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Chemistry, biological activities and toxic effects of alkaloidal constituents of genus *Delphinium* - A mini review

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ABSTRACT

The genus *Delphinium* is one of the essential members of the family Ranunculaceae. These species grow wild in North America, Europe, and Asia. They have demonstrated antioxidant, antimicrobial, and cytotoxic activities. Diterpenoid alkaloids are their main constituents and seem to be responsible for medicinal and toxic properties. The primary purpose of this paper is to review the therapeutic benefits of *Delphinium* species, chemical composition, and its medicinal uses, in addition to the reported toxic effects of these plants influencing different animals and humans.

Implication for health policy/practice/research/medical education:
This review provides a comprehensive insight into the *Delphinium* sp. phytochemistry, medicinal uses, and toxic effects. It shows the traditional application and distribution of alkaloids on the species of this genus as a reliable source for the preparation of new drugs.

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Introduction

The genus *Delphinium* sp. (larkspur) belongs to the family Ranunculaceae. This genus consists of approximately 356 species commonly spread in the northern hemisphere, North America, Europe, and Asia; however, 113 species are generally grown in China (1,2). The *Delphinium* genus encompasses diterpenoid alkaloids with a variety of medicinal uses (3,4). Ewan (1945) and Warnock (1995, 1997) were the first and second researchers who published the *Delphinium* genus' two synopses. So far, 427 alkaloids have been detected based on Yin et al studies (1,2). The toxicity of larkspurs has been attributed to two types of toxic alkaloids: 1) N-methylsuccinimidoanthranoyllycoctonine (MSAL) type that is toxic and 2) a less toxic type called methylenedioxy lycoctonine (MDL) (5, 6). However, tall larkspur species have high levels of MSAL-type alkaloids, namely methyllycaconitine (7), which have been declared responsible for the toxicity of these species.

Additionally, they are 20 times more toxic than MDL-type alkaloids (6). Several factors are known to influence the alkaloid content of larkspur and its toxicity: plant species, stage of plant growth, plant part, and environment (8). Cook et al identified two distinct chemotypes of *D. ramosum* and concluded botanical classification alone was not suitable for estimating the relative risk of toxicity in populations like *D. ramosum*. These two chemotypes will probably differ in their toxic potential and finally pose different risks of toxicity when grazed by livestock species. Knowledge in this area has important implications in grazing management decisions on *D. ramosum* infested

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rangelands and exhibits that botanical classification alone is not a helpful indicator of relative risk of toxicity (9). We suggest that some other factors, such as factors mentioned earlier in the text, play essential roles in plant poisoning. The *Delphinium* sp. alkaloid components have been found to inhibit the formation of action potential in muscle, decreasing synaptic functionality, and block neuromuscular transmission probably by antagonizing nicotinic receptors, suggesting a possible mechanism for poisonous effects of these chemical toxins (10). Apart from these poisoning activities of *Delphinium* sp., this genus has some medicinal uses such as antimicrobial, anti-inflammatory, antineoplastic, and antifeedant, as well as cholinesterase inhibition effects (2,11-13). Additionally, the medicinal uses of this genus showed diverse pharmacological potentials, including antiarrhythmic, arrhythmogenic, neurotropic, analgesic, anti-inflammatory, muscular relaxant, hypotensive, local anesthetic, psychotropic, and spasmolytic activities. Its alkaloids are divided into two groups according to their diverse effects, varying from poisonous (e.g., aconitine) to therapeutic (e.g., lappaconitine) (14,15). On the other hand, it has been traditionally observed that the aqueous extracts of the roots of this species are beneficial for treating fungal infections, epilepsy, paralysis, cholera, jaundice, and cardiac diseases (16-18). The main goal of this review is to provide an updated overview of the literature on the medicinal uses of different *Delphinium* sp., their alkaloidal components, and the therapeutic benefits in addition to numerous toxic effects of the plant influencing animals and humans.

Materials and Methods

Records identified through databases of PubMed, Scopus, Web of Science, Cochrane Library, and Embase electronic databases up to June 2021, using keywords: *Delphinium*, larkspur, alkaloid, pharmacological, pharmaceutical, and biological were included in the study. This study summarizes the findings of alkaloidal content and pharmacological studies. We also checked review articles for additional studies. The references of articles selected were also reviewed for potentially eligible ones. Reviews, records not relevant, without pharmacological data, not accessible, or not eligible with reasons were excluded from the study. Two independent authors without knowledge of existing scores examined the selected studies based on the above criteria to resolve discrepancies.

Results

Botany

The Ranunculaceae family comprises 59 genera and 2500 shrubs, herbs, or woody climbers, generally known as the crowfoot or buttercup family. This family is found all over the world. In addition, several members of this family are in tropical and subtropical areas except in the Montane region (19).

The *Delphinium* genus is a prolific source of complex biologically active molecules, mostly norditerpenoid and diterpenoid alkaloids. This genus consists of 370 species distributed all over the world in northern temperate areas. Munz (1967 & 1968) recorded 244 species from Asia, with a diversity center in the Eastern Himalayas and South-West China that 150 species live (19).

The *Delphinium* leaves are 5-15 cm in diameter, rounded shape, segregated into 3-5 mainly obviated parts, divided into 2-3 mm wide teeth or oblong lobes. Flowers' characteristics are bisexual, regular, and approximately 2.5 cm long, with a 1.4-1.5 cm spur and upper outer petals of 1.2 cm. The upper inner petals are white, provided the other petals are blue. The perianth is simple or splits into a calyx and a corolla. It has several free stamens. In arranged flowers, the carpels, in most cases, are numerous. The fruit is an etaerio of achenes, follicles, or a berry. It has a squamulose leaf bud at the crown part. Some flowers are scattered, seeds are light blue, small, and endospermic (19). The herb has a blackish-brown rhizome, 1 cm to 2 cm wide and 3 cm to 6.5 cm long at the crown in length, very hard with conical shape and externally covered by a suberised metaderm, with numerous small circular scars that are the residues of lateral roots. The parenchymatic cells of the root part consist of the starch grains that occur in groups. These have no calcium oxalate, fibers, or cork cells (19).

Traditional application

There are minimal data on traditional uses of the genus *Delphinium* in the literature, and a few species of *Delphinium* have traditionally medicinal benefits. However, we present available published papers that show the applications of this genus in traditional medicine (Table 1). *D. denudatum*, known as Jadwar, is an herbal plant generally grown at high altitude habitats in the western Himalayas. Aqueous extract of the roots of this species is beneficial for treating fungal infections, epilepsy, paralysis, cholera, jaundice, and cardiac diseases (16-18).

Additionally, *D. denudatum* root is an invigorating supplementation and therapeutic approach for treating rheumatism, syphilis, toothache, and an antidote against aconite poisoning and snakebite (20,21). The indigenous traditional healers involved in the conventional Unani medical care system prescribe this species to treat epilepsy (22). In this medicinal system, the root extract of *D. denudatum* is a drug with effects on the central nervous system (CNS) as a sedative, analgesic, brain tonic, and therapy for tremors, hysteria, atony, numbness, paralysis, morphine addiction (23). Various extracts of *Delphinium* are used as a vomiting agent, relieving epileptic symptoms and tremors of tetanus and treating rabies in Turkish traditional medicine (24). Roots and flowers of Indian species of *Delphinium* sp. have been

Table 1. Traditional uses of different *Delphinium* species in the traditional system

Delphinium species	Traditional uses	Used part	Regional traditional system
D. albocoeruleum Maxim	Anti-inflammation, reducing fever, and analgesic	Whole herb	Chinese traditional medicine
D. anthriscifolium var. Majus	Clearing heat, detoxification, healing cough, and treating phlegm	Whole herb	Chinese traditional medicine
D. tianshanicum W.T. Wang	Relieving pain and treating rheumatoid arthritis	Whole herb	Chinese traditional medicine
D. trifoliolatum	Treating rheumatism and neuralgia	Root extracts	Chinese traditional medicine
D. caeruleum	Anti-inflammation, diarrhea, and edema	Aerial parts	Tibetan traditional medicine
D. scabriflorum	Treating rheumatism, reducing fever, and wound healing	Roots and the juice of the leaves	Nepal's traditional medicine
D. denudatum	Sedative, analgesic, brain and nervine tonic, tremors, hysteria, atony, numbness, paralysis, morphine addiction, and epilepsy	Root extract	Unani medical care system
Turkish species of *Delphinium*	To treat vomiting, relieving epileptic symptoms and tremors of tetanus, and treating rabies	Various extracts	Turkish traditional medicine
Indian species of *Delphinium*	Insecticides, anti-maggot in wounds, cardiac and respiratory depressants, stimulants, and treating diarrhea and dysentery	Roots and flowers	Indian Traditional medicine
Mongolian species of *Delphinium*	Therapy for infectious fever, diarrhea due to defects in the biliary system, and relieving toothache	Decoctions	Mongolian traditional medicine

applied as insecticides, anti-maggot in wounds, cardiac and respiratory depressants, stimulants, and treatment for patients with diarrhea and dysentery (22,25). Mongolian traditional healers have used the decoctions of some species of the *Delphinium* genus as antipyretics in cases of relieving toothache, infectious fever, and diarrhea therapy due to defects in the biliary system (26). In Chinese traditional medicine, *D. albocoeruleum* Maxim has reduced inflammation, fever, and pain. This species is a perennial plant grown at an altitude of 3600–4700 m in the Northwest areas of China (1). *D. anthriscifolium* var. *majus* is a traditional Chinese remedy with therapeutic effects on clearing heat, detoxification, healing cough, and treating phlegm (27). In Tibetan folk medicine, the aerial portions of *D. caeruleum*, known as Daimusa, have been revealed to have therapeutic potential against inflammation, diarrhea, and edema (28).

