exhibiting the symptoms prior to their mothers, indicating that the toxin is transferred in the milk. The primary toxic constituent of locoweeds was determined to be the indolizidine alkaloid swainsonine [24]. Swainsonine was first isolated and identified as the toxic constituent of *Swainsona* species, known as “poison peas,” a genus of legumes restricted to Australia but closely related to the locoweeds both in physical appearance and also in their effects on livestock. The toxin has also been found in a number of *Astragalus* and *Oxytropis* species in other parts of the world, such as South America and areas of China. It has also been shown to occur in members of the morning glory family (*Ipomoea* species) that are poisonous to sheep in Australia and to goats in Mozambique and Brazil [25].

The chemical structure of swainsonine has many similarities to the simple sugar mannose, which it appears to mimic.

As a result of this mimicry, swainsonine inhibits the enzyme α -mannosidase, which trims sugar molecules from glycoproteins within the cell and thus is essential for the proper cellular function. Failure of the trimming process results in an accumulation of the glycoproteins within the cell until vacuolation results. After a sufficient number of cells have been damaged in this way, the signs of poisoning appear in the animal. Because all cells depend on the proper functioning of α -mannosidase, many different organs can be damaged, including the brain, heart, reproductive system, and digestive system. The particular organs affected and signs of poisoning depend on the amount of swainsonine consumed and the period of exposure, as well as external factors such as nutritional status, grazing altitude, and pregnancy. Swainsonine is very water soluble and therefore distributed to many parts of the body. It is also rapidly excreted, primarily in the urine, but in lactating animals, a portion of it is transferred to the milk, such that nursing animals may become locoed [26, 27]. The fast excretion rate suggests that occasional consumption of locoweeds for short periods is unlikely to have serious adverse effects but that continuous consumption, even at relatively low levels, can result in poisoning.

The recognition that swainsonine was an inhibitor due to its mimicry of mannose suggested that structurally similar alkaloids might have similar properties. One such alkaloid, castanospermine, has been isolated from seeds of Moreton Bay chestnut (*Castanospermum australe*). Other scientists found that castanospermine was a potent inhibitor of glucosidases, enzymes that are also essential for glycoprotein processing [28]. The large leguminous seeds, which litter the ground beneath the trees, had been known to be toxic to livestock, especially cattle and horses and, even on occasion, humans [29]. The discovery of the biochemical activity of this alkaloid therefore suggested a close analogy to locoweed poisoning. Glycosidase-inhibitory alkaloids, which were initially represented by swainsonine alone, have now expanded to encompass a number of structural classes including indolizidines, pyrrolizidines, tropanes, and simpler pyrrolidine and piperidine analogues. Their bioactivity has stimulated the synthesis of counterparts. To include so many disparate structural types under a single classification, the generic name of “polyhydroxy alkaloids” has been applied because the presence of multiple hydroxyl groups is a consistent feature [30].

The fundamental cellular function of glycoprotein processing primarily affects N-linked glycoproteins, which are involved in numerous essential physiological functions, especially cell–cell recognition reactions critical to pathogenesis, inflammation, parasitism, development, cell adhesion, and symbiosis. Consequently, as might be expected from a class of compounds that inhibits glycosidases, the polyhydroxy alkaloids exhibit an exceptional diversity of biological effects, including insecticidal, herbicidal, antimicrobial, and therapeutic activities. Discovery and isolation of many

of the alkaloids has been a result of observations of the ultimate clinical effects that result from the consumption by animals of plants containing these bioactive compounds. The capability of polyhydroxy alkaloids to disrupt the general cellular function of glycoprotein processing leads to the expectation that these compounds may have therapeutic potential for the treatment of various diseases. Investigation of the alkaloids for therapeutic potential has so far concentrated on the treatment of cancer and inhibition of metastasis, as antiviral agents and as antiparasitics. Structurally related compounds have also been used as antidiabetic drugs [31]. Awareness of these alkaloids first arose because of their effects as the toxic components of poisonous plants, but many are now being identified in plants not established as toxic and even in common food plants for humans, including tomatoes, potatoes, eggplants, and sweet potatoes [32].

Summary

The PPRL is a USDA research laboratory that studies plants that poison livestock; however, many of these plants are also toxic to humans. Additionally, many plant toxins have been adopted as tools for biomedical investigations from plants that were first investigated for the problems inflicted on the livestock industry. It is probable that more toxins from poisonous plants, only now being investigated, will also prove to have biomedical applications. Scientists at the PPRL are willing to help with poisoning incidences in any manner they can including plant identification and toxin analysis.

Source of Funding The PPRL is a federal research laboratory which is funded by congressional mandate.

Conflicts of Interest None.

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