Another Chinese folk medicinal herb is *D. tianshanicum* W.T. Wang, distributed in the Xinjiang of China, which has beneficial effects on relieving pain and treating rheumatoid arthritis (29). The other Chinese medicinal plant is *D. trifoliolatum,* whose root extracts treat rheumatism and neuralgia (30). In Nepal's traditional medical system, roots and leaves extracts or juices of *D. scabriflorum* are used to treat rheumatism, fever, and wound (31).

Delphinium denudatum is a 40–80 cm tall herb that is found over a wide area from mountainous northern regions of Pakistan to Kashmir and northwest India. Biochemical and phytochemical studies showed that alkaloids, sterols, fatty acids, sugar, protein, and starch are the main constituents of the root extracts of *D. denudatum*. Moreover, these extracts effectively treat a wide range of ailments such as cardiac weakness, palpitation, rheumatism, epilepsy, facial palsy, insanity, mania, migraine, opium addiction, paralysis, snakebite, scorpion sting, toothache etc. (32). The extracts obtained from whole plants of *D. vestitum* species have also been traditionally prescribed for the treatment of Snake bite, cuts, wounds, fever, diarrhea (33).

Another traditionally used herb is *D. staphisagria*. The seed extracts of this species have been utilized for the treatment of human hair loss. Accordingly, Koparal and Bostancıoğlu conducted an in-vitro study to evaluate the cytotoxic and pro-angiogenic activities of *D. staphisagria* seeds extracts in endothelial cells and human keratinocytes (HaCaT) cells. Interestingly, they could show that these extracts' hair growth promoting effects occurred by inducing angiogenesis (34). The poisonous property of *D. staphisagria* L. caused it to be used as a good choice against body lice by the British army during Waterloo and Great Wars (35). However, to confirm the beneficial properties of these traditionally used plants, their biological effects must be precisely investigated in meticulous, well-controlled clinical trials.

Alkaloidal distribution of Delphinium sp.

As mentioned earlier, the genus *Delphinium* has valuable, healthy properties, generally attributable to its chemical components. The main chemical components of this genus are diterpenoid alkaloids (36). Cook et al measured the levels of alkaloids in vegetative and floral tissues and pollen and nectar in *D. barbeyi* and *D. nuttallianum* species. Interestingly, they found that the alkaloid contents of nectar were considerably low compared to other tissues and proposed that these decreased levels could help plant compatibility via reducing harmful effects on the activity

of pollinators. They also discovered that fruits, flowers, and anthers had significant alkaloids in both species (37). This is inconsistent with optimal defense theory and may suggest that the latter tissues are the most heavily defended because they contain high concentrations of poisonous MSAL and MDL alkaloids, which protect them from herbivorous insects and mammals (38,39). Then, they assessed the alkaloid content of vegetative and reproductive tissues in *D. nuttallianum* at various phases of plant growth to uncover the effect of plant growth on alkaloid levels during a growing season. They observed high levels of alkaloids in reproductive tissues compared to vegetative ones with no remarkable variation in alkaloid contents of the plant during maturity stages across the growing season, suggesting the impact of the plant's life history on alkaloid allocation in its different parts (40). These alkaloid contents are useful to evaluate the chemical taxonomic diversity and classification of different species of larkspurs (41). Xue et al isolated 18 aconitine-type C19- diterpenoid alkaloids from the whole plant of *D. pseudoaemulans*, of which tianshanisine E, sharwuphinine B, potanisine A, lycoctonine, delbruline, isondelpheline, delavaines A–B, and shawurenines A–B, have been described previously, and pseudophnines A–D, pseudorenines A–B, and pseudonidines A–B were found later. They performed an MTT assay to assess the effects of these compounds on cellular viability and consequently found that none of their compounds had cytotoxic potentials (42). Batbayar et al also isolated 14 norditerpenoid alkaloids from the aerial parts of 4 distinct species of *Delphinium*, including *D. dissectum* Huth, D. *excelsum* Reichenb., *D. grandiflorum* L., and *D. triste* Fisch. Eleven out of 14 alkaloids were previously reported, but 3 new ones have also been isolated from *D. dissectum* Huth and *D. excelsum* Reichenb. Known compounds were delavaine A/B, deoxylycoctonine, methyllycaconitine, delcaroline, delectinine, delterine, delcosine, deltatsine, grandiflorine, macrocentridine, and 14-dehydrodelcosine, but new cases were 10-hydroxymethyllycaconitine, 18-O-methyldelterine, and 10-hydroxynudicaulidine (26). In another phytochemical study, six alkaloids from *D. elatum* cv. Pacific Giant were isolated and identified. These C19-norditerpenoid alkaloids included: N-formyl-4,19-secopacinine, iminoisodelpheline, iminodelpheline, iminopaciline, 6-dehydroeladine, and elapacidine (43). Lin et al. provided evidence to declare that the extracts obtained from whole plants of *D. trichophorum* Franch contained unknown hetisane-type C20-diterpenoid alkaloids (trichodelphinines A–E) and one delnudine-type C20-diterpenoid alkaloid (trichodelphinine F). They also analyzed the structures of their compounds by spectroscopic methods and X-ray crystallography and measured their cytotoxicity using MTT assay. Finally, they discovered trichodelphinine F as the first example of a natural diterpene alkaloid having a phenyl acetyl group (44). A study conducted to evaluate the phytoconstituents of *D. denudatum* confirmed three new norditerpenoid alkaloids such as 1β-hydroxy,14β-acetyl condelphine, jadwarine-A, jadwarine-B, and known alkaloids like isotalatizidine hydrate and dihydropentagynine.

Molecular docking studies and enzyme assays showed that jadwarine-A, isotalatizidine hydrate, and dihydropentagynine competitively inhibited acetylcholinesterase (AChE), and butyrylcholinesterase (BChE), while 1β-hydroxy,14β-acetyl condelphine, and jadwarine-B noncompetitively blocked these enzymes. These results suggested that the isolated compounds could act as therapeutic agents against cerebral dementia and Alzheimer diseases (45). Another study conducted to investigate the phytochemicals analysis of the seeds of *D. elatum* cv. Pacific Giant. The results revealed 11 previously found C19-diterpenoid alkaloids, together with 4 new C19-diterpenoid alkaloids, which included elapacigine, N-deethyl-N-formylpaciline, N-deethyl-N-formylpacinine, and N-formyl-4,19-secoyunnadelphinine. Additional data showed no cytotoxic ability of these new and known alkaloids against five different cancer cell lines (46). He, et al investigated the chemical constituents of *D. albocoeruleum* Maxim and isolated two C20-diterpenoid alkaloids, and five C19-diterpenoid alkaloids, from the whole herb of this species (1). The whole plant material of *D. tianshanicum* showed the presence of 6 previously discovered C19-diterpenoid alkaloids in addition to 3 novel C19-diterpenoid alkaloids including tianshanisine, tianshanine, and tianshanidine (29). Zhou, et al isolated three new norditerpenoid alkaloids, trifoliolasines A-C, from the extracts of the whole herb of *D. trifoliolatum*, and determined their structures by interpreting spectral data (47). One year later, they reported that the extracts obtained from aerial parts of *D. trifoliolatum* had three previously unknown C20-diterpenoid alkaloids, including trifoliolasines D-F. Additionally, the structures of all three alkaloids were estimated using spectroscopic and X-ray crystallographic techniques (30). Fang Sun and Michael Benn examined the alkaloid content of *D. zalil*, and found seven norditerpenoid alkaloids in seed extracts of the plant. Six out of 7 isolated alkaloids were anhweidelphinine, browniine, desacctylnudicauline, lycoctonine, methyllycaconitine, and nudicauline, which all had been discovered in previous studies. They found that the seventh was an unknown compound and named it "Zaliline" (48). The phytoconstituents of the whole plant of *D. majus* have been reported by Chen and coworkers. Fifteen known alkaloids and three new C19-diterpenoid alkaloids, majusines A-C, and six new C20-diterpenoid alkaloids, majusimines A-D and majusidine A-B, were isolated from this plant (14). A study by Pradeep Man Shrestha and Alfred Katz indicated that chloroform-based extractions obtained from the roots of *D. cabriflorum* led to the isolation of a

new diterpenoid alkaloid, named 13-(2-methylbutyryl) azitine, together with 11 previously found alkaloids (31). Chemical investigation of the extracts from aerial parts of *D. nordhagenii* has resulted in the isolation of four norditerpenoid alkaloids, including nordhagenine A-C, and lycoctonine (49). There are two different alkaloid isolates from the species *D.anthriscifolium* var. majus, anthriscifolcones A and B, which both have a lycoctonine skeleton from the whole plant of this species (27). Another study on *D. anthriscifolium* was performed by Shan, et al. to evaluate the alkaloid content of the species. They found that the whole plant of *D. anthriscifolium* contained five C18-diterpenoid alkaloids such as anthriscifoltine A, anthriscifoltine B, deoxydelcorine, anthriscifolcine A, and anthriscifolcine G (50). Aerial parts of *D. uralense* have been described to have alkaloids methyllycaconitine and delcorine, as well as a norditerpenoid alkaloid, which was called Uraline (51). Using chromatographic techniques and spectroscopic method, to isolate the phytoconstituents and structures of compounds from *D. grandiflorum* were performed. The results showed that the roots of the plant encompass 5 diterpenoid alkaloids such as methyllycaconitine, lycoctonine, delavaine A, delsemine A, and delajadine (52). Zhan, et al designed a study to determine the chemical composition of *D. caeruleum*. Their data demonstrated the presence of 10 diterpenoid alkaloids in the extracts of the plant, including lycoctonine, caerudelphinine A, talitine A-C, tatsienine-V, d-magnoflorine, vakhmatine, delatisine, and a propionate derivative (28). Kurbanov et al revealed that the aerial parts of *D. leptocarpum* during flowering season possessed a new alkaloid compound with a formula of $C_{34}H_{43}N_3O_3$ that was called leptanine (53). The genus *D. delavayi* Franch has been described to contain several diterpenoid alkaloid compounds; however, deltaline with formula $C_{27}H_{41}NO_8$ is a major C19-diterpenoid alkaloid isolated from the root extracts of *Delphinium* sp. (54). Another typical C19-diterpenoid alkaloid, called bonvalotidine A, was also isolated from *D. bonvalotii* Franch. Its structure was determined using the crystallographic method (54). *D. formosum* roots collected from Trabzon, Turkey, were studied by Utsukarci et al to determine the plant's alkaloid content. After different extraction steps, 7 norditerpenoid alkaloids (methyllycaconitine, avadharidine, anthranoyllycoctonine, delsemine A/B, browniine and lycoctonine) were isolated from root extracts of the plant (55). The name and chemical class of alkaloids isolated from *Delphinium* sp. are mentioned briefly in Table 2.

Chemical classification of *Delphinium* alkaloids

Based on a dictionary of natural products published

Table 2. Name and the chemical class of alkaloids isolated from *Delphinium* sp.

Alkaloid constituennts name	Chemical class	*Dephinium* sp.	Ref.
Tianshanisine E, sharwuphinine B, potanisine A, lycoctonine, delbruline, isondelpheline, delavaines A–B, shawurenines A–B pseudophnines A–D, pseudorenines A–B, and pseudonidines A–B	18 aconitine-type C19-diterpenoid alkaloids	*D. pseudoaemulans*	(42)
Delavaine A/B, deoxylycoctonine, methyllycaconitine, delcaroline, delectinine, delterine, delcosine, deltatsine, grandiflorine, macrocentridine, and 14-dehydrodelcosine, hydroxymethyllycaconitine, 18-O-methyldelterine and 10- hydroxynudicaulidine	14 norditerpenoid alkaloids	*D. dissectum* Huth, D. *excelsum* Reichenb., *D. grandiflorum* L., and *D. triste* Fisch	(26)
N-formyl-4,19-secopacinine, iminoisodelpheline , iminodelpheline, iminopaciline, 6-dehydroeladine, and elapacidine,	C19-norditerpenoid alkaloids	*D. elatum* cv. Pacific Giant	(43)
Trichodelphinines A–E and trichodelphinine F	Hetisane-type C20-diterpenoid and one delnudine-type C20-diterpenoid alkaloids	*D. trichophorum Franch*	(44)
1β-hydroxy,14β-acetyl condelphine, jadwarine-A, jadwarine-B, isotalatizidine hydrate and dihydropentagynine.	Norditerpenoid alkaloids	*D. denudatum*	(45)
Elapacigine, N-deethyl-N-formylpaciline, N-deethyl-N-formylpacinine, and N-formyl- 4,19-secoyunnadelphinine.	C19-diterpenoid alkaloids	*D. elatum* cv. Pacific Giant	(46)
-	C19 and C20-diterpenoid alkaloids	*D. albocoeruleum* Maxim	(1)
Tianshanisine, tianshanine, and tianshanidine	C19-diterpenoid alkaloids	*D. tianshanicum*	(29)
Trifoliolasines A-C	Norditerpenoid alkaloids	*D. trifoliolatum*	(47)
Trifoliolasines D-F	C20-diterpenoid alkaloids	*D.trifoliolatum*	(30)
Anhweidelphinine, browniine, desacctylnudicauline, lycoctonine, methyllycaconitine nudicauline, and Zaliline	Norditerpenoid alkaloid	*D. Zalil*	(48)
Majusines A-C, majusimines A-D and majusidine A and B	C19 and C20-diterpenoid alkaloids	*D. majus*	(14)
13-(2-methylbutyryl)azitine , together with 11 previously found alkaloids	Diterpenoid alkaloid	*D. cabriflorum*	(31)
Nordhagenine A-C, and lycoctonine	Norditerpenoid alkaloids	*D. nordhagenii*	(49)

Table 2. Continued

Alkaloid constituennts name	Chemical class	*Dephinium* sp.	Ref.
Anthriscifolcones A and B	Lycoctonine skeleton	*D. anthriscifolium* var. majus,	(27)
Anthriscifoltine A, anthriscifoltine B, deoxydelcorine, anthriscifolcine A, and anthriscifolcine G	C18-diterpenoid alkaloids	*D. anthriscifolium*	(50)
Methyllycaconitine, delcorine, and Uraline	Norditerpenoid alkaloid	*D. uralense*	(51)
Methyllycaconitine, lycoctonine, delavaine A, delsemine A, and delajadine	Diterpenoid alkaloids	*D. grandiflorum*	(52)
Lycoctonine, caerudelphinine A, talitine A-C, tatsienine-V, d-magnoflorine, vakhmatine, delatisine, and a propionate derivative	Diterpenoid alkaloids	*D. caeruleum*.	(28)
Leptanine	C_{34} alkaloid	*D. leptocarpum*	(53)
Deltaline	C19-diterpenoid alkaloid	*D. delavayi* Franch	(54)
Bonvalotidine A	C19-diterpenoid alkaloid	*D. bonvalotii* Franch	(56)
Methyllycaconitine, avadharidine, anthranoyllycoctonine, delsemine A/B, browniine and lycoctonine	Norditerpenoid alkaloids	*D. formosum*	(55)

in June 2018, the main bioactive compounds found in *Delphinium* species were alkaloids divided into four major structural types, including C18-dinorditerpenoid, C19-norditerpenoid, C20-diterpenoid, and miscelanous alkaloids. There are a few C-18 alkaloid diterpenes in *Delphinium* sp. which lack C-18 compared to the C-19 aconitine ring system and vary mostly in oxidation pattern of C-1, 3, 4, 6, 7, 8, 9, 14, and 16 and alkylation of the nitrogen atom. C-19 alkaloids differ mainly with C-18 alkaloids in an excess methyl group (C-18) on C-4. C-19 alkaloids vary in oxidation pattern at 1, 3, 6, 7, 8, 9, 14, 15, 16, and 18 or double bonds, especially at $\Delta^{8(15)}$. The majority of C-19 diterpene alkaloids are based on the aconitane ring system. A few alkaloids belong to 13,14-seco or 7,17-seco aconitine. 13, 14-Seco types form 13, 14- δ-lactone or tetrahydropyran ring, and 7, 17-secoaconitines usually have a double bond at C-7 (57). A few others have rearranged the framework and differed with aconitane in C8–C17 bridge instead of the C7–C17 bridge. The third major group is the C20 alkaloids, which are around 116 alkaloids in *Delphinium* sp. They are mainly based on atidane structure (Atisines or Atidines) derived from atisane tetracyclic diterpenes with additional 19,20-pentazine ring. Other variations are Denudatine by the formation of C-7 to C-20 bond; Hetidines with C-14 to C-20 bond; Hetisines or Hetisanes with C-14 to C-20, and C-6 to C-20 bonds (17 cases); Vakognavines or N,19 secohetisanes with cleaved N, C-19 bond and with C-14 to C-20, and C-6 to C-20 bonds (15 cases); Staphisagnines with dimeric C-20 diterpene units. Napelline types are different C-20 alkaloids derived from Ent-kaurane tetracyclic diterpene with N to C-20, N to C-19, and C-7 to C-20 bonds, rare and reported in a few numbers in *Delphinium* sp. Minor alkaloids are from aporphine, benzylisoquinoline, or anthranilic derivatives. A rare and undescribed alkaloid is also reported from *D. caeruleum* with 2-Methyl-4-(3,6,7-trimethoxy-2-naphthalenyl)-2-azabicyclo[2.2.1]heptan-3-one structure (71,72). The alkaloids classification of these terpenoids are shown in Figure 1.

Extraction and purification of the alkaloids

Alkaloids are alkaline and are present in salt form with organic acids in the plant. After the extraction of alkaloids, the salt is replaced by inorganic acid salt. Acid extraction methods usually use 0.1% to 3% sulfuric acid or hydrochloric acid to pH 3, followed by washing with chloroform to remove nonalkaloidal nonpolar components like fats, chlorophylls, and terpenoids. It becomes basic to pH 9, and alkaloidal material is extracted with CHCl3 (57). For purification, mostly alkaloid fraction is submitted on silica gel or alumina column using chloroform: methanol with increasing polarity. In a gradient system, fractions rich in alkaloids are submitted on a silica gel column chromatography (CC) with chloroform: methanol or hexane: ethyl acetate. Finally, subfractions are purified by repeated recrystallization, PTLC on alumina plate with hexane: ethyl acetate or Silica gel PTLC or CC using hexane: ethyl acetate: diethylamine in optimized ratio. For example, Gabbasov et al loaded alkaloid fraction of *D. flexuosum* over silica gel CC with benzene: methanol from 0.5% to 2% followed by silica gel CC using tetrachloride carbon: isopropanol from 0.5 to 1.5 l% (57). Chen et al separated crude alkaloids of *D. majus* by CC over a silica gel column using cyclohexane: acetone with increasing polarity. In different ratios, fractions were purified on a silica gel CC using cyclohexane: acetone or CHCl3-CH3OH (57). Reina et al isolated five diterpenoid alkaloids from *D. cardiopetalum* by CC of crude alkaloid over alumina eluting with a gradient system of hexane: ethyl acetate. Preparative alumina TLC purified fractions using hexane: ethyl acetate (4:1) six times (8). He et al isolated three C19-diterpenoid alkaloids from *D. bonvalotii* by repeated CC of alkaloid fraction on silica gel H using cyclohexane: acetone (5:1 to 1:1) followed by PTLC on silica gel plates by petroleum: acetone: ammonium

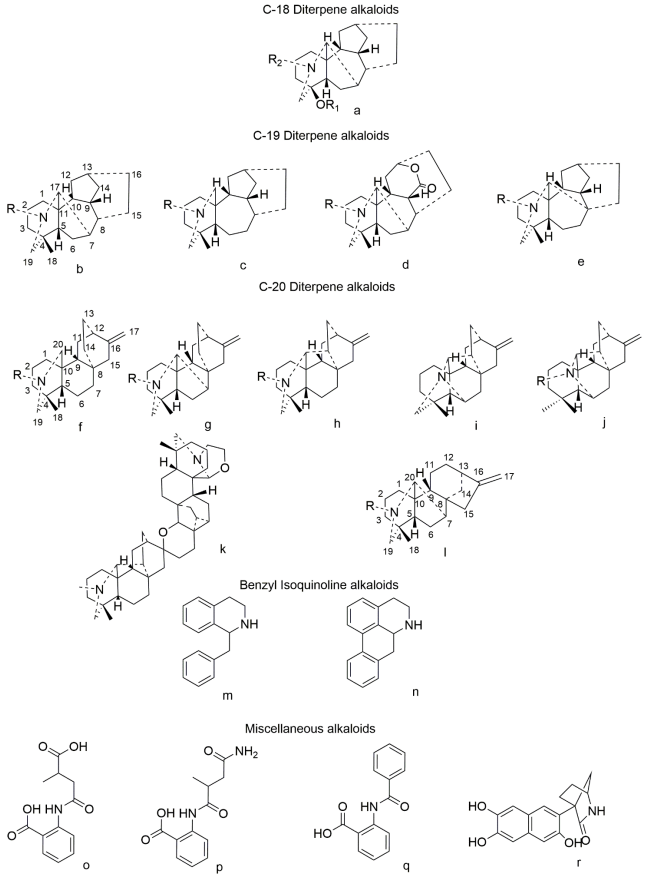

Figure 1. Structure of alkaloids found in *Delphinium* species. a) 18-noraconitines; b) aconitines; c) 13,14-seco aconitines; d) 7,17-seco aconitines; e) rearranged acconitines; f) atidanes; g) denudatines; h) hetidines; i) Hetisines or Hetisanes; j) vakognavines or N,19 secohetisanes; k) Staphisagnines with dimeric C-20 diterpene units; l) napelline; m) benzylisoquinolines; n) aporphines; o, p, q) anthranilic derivatives.

(75:25:2)] (22). Weda et al separated six norditerpenoid alkaloids from *D. elatum* by CC on silica gel eluting with a gradient system of hexane: diethyl ether saturated with 28% ammonia. They purified subfractions by repeated CC with chloroform: methanol (0–10%) saturated with 28% ammonia (43). Yang et al separated alkaloids from *D. ajacis* by silica gel column chromatography with chloroform: methanol (100:0, 100:1, 50:1, 40:1, 30:1, 20:1, 10:1, 0:1). Sub-fractions were submitted over C-18 CC by methanol: water (50:50, 60:40, 70:30, 80:20, 90:10, 100:0) followed by HPLC on an ODA column using acetonitrile: water with different ratios (7).

Biological activity of alkaloids isolated from *Delphinium* sp.

As mentioned before, alkaloid compounds are the most investigated and discovered chemical components of *Delphinium* sp. Diterpenoid alkaloids isolated from the plant of *Delphinium* species are mainly C18, C19, and C20 diterpenoids. Medicinal chemists have received considerable attention for their diverse pharmacological potentials, including antiarrhythmic, arrhythmogenic, neurotropic, analgesic, anti-inflammatory, muscular relaxant, hypotensive, local anesthetic, and psychotropic spasmolytic activities. These researchers have subdivided these alkaloids into two groups according to their diverse effects, varying from poisonous (e.g., aconitine) to therapeutic (e.g., lappaconitine) (14,15). Interestingly, several reports on the antiproliferative properties of the diterpenoid alkaloids against cancer cells have appeared in recent years (58-62). *D. staphisagria* seeds have been observed to have pediculicide, vermin-destroying, and parasiticidal properties (63). Antibacterial activities of the plant have been repeatedly proven in several studies. Methanolic extracts of aerial parts of *D. uncinatum* have been highlighted against gram positive bacterial strains (*Bacillus cereus* and *Staphylococcus aureus*) and gram-negative bacterial strains (*Escherichia coli* and *Klebsiella pneumonia*) with higher efficiency against *B. cereus, E. coli*, and *S. aureus* (64). *D. brunonianum* Royle, a plant indigenous to Afghanistan, has previously been reported as an antibacterial herb that affects *S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa* strains (65). Suresh et al synthesized silver nanoparticles (AgNPs) from water extracts of *D. denudatum* roots. Subsequently, they provided evidence to declare that these green synthesized AgNPs had antibacterial properties against four strains of bacteria such as *S. aureus*, *B. cereus*, *E.*

coli, and *P. aeruginosa,* together with destroying effect on *Aedes aegypti* larvae (66). 8-Acetylheterophyllisine, vilmorrianone, panicutine are three diterpenoid alkaloids that revealed potential antifungal properties against four types of disease-caused by fungi (16). A study by Kolar, et al revealed different phenolics, flavonoids, and alkaloid components in the roots, stems, and leaves of *D. malabaricum*. Furthermore, the authors have highlighted that the root had higher antioxidant activity than the other parts of the plant, which might be attributed to alkaloids (67). Alhalil and colleagues did another antifungal study of the aerial parts of *Delphinium* species. It was reported that isolated diterpenoid alkaloids including Hydrodavisine, Delcarpum, Delphitisine, and Peregrine from *D. Peregrinum eriocarpum* revealed antifungal activity (68). Kolak et al isolated eight norditerpene alkaloids from the root extracts of *D. linearilobum*. Further experiments clarified that all the diterpenoid alkaloids possess radical scavenging capacity (24). There are multiple pieces of evidence suggesting the effects of *Delphinium* isolates on CNS. Four studies performed by Raza et al indicated anticonvulsant activities of the root extracts obtained from *D. denudatum*. In 2001, they carried out an experiment to derive ethanolic and aqueous extracts of dried roots of *D. denudatum*, and reported that ethanolic extract showed weaker dose-dependent anticonvulsant activity on epileptic seizures; however, aqueous extract of the roots significantly displayed anticonvulsant property. Accordingly, they proposed further studies to affirm these anticonvulsant effects and description of underlying mechanism (69). Based on these results, the study implied a remarkable inhibiting effect of this fraction on sustained repetitive firing in hippocampal neurons similar to phenytoin. Moreover, further investigations unraveled the interaction of aqueous fraction with both inactive and resting state of the Na^+ channels compared to phenytoin. This anticonvulsant drug binds to the sodium channel during an inactive state (70). Afterward, they tried to conduct a study to purify aqueous fraction of *D. denudatum* roots, so-called FS-1 subfraction, and assessing the effect of this subfraction on mouse models of induced seizure. According to their obtained data, FS-1 subfraction had compounds that significantly decreased the onset of seizure in mice, suggesting the strong anticonvulsant property of the compounds in FS-1 subfraction extracted from *D. denudatum* roots (71). Two years later, they investigated the effect of FS 1 subfraction of *D. denudatum* on SRF (sustained repetitive firing) in cultured neonatal rat hippocampal pyramidal neurons *in vitro* compared to phenytoin. Interestingly, they could prove that FS-1 subfraction had compounds, which have suppressing effects on SRF of their tested neurons similar to phenytoin and could act as a potent antiepileptic agent (18). Isotalatazidine hydrate isolated from the aerial parts of *D. denudatum* has been unraveled to block AChE competitively and BChE enzymes, implying this isolate is an efficient cholinesterase inhibitor a therapeutic agent for Alzheimer disease (72). The other activity of *D. denudatum* on CNS was observed in Abid et al. They identified that hydroalcoholic isolates extracted from *D. denudatum* root and *Amaranthus spinosus* leaves could have potential antianxiety activities in experimental rats. However, the results showed that *A. spinosus* was more potent than *D. denudatum* (73). Some studies have shown that *D. denudatum* extracts may act against morphine-induced tolerance and dependence symptoms via reducing morphine withdrawal syndrome signs could be an alternative therapy for morphine de-addiction (74-76).

Hair loss is a complication that has been proven to be treated by using seed extracts of *D. staphisagria*; however, the underlying mechanism was not known until Koparal and Bostancıoğlu reported that water and vinegar extract of *D. staphisagria* seeds could promote the hair growth by increasing the proliferation of human keratinocyte cells and inducing the angiogenesis *in-vitro* (77). The anti-inflammatory activity of isolated compounds from the root extract of *D. tatsienense* was examined by Yin et al. The isolated compounds comprising vakognavine-type C20-DA tatsiedine C and lycaconitine-type C19-DAs tatsiedines A and B were observed to display the inhibitory activity on NO production *in vitro* experiments. Although they had not remarkable inhibitory activities (78). The cytotoxic activity of herbaceous plant *D. aemulans* was checked. The isolated diterpene alkaloids showed significant cytotoxic activity in comparison with the positive control group (79). Table 3 lists the medicinal applications of *Delphinium* species.

Toxic activity of alkaloids isolated from Delphinium species

Ample evidence indicates the poisoning and toxic effects of *Delphinium* sp. to different mammalians (8,9,80). A vast body of literature implies that the toxic effects of different species of this genus mainly occur due to their alkaloid components. Pfister et al showed that young *D. glaucescens* leads to death loss in grazing cattle due to the high alkaloid compounds in these plants (80). Gardner and Pfister reported that the toxic alkaloids were isolated from D. nuttallianum, D. andersonii, and *D. geyeri* in North America, including methyllycaconitine, nudicauline, and geyerline compounds (7). They also asserted that toxic alkaloid concentrations ≥3 mg/g could risk death to grazing cattle, and all their investigated plants contained more than 3 mg/g of poisonous alkaloid content (81). Pfister et al suggested that the cattle should be returned to the area when alkaloid concentrations decreased to 3 mg/g during pod shatter (38). Manners et al examined 14 different norditerpenoid alkaloids of *Delphinium* sp. related to cattle intoxication. They reported that the tertiary nitrogen atom, the esterified anthranilic acid, and variation in C-14 functionality of the poisonous alkaloids are the key factors in toxic effects (82). *Delphinium*

Table 3. Medicinal application of different *Delphinium* species

Delphinium species	Biological activity	Used part/ constituents	Affected *in vitro*/*in vivo* model	Ref.
D. Staphisagria	Pediculicide, vermin-destroying, and parasiticidal	Seed extracts	Lice, parasites, and vermins	(63)
D. uncinatum	Antibacterial	Methanolic extracts of aerial parts	Gram-positive bacterial strains (*B. cereus* and *S. aureus*) and gram-negative bacterial strains (*E. coli* and *K. pneumonia*); Higher efficiency against *B. cereus*, *E. coli*, and *S. aureus*	(64)
D. brunonianum Royle	Antibacterial	Herba	*S. aureus*, *E. coli*, *B. subtilis*, and *P. aeruginosa* strains	(65)
D. denudatum	Antibacterial and insecticide	Silver nanoparticles (AgNPs) synthesized from water extracts roots	*S. aureus*, *B. cereus*, *E. coli*, and *P. aeruginosa*. *Aedes aegypti* larvae	(66)
D. staphisagria	Parasiticidal	Flavonoid compounds isolated from the aerial parts	*Trypanosoma cruzi*	(63)
D. denudatum	Antifungal	diterpenoid alkaloids: 8-acetylheterophyllisine, vilmorrianone, panicutine	Disease caused by fungi	(16)
D. Peregrinum eriocarpum	Antifungal activity	diterpenoid alkaloids including Hydrodavisine, Delcarpum, Delphitisine, and Peregrine	*In vitro*	(68)
D. malabaricum	Antioxidant activity	Phenolics, flavonoids, and alkaloid components in the roots, stems, and leaves of the plant	DPPH free radical-scavenging assay and Ferric reducing antioxidant power assay (FRAP assay)	(67)
D. linearilobum	Antioxidant activity	Norditerpene alkaloids from the root extracts	DPPH free radical-scavenging assay	(24)
D. denudatum	Anticonvulsant	ethanolic and aqueous extracts of dried roots	CF-1 mice and neonatal rat hippocampal pyramidal neurons	(71)
D. denudatum	Cholinesterase inhibitor	Isotalatazidine hydrate isolated from the aerial parts	*In vitro* enzyme inhibition assay	(72)
D. denudatum	Antianxiety	Hydroalcoholic isolates of root extract	Rat	(73)
D. denudatum	Morphine-induced tolerance and dependence symptoms and by reducing morphine withdrawal syndrome	Root extract	Mice	(74-76)
D. staphisagria	Promote the hair growth	Seed extract	Human keratinocyte cells	(77)
D. tatsienense	Anti-inflammatory	vakognavine-type C20-DA tatsiedine C and lycaconitine-type C19-DAs tatsiedines A and B	LPS-stimulated RAW 264.7 macrophages	(78)
D. aemulans	Cytotoxic activity	Aerial part extract		(79)

alkaloids inhibit muscular action potential formation, decrease synaptic functionality and block neuromuscular transmission probably by antagonizing nicotinic receptors (10). On the contrary, Raza et al reported that the isolated subfractions from these plants with anticonvulsant activity were safe at the doses lower than 100 mg/kg with no significant change in behavior and neurotoxic activities (83). Some studies have introduced different ways of noninvasive samples collection from poisoned animals and suggested developing immunological techniques such as enzyme-linked immunosorbent assays (ELISA) to diagnose the animals intoxicated by alkaloid components of *Delphinium* sp. (84,85). Here (Figure 2), we summarized *Delphinium* sp. toxic effects in animals and humans.

In cattle, Welch et al conducted a study using Hereford steers administered with different doses of *D. barbeyi*. According to their data, the toxic effects of methyllycaconitine occurred when the serum levels of this alkaloid reached 355 ng/mL. They suggested that the consumption of 1.25 kg-day of *D. barbeyi* by a 500-kg steer did not lead to severe intoxication (86). On the other hand, the poisonous impacts of *D. barbeyi* appear to be also age-dependent. A study revealed that this species' oral consumption of yearling Angus steers had more significant toxic effects than two years of animals (87).

Moreover, the susceptibility of cattle to standardized amounts of *D. barbeyi* seems to be also sex-dependent. Yearling Angus heifers are more susceptible to *Delphinium* sp. alkaloids than steers and bulls (88). The cattle that consume *D. andersonii* during the growing season are

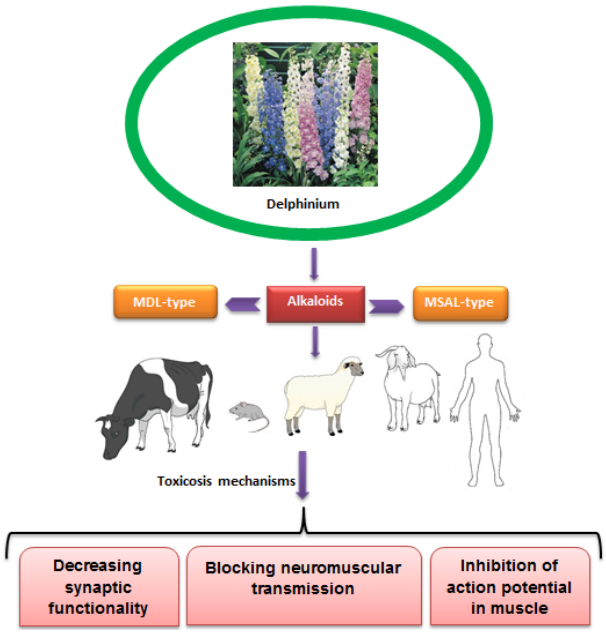

Figure 2. Toxic effects of alkaloid compounds of *Delphinium* sp. on humans and some animals.

generally at risk of death. Yearling heifers consume more than mature cattle and are more susceptible to plant intoxication (89). Toxicokinetics studies of *D. andersonii* with alkaloid constituents include MLA, geyerline, nudicauline, and 16-deacetylgeyerline in Angus steer, showed severe toxicities occur about 18 hours after consumption (90). Therefore, the animals are better to examined for at least 36 hours after the first consumption. The MSAL-type alkaloids in *Delphinium* sp. were more toxic to cattle and mice 24 hours after treatment; thus, this type of alkaloid might be responsible for the lethal effects of the plant (91). However, non-MSAL-containing plants have a shallow risk for poisoning cattle, and consuming more than 70% of non MSAL containing diet was required to poison the cattle (92). Welch et al claimed decreased ratio of MDL- to MSAL-type alkaloids in cattle intoxication; however, less poisonous MDL-type alkaloids could lead to the more severe toxicity of the MSAL-type alkaloids. Therefore, estimating the concentrations of MSAL-type alkaloid compounds and the total alkaloids is required to assess the relative toxicity of tall larkspurs (5,6). Pfister et al also affirmed that MDL-type alkaloids have a prominent role in poisoning properties of *Delphinium* sp. and may potentiate cattle intoxication (93). Green et al examined the alkaloid content of *D. nuttallianum* and *D. andersonii*. This alkaloid profile did not influence the kinetic parameters. However, they suggested examining the types and concentration of these chemical compounds are necessary to determine the risk of plant intoxication in cattle (94). Interestingly, administering an anticholinesterase inhibitor, physostigmine, to grazing cattle could rapidly reverse the toxic effects of larkspurs (95). Results of a previous investigation have established that MSAL-type alkaloids increase heart rate in cattle, but physostigmine and another anticholinesterase inhibitor, neostigmine, could reverse adverse effects of these alkaloids on cardiac function (96).

In 1991, Olsen and Sisson designed a study to establish an appropriate laboratory animal model in mice, hamsters, rats, and sheep. They found that mice were the best model to measure the toxicity of larkspur because these animals showed high susceptibility, immediate response time, and needed small doses for delivering the response (97). MLA is an MSAL-type diterpenoid alkaloid with toxic effects in larkspurs. There is an investigation of the clinical impacts of MLA intoxication and its excretion in mice. The results showed that MLA is immediately distributed in the body and excreted. It could cause CNS-related disorders such as convulsions, abnormal muscle contraction, and dyspnea (98).

Delphinium barbeyi plants have high concentrations of norditerpenoid alkaloids deltaline 14-O-acetyldictyocarpine (14-OAD), which both are MDL-type alkaloids compounds. As previously described in the case of cattle, consumption of MDL-type alkaloids in addition to MSAL-types exerts an additive adverse effect on MSAL-type alkaloid intoxication in mice (99). However, *Delphinium* sp. containing MSAL-type alkaloids has more toxic effects on mice and cattle, suggesting more severe death by consuming these plants (91). Some species of *Delphinium* have various harmful effects on different animals. For example, *D. stachydeum* exerts lower toxic effects on mice than *D. barbeyi and D. occidentale* because this species has only MDL-type alkaloids. However, it severely affects the heart rate and time of exercise in cattle compared with *D. occidentale* (100).

In the case of toxicity reported in sheep and goats, *Toxicoscordion venenosum* is a species of the Melanthiaceae family that cause toxicosis-related symptoms such as cardiovascular failure and muscle weakness in sheep. However, the study results affirmed that co-treatment of *Delphinium* sp. with this species could not exert additive toxic effects in these animals. This suggests that sheep are resistant to acute poisoning with *Delphinium* species (101). There appear to be some reasons for the difference in susceptibility between sheep, goats, and cattle to larkspur. One probable description could originate from variations in the toxicokinetics of the toxic alkaloids. For example, cattle absorb more poisonous alkaloids, and these chemicals are more bioavailable in cattle than the sheep and goats. High concentrations in the neuromuscular junctions cause an increased blocking of the nicotinic acetylcholine receptors (nAChR) and worsening the toxic effects. The other cause for this difference may arise from the lower binding affinity of the poisonous alkaloids for nAChRs in sheep compared to cattle (102). A study by Welch et al. indicated that the oral consumption of *D. barbeyi* by the goat is more effective in eliciting toxicities than the injection of its alkaloid extracts such as deltaline and MLA to the animal. A possible reason for this

effect may form flip-flop kinetics of deltaline and MLA contained in the plant, the kinetic that leads to a slower absorption rate of alkaloids than the rate of elimination (103).

Delphinium sp. toxic effect on humans is rare; Tomassoni et al in 1996 reported the first case of a teenager with ventricular tachycardia and convulsion, who consumed 1 gram of *Delphinium* root (104). The other case was a 13-year-old girl poisoned by drinking about 250 mL of *D. peregrinum* solution to treat migraine. She had clinical symptoms, including confusion, low respiration rate (eight breaths per minute), areflexia, and headache (105).

Conclusion

Diterpenoid alkaloids are the most investigated and the major chemical components of *Delphinium* species and are possibly responsible for both toxic and medicinal effects of this genus. Although phytochemical, biological, and toxicological studies on the alkaloidal extract, fractions, and chemical constituents of *Delphinium* sp. have attracted considerable interest, some points need more explanation. First, the biological activity of most of the isolated alkaloids wasn't evaluated. Second, all of the biological activities of the isolated alkaloids were investigated by using *in vitro* tests and rarely have done *in vivo* animal models or on their pharmacological mechanisms. To date, there is no clinical trial examining the effects of safer doses of the plant or its isolated compounds on humans, and this lack of information does not allow us to fully comprehend the beneficial medicinal impacts of this genus and comparing its various species together. Accordingly, further preclinical and clinical experiments are required to thoroughly understand all aspects of the toxic and medical influences of the plant.

Limitation of the study

It is a short narrative review describing phytochemistry and biological data of *Delphinium* sp., which does not cover all of the data extracted so far about them. On the other hand, there are about 365 *Delphinium* species worldwide, but the natural compounds of only 87 species and 10 varietal have been investigated in the last decades. The natural chemical constituents of many of *Delphinium* species remain unexploited. Most of the medicinal activities of the *Delphinium*-derived constituents were also discovered by applying in vitro tests, and there is not many studies in animal models.

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Authors' contributions

Supervision, conceptualization, and methodology: GM; investigation and original draft preparation: LM; writing-review and editing: LM, MPM, ASA, and KF.

Conflict of interests

The authors declare that they have no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication, etc.) have been completely observed by the authors.

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References

1. He YQ, Ma ZY, Yang Q, Du BZ, Jing ZX, Yao BH, et al. Diterpenoid alkaloids and flavonoids from *Delphinium albocoeruleum* Maxim. Biochem Syst Ecol. 2010;38(4):554-6. doi: 10.1016/j.bse.2010.06.004.
2. Yin T, Cai L, Ding Z. An overview of the chemical constituents from the genus *Delphinium* reported in the last four decades. RSC Adv. 2020;10(23):13669-86. doi: 10.1039/d0ra00813c.
3. Agnihotri P, Jena SN, Husain D, Husain T. Perspective of the genus *Delphinium* Linnaeus (Ranunculaceae) in India. Pleione. 2014;8(2):344-52.
4. Kolar F, Pawar N, Dixit G. Induced chlorophyll mutations in *Delphinium malabaricum* (Huth) Munz. J Appl Hortic. 2011;13(1):18-24.
5. Welch KD, Green BT, Gardner DR, Cook D, Pfister JA, Stegelmeier BL, et al. Influence of 7,8-methylenedioxylycoctonine-type alkaloids on the toxic effects associated with ingestion of tall larkspur (*Delphinium* spp) in cattle. Am J Vet Res. 2010;71(4):487-92. doi: 10.2460/ajvr.71.4.487.
6. Welch KD, Green BT, Gardner DR, Cook D, Pfister JA, Panter KE. The effect of 7, 8-methylenedioxylycoctonine-type diterpenoid alkaloids on the toxicity of tall larkspur (*Delphinium* spp.) in cattle. J Anim Sci. 2012;90(7):2394-401. doi: 10.2527/jas.2011-4560.
7. Mlakar J, Korva M, Tul N, Popović M, Poljšak-Prijatelj M, Mraz J, et al. Zika virus associated with microcephaly. N Engl J Med. 2016;374(10):951-8. doi: 10.1056/NEJMoa1600651.
8. Olsen JD, Manners GD, Pelletier SW. Poisonous properties of larkspur (*Delphinium* spp.). Collect Bot. 1990(19):141-51.
9. Cook D, Gardner DR, Lee ST, Stonecipher CA, Pfister JA, Welch KD, et al. Two *Delphinium ramosum* chemotypes, their biogeographical distribution and potential toxicity. Biochem Syst Ecol. 2017;75:1-9. doi: 10.1016/j.bse.2017.09.002.
10. Dobelis P, Madl JE, Pfister JA, Manners GD, Walrond JP. Effects of *Delphinium alkaloids* on neuromuscular transmission. J Pharmacol Exp Ther. 1999;291(2):538-46.
11. Pfister JA, Green BT, Gardner DR, Welch KD, Cook D, Stonecipher CA. Grazing of *Delphinium occidentale* (duncecap larkspur) by susceptible and resistant cattle. Prof Anim Sci. 2018;34(6):581-93. doi: 10.15232/pas.2018-01775.
12. Shan L, Chen L, Gao F, Zhou X. Diterpenoid alkaloids

from *Delphinium naviculare* var. *lasiocarpum* with their antifeedant activity on *Spodoptera exigua*. Nat Prod Res. 2019;33(22):3254-9. doi: 10.1080/14786419.2018.1475382.
13. Pereira F. Polypharmacology of *Aconitum* and *Delphinium* sp. diterpene alkaloids: antiarrhythmic, analgesic and anti-inflammatory effects. Mini Rev Org Chem. 2017;14(4):304-10. doi: 10.2174/1570193x14666170519143252.
14. Chen FZ, Chen DL, Chen QH, Wang FP. Diterpenoid alkaloids from *Delphinium majus*. J Nat Prod. 2009;72(1):18-23. doi: 10.1021/np800439a.
15. Faridi B, Alaoui K, Alnamer R, Cherrah Y, Zellou A. Analgesic activity of ethanolic and alkaloidic extracts of *Delphinium staphysagria* seed. Int J Univ Pharm Bio Sci. 2013;2(5):102-12.
16. Atta-ur-Rahman, Nasreen A, Akhtar F, Shekhani MS, Clardy J, Parvez M, et al. Antifungal diterpenoid alkaloids from *Delphinium denudatum*. J Nat Prod. 1997;60(5):472-4. doi: 10.1021/np960663n.
17. Choudhary MI. Recent studies on bioactive natural products. Pure Appl Chem. 1999;71(6):1079-81. doi: 10.1351/pac199971061079.
18. Raza M, Shaheen F, Choudhary MI, Rahman AU, Sombati S, Suria A, et al. Anticonvulsant effect of FS-1 subfraction isolated from roots of *Delphinim denudatum* on hippocampal pyramidal neurons. Phytother Res. 2003;17(1):38-43. doi: 10.1002/ptr.1072.
19. Aleem M, Ahmad E, Anis M. Botany, phytochemistry, pharmacology and Unani traditional uses of Jadwar (*Delphinium denudatum* Wall.): a review. J Phytopharmacol. 2020;9(5):378-83. doi: 10.31254/phyto.2020.9516.
20. Kirtikar KR, Basu BD. Indian medicinal plants. Allahabad, India: Apura Krishna Bose Indian Press; 1918. p. 8.
21. Kapoor LD. Handbook of Ayurvedic Medicinal Plants: Herbal Reference Library. Boca Raton, Florida: CRC Press; 1990. p. 159.
22. Said HM, editor. Hamdard Pharmacopoeia of Eastern Medicine. Karachi, Pakistan: Hamdard National Foundation, Times Press; 1970. p. 49.
23. Nizami Q, Jafri M. Unani drug, Jadwar (*Delphinium denudatum* Wall.): a review. Indian J Tradit Knowl. 2006;5(4):463-7.
24. Kolak U, Oztürk M, Ozgökçe F, Ulubelen A. Norditerpene alkaloids from *Delphinium linearilobum* and antioxidant activity. Phytochemistry. 2006;67(19):2170-5. doi: 10.1016/j.phytochem.2006.06.006.
25. Prajapati ND, Kumar U. Argo's Dictionary of Medicinal Plants. Jodhpur, India: Agrobios; 2003. p. 107.
26. Batbayar N, Enkhzaya S, Tunsag J, Batsuren D, Rycroft DS, Sproll S, et al. Norditerpenoid alkaloids from *Delphinium* species. Phytochemistry. 2003;62(4):543-50. doi: 10.1016/s0031-9422(02)00514-9.
27. Wang S, Zhou XL, Gong XM, Fan XY, Lan MS. Norditerpenoid alkaloids from *Delphinium anthriscifolium*. J Asian Nat Prod Res. 2016;18(2):141-6. doi: 10.1080/10286020.2015.1056522.
28. Lin CZ, Liu ZJ, Bairi ZD, Zhu CC. A new diterpenoid alkaloid isolated from *Delphinium caeruleum*. Chin J Nat Med. 2017;15(1):45-8. doi: 10.1016/s1875-5364(17)30007-9.
29. Zhao B, Usmanove S, Aisa HA. Three new C19-diterpenoid alkaloids from *Delphinium tianshanicum* WT Wang. Phytochem Lett. 2014;10:189-92. doi: 10.1016/j.phytol.2014.09.010.
30. Zhou XL, Chen DL, Chen QH, Wang FP. C20-diterpenoid alkaloids from *Delphinium trifoliolatum*. J Nat Prod. 2005;68(7):1076-9. doi: 10.1021/np0401922.
31. Shrestha PM, Katz A. Diterpenoid alkaloids from the roots of *Delphinium scabriflorum*. J Nat Prod. 2004;67(9):1574-6. doi: 10.1021/np0305066.
32. Zafar S, Ahmad MA, Siddiqui TA. Jadwar (*Delphinium denudatum* Wall.) roots: a boon in Unani medicine. Hamdard Med. 2003;6(2):9-14.
33. Joshi B, Tyagi V. Traditional knowledge and utilization of medicinal plants of Himalayan region. Nat Sci. 2011;9(5):1-6.
34. Koparal AT, Bostancıoğlu RB. Promotion of hair growth by traditionally used *Delphinium staphisagria* seeds through inducti on of angiogenesis. Iran J Pharm Res. 2016;15(2):551-60.
35. Ulubelen A, Kolak U. Chemical and biological studies with an *Aconitum* and a *Delphinium* species. In: Şener B, ed. Innovations in Chemical Biology. Dordrecht: Springer; 2009. p. 39-49. doi: 10.1007/978-1-4020-6955-0_4.
36. Tripathee HP, Sharma RP, Timilsina YP, Pathak R, Devkota KP. An assessment of ethnomedicinal use, chemical constituents analysis and bioactivity evaluation on high altitude medicinal plant *Delphinium brunonianum* of Manang district. Nepal J Sci Technol. 2011;12:111-8. doi: 10.3126/njst.v12i0.6488.
37. Cook D, Manson JS, Gardner DR, Welch KD, Irwin RE. Norditerpene alkaloid concentrations in tissues and floral rewards of larkspurs and impacts on pollinators. Biochem Syst Ecol. 2013;48:123-31. doi: 10.1016/j.bse.2012.11.015.
38. Pfister JA, Ralphs MH, Gardner DR, Stegelmeier BL, Manners GD, Panter KE, et al. Management of three toxic *Delphinium* species based on alkaloid concentrations. Biochem Syst Ecol. 2002;30(2):129-38. doi: 10.1016/s0305-1978(01)00124-7.
39. Alba C, Bowers MD, Hufbauer R. Combining optimal defense theory and the evolutionary dilemma model to refine predictions regarding plant invasion. Ecology. 2012;93(8):1912-21. doi: 10.1890/11-1946.1.
40. Cook D, Slominski A, Gardner DR, Pfister JA, Irwin RE. Seasonal variation in the secondary chemistry of foliar and reproductive tissues of *Delphinium nuttallianum*. Biochem Syst Ecol. 2016;65:93-9. doi: 10.1016/j.bse.2016.02.006.
41. Gardner DR, Ralphs MH, Turner DL, Welsh SL. Taxonomic implications of diterpene alkaloids in three toxic tall larkspur species (*Delphinium* spp.). Biochem Syst Ecol. 2002;30(2):77-90. doi: 10.1016/s0305-1978(01)00120-x.
42. Xue WJ, Zhao B, Ruzi Z, Zhao JY, Aisa HA. Norditerpenoid alkaloids from *Delphinium pseudoaemulans* CY Yang et B. Wang. Phytochemistry. 2018;156:234-40. doi: 10.1016/j.phytochem.2018.09.010.
43. Wada K, Chiba R, Kanazawa R, Matsuoka K, Suzuki M, Ikuta M, et al. Six new norditerpenoid alkaloids from Delphinium elatum. Phytochem Lett. 2015;12:79-83. doi: 10.1016/j.phytol.2015.02.010.
44. Lin CZ, Zhao ZX, Xie SM, Mao JH, Zhu CC, Li XH, et al. Diterpenoid alkaloids and flavonoids from *Delphinium trichophorum*. Phytochemistry. 2014;97:88-95. doi: 10.1016/j.phytochem.2013.10.011.
45. Ahmad H, Ahmad S, Ali M, Latif A, Shah SAA, Naz H, et al. Norditerpenoid alkaloids of *Delphinium denudatum* as cholinesterase inhibitors. Bioorg Chem. 2018;78:427-35. doi: 10.1016/j.bioorg.2018.04.008.

46. Yamashita H, Katoh M, Kokubun A, Uchimura A, Mikami S, Takeuchi A, et al. Four new C19-diterpenoid alkaloids from *Delphinium elatum*. Phytochem Lett. 2018;24:6-9. doi: 10.1016/j.phytol.2017.12.013.
47. Zhou XL, Chen QH, Wang FP. New C19-diterpenoid alkaloids from *Delphinium trifoliolatum*. Chem Pharm Bull (Tokyo). 2004;52(4):381-3. doi: 10.1248/cpb.52.381.
48. Sun F, Benn M. Norditerpenoid alkaloids from seeds of *Delphinium zalil*. Phytochemistry. 1992;31(9):3247-50. doi: 10.1016/0031-9422(92)83485-h.
49. Shaheen F, Zeeshan M, Ahmad M, Anjum S, Ali S, Fun HK, et al. Norditerpenoid alkaloids from *Delphinium nordhagenii*. J Nat Prod. 2006;69(5):823-5. doi: 10.1021/np050478m.
50. Shan L, Zhang J, Chen L, Wang J, Huang S, Zhou X. Two new C18-diterpenoid alkaloids from *Delphinium anthriscifolium*. Nat Prod Commun. 2015;10(12):2067-8.
51. Gabbasov TM, Tsyrlina EM, Spirikhin LV, Danilov VT, Iunusov MS. [Uraline, a new norditerpenoid alkaloid from aerial parts of *Delphinium uralense* Nevski]. Bioorg Khim. 2005;31(4):425-9. doi: 10.1007/s11171-005-0053-1. [Russian].
52. Yang XH, Jia ZP, Li MX, Zhang RX, Li C. [Study on chemical constituents of *Delphinium grandiflorum*]. Zhong Yao Cai. 2008;31(4):524-7. [Chinese].
53. Kurbanov UK, Tashkhodzhaev B, Turgunov KK, Mukarramov NI. A new alkaloid from *Delphinium leptocarpum*. Chem Nat Compd. 2019;55(1):197-9. doi: 10.1007/s10600-019-02653-3.
54. Wang XQ, Song Q, Guo XQ, Yan J. Deltaline from *Delphinium delavayi* Franch. Acta Crystallogr Sect E Struct Rep Online. 2011;67(Pt2):o439.doi:10.1107/s1600536811001681.
55. Sen-Utsukarci B, Zapp J, Kiemer AK, Mericli AH. A reinvestigation of norditerpenoid alkaloids from the roots of *Delphinium formosum*. Chem Nat Compd. 2018;54(2):405-6. doi: 10.1007/s10600-018-2363-6.
56. Li SH, Chen FZ. Bonvalotidine A acetone solvate from *Delphinium bonvalotii* Franch. Acta Crystallogr Sect E Struct Rep Online. 2010;66(Pt 12):o3319. doi: 10.1107/s1600536810047562.
57. Kohno S, Kida H, Mizuguchi M, Shimada J. Efficacy and safety of intravenous peramivir for treatment of seasonal influenza virus infection. Antimicrob Agents Chemother. 2010;54(11):4568-74. doi: 10.1128/aac.00474-10.
58. Ren MY, Yu QT, Shi CY, Luo JB. Anticancer activities of C18-, C19-, C20-, and bis-diterpenoid alkaloids derived from genus *Aconitum*. Molecules. 2017;22(2):267. doi: 10.3390/molecules22020267.
59. Habli Z, Toumieh G, Fatfat M, Rahal ON, Gali-Muhtasib H. Emerging cytotoxic alkaloids in the battle against cancer: overview of molecular mechanisms. Molecules. 2017;22(2):250. doi: 10.3390/molecules22020250.
60. Huang L, Feng ZL, Wang YT, Lin LG. Anticancer carbazole alkaloids and coumarins from *Clausena* plants: a review. Chin J Nat Med. 2017;15(12):881-8. doi: 10.1016/s1875-5364(18)30003-7.
61. Sallam AA, Ayoub NM, Foudah AI, Gissendanner CR, Meyer SA, El Sayed KA. Indole diterpene alkaloids as novel inhibitors of the Wnt/β-catenin pathway in breast cancer cells. Eur J Med Chem. 2013;70:594-606. doi: 10.1016/j.ejmech.2013.09.045.
62. He YQ, Ma ZY, Wei XM, Du BZ, Jing ZX, Yao BH, et al. Chemical constituents from *Delphinium chrysotrichum* and their biological activity. Fitoterapia. 2010;81(7):929-31. doi: 10.1016/j.fitote.2010.06.008.
63. Duke JA. Handbook of Medicinal Herbs. 2nd ed. CRC Press; 2002. p. 896.
64. Zahoor M, Nisar ud Din, Khan N. Antibacterial activities of methanolic extracts of aerial parts of *Delphinium uncinatum* Hook's and Thoms. Sci Technol Dev. 2014;33(2):77-9.
65. Jeppesen AS, Soelberg J, Jäger AK. Antibacterial and COX-1 inhibitory effect of medicinal plants from the Pamir Mountains, Afghanistan. Plants (Basel). 2012;1(2):74-81. doi: 10.3390/plants1020074.
66. Suresh G, Gunasekar PH, Kokila D, Prabhu D, Dinesh D, Ravichandran N, et al. Green synthesis of silver nanoparticles using *Delphinium denudatum* root extract exhibits antibacterial and mosquito larvicidal activities. Spectrochim Acta A Mol Biomol Spectrosc. 2014;127:61-6. doi: 10.1016/j.saa.2014.02.030.
67. Kolar FR, Ghatge SR, Kedage VV, Dixit GB. An assessment of phytochemical constituents and antioxidant potential of *Delphinium malabaricum* (Huth) Munz. Turk J Biochem. 2014;39(3):277-84. doi: 10.5505/tjb.2014.47965.
68. Alhilal M, Sulaiman YAM, Alhilal S, Gomha SM, Ouf SA. Antifungal activity of new diterpenoid alkaloids isolated by different chromatographic methods from *Delphinium peregrinum* L. var. *eriocarpum* Boiss. Molecules. 2021;26(5):1375. doi: 10.3390/molecules26051375.
69. Raza M, Shaheen F, Choudhary MI, Sombati S, Rafiq A, Suria A, et al. Anticonvulsant activities of ethanolic extract and aqueous fraction isolated from *Delphinium denudatum*. J Ethnopharmacol. 2001;78(1):73-8. doi: 10.1016/s0378-8741(01)00327-0.
70. Raza M, Shaheen F, Choudhary MI, Sombati S, Rahman AU, DeLorenzo RJ. Inhibition of sustained repetitive firing in cultured hippocampal neurons by an aqueous fraction isolated from *Delphinium denudatum*. J Ethnopharmacol. 2004;90(2-3):367-74. doi: 10.1016/j.jep.2003.10.017.
71. Raza M, Shaheen F, Choudhary MI, Suria A, Rahman AU, Sombati S, et al. Anticonvulsant activities of the FS-1 subfraction isolated from roots of *Delphinium denudatum*. Phytother Res. 2001;15(5):426-30. doi: 10.1002/ptr.792.
72. Ahmad H, Ahmad S, Khan E, Shahzad A, Ali M, Tahir MN, et al. Isolation, crystal structure determination and cholinesterase inhibitory potential of isotalatizidine hydrate from *Delphinium denudatum*. Pharm Biol. 2017;55(1):680-6. doi: 10.1080/13880209.2016.1240207.
73. Abid M, Gosh AK, Khan NA. In vivo psychopharmacological investigation of *Delphinium denudatum* and *Amaranthus spinosus* extracts on Wistar rats. Basic Clin Neurosci. 2017;8(6):503-12. doi: 10.29252/nirp.bcn.8.6.503.
74. Zafar S, Ahmad MA, Siddiqui TA. Protective role of *Delphinium denudatum* (Jadwar) against morphine induced tolerance and dependence in mice. J Ethnopharmacol. 2001;78(1):95-8. doi: 10.1016/s0378-8741(01)00317-8.
75. Zafar S, Ahmad MA, Siddiqui TA. Effect of roots aqueous extract of *Delphinium denudatum* on morphine-induced tolerance in mice. Fitoterapia. 2002;73(7-8):553-6. doi: 10.1016/s0367-326x(02)00223-x.
76. Rahman S, Ali Khan R, Kumar A. Experimental study of the morphine de-addiction properties of *Delphinium denudatum* Wall. BMC Complement Altern Med. 2002;2:6. doi: 10.1186/1472-6882-2-6.
77. Shen YL, Li XQ, Pan RR, Yue W, Zhang LJ, Zhang H. Medicinal plants for the treatment of hair loss and the

suggested mechanisms. Curr Pharm Des. 2018;24:3090-100.
78. Yin T, Yan Y, Li X, Gong G, Wang W. Three new diterpenoid alkaloids from *Delphinium tatsienense*. Phytochem Lett. 2021;41:142-6. doi: 10.1016/j.phytol.2020.11.017.
79. Ablajan N, Zhao B, Zhao JY, Kodirova DR, Sagdullaev SS, Aisa HA. Alkaloids from *Delphinium aemulans*. Chem Nat Compd. 2020;56(5):977-80. doi: 10.1007/s10600-020-03207-8.
80. Pfister JA, Cook D, Gardner DR, Baker SD. Early season grazing by cattle of waxy larkspur (*Delphinium glaucescens*) in central Idaho. Rangelands. 2013;35(4):2-5. doi: 10.2111/rangelands-d-13-00014.1.
81. Gardner DR, Pfister JA. Toxic Alkaloid Concentrations in *Delphinium nuttallianum*, *Delphinium andersonii*, and *Delphinium geyeri* in the Intermountain Region. Rangel Ecol Manag. 2007;60(4):441-6. doi: 10.2111/1551-5028(2007)60[441:tacidn]2.0.co;2.
82. Manners GD, Panter KE, Pelletier SW. Structure-activity relationships of norditerpenoid alkaloids occurring in toxic larkspur (*Delphinium*) species. J Nat Prod. 1995;58(6):863-9. doi: 10.1021/np50120a007.
83. Raza ML, Zeeshan M, Ahmad M, Shaheen F, Simjee U. Toxicity testing behavior assessment of anticonvulsant fraction isolated from *Delphinium* specie. J Pharmacol Toxicol Methods. 2008;58(2):152. doi: 10.1016/j.vascn.2008.05.030.
84. Stonecipher CA, Lee ST, Green BT, Cook D, Welch KD, Pfister JA, et al. Evaluation of noninvasive specimens to diagnose livestock exposure to toxic larkspur (*Delphinium* spp.). Toxicon. 2019;161:33-9. doi: 10.1016/j.toxicon.2019.02.013.
85. Lee ST, Schoch TK, Gardner DR, Stegelmeier BL. Development of enzyme-linked immunosorbent assays for toxic larkspur (*Delphinium* spp.) alkaloids. J Agric Food Chem. 2000;48(10):4520-8. doi: 10.1021/jf000600h.
86. Welch KD, Green BT, Gardner DR, Cook D, Pfister JA. The effect of administering multiple doses of tall larkspur (*Delphinium barbeyi*) to cattle. J Anim Sci. 2015;93(8):4181-8. doi: 10.2527/jas.2015-9101.
87. Green BT, Gardner DR, Cook D, Pfister JA, Welch KD, Keele JW. Age-dependent intoxication by larkspur (*Delphinium*) in Angus steers. Toxicon. 2018;152:57-9. doi: 10.1016/j.toxicon.2018.07.020.
88. Green BT, Keele JW, Gardner DR, Welch KD, Bennett GL, Cook D, et al. Sex-dependent differences for larkspur (*Delphinium barbeyi*) toxicosis in yearling Angus cattle1. J Anim Sci. 2019;97(3):1424-32. doi: 10.1093/jas/skz002.
89. Pfister JA, Cook D, Gardner DR. Cattle grazing toxic *Delphinium andersonii* in south-central Idaho. Rangel Ecol Manag. 2011;64(6):664-8. doi: 10.2111/rem-d-11-00001.1.
90. Green BT, Welch KD, Gardner DR, Stegelmeier BL, Pfister JA, Cook D, et al. Toxicokinetics of norditerpenoid alkaloids from low larkspur (*Delphinium andersonii*) orally administered to cattle. Am J Vet Res. 2012;73(8):1318-24. doi: 10.2460/ajvr.73.8.1318.
91. Cook D, Green BT, Welch KD, Gardner DR, Pfister JA, Panter KE. Comparison of the toxic effects of two duncecap larkspur (*Delphinium occidentale*) chemotypes in mice and cattle. Am J Vet Res. 2011;72(5):706-14. doi: 10.2460/ajvr.72.5.706.
92. Welch KD, Stonecipher CA, Green BT, Gardner DR, Cook D, Pfister JA. Administering multiple doses of a non N-(methylsuccinimido) anthranoyllycoctonine (MSAL)-containing tall larkspur (*Delphinium occidentale*) to cattle. Toxicon. 2017;128:46-9. doi: 10.1016/j.toxicon.2017.01.020.
93. Green BT, Welch KD, Gardner DR, Stegelmeier BL, Pfister JA, Cook D, et al. A toxicokinetic comparison of norditerpenoid alkaloids from *Delphinium barbeyi* and *D. glaucescens* in cattle. J Appl Toxicol. 2011;31(1):20-6. doi: 10.1002/jat.1563.
94. Green BT, Welch KD, Gardner DR, Stegelmeier BL, Lee ST. A toxicokinetic comparison of two species of low larkspur (*Delphinium* spp.) in cattle. Res Vet Sci. 2013;95(2):612-5. doi: 10.1016/j.rvsc.2013.04.018.
95. Pfister JA, Panter KE, Manners GD, Cheney CD. Reversal of tall larkspur (*Delphinium barbeyi*) poisoning in cattle with physostigmine. Vet Hum Toxicol. 1994;36(6):511-4.
96. Green BT, Pfister JA, Cook D, Welch KD, Stegelmeier BL, Lee ST, et al. Effects of larkspur (*Delphinium barbeyi*) on heart rate and electrically evoked electromyographic response of the external anal sphincter in cattle. Am J Vet Res. 2009;70(4):539-46. doi: 10.2460/ajvr.70.4.539.
97. Olsen JD, Sisson DV. Toxicity of extracts of tall larkspur (*Delphinium barbeyi*) in mice, hamsters, rats and sheep. Toxicol Lett. 1991;56(1-2):33-41. doi: 10.1016/0378-4274(91)90087-m.
98. Stegelmeier BL, Hall JO, Gardner DR, Panter KE. The toxicity and kinetics of larkspur alkaloid, methyllycaconitine, in mice. J Anim Sci. 2003;81(5):1237-41. doi: 10.2527/2003.8151237x.
99. Welch KD, Panter KE, Gardner DR, Green BT, Pfister JA, Cook D, et al. The effect of 7,8-methylenedioxylycoctonine-type diterpenoid alkaloids on the toxicity of methyllycaconitine in mice. J Anim Sci. 2008;86(10):2761-70. doi: 10.2527/jas.2008-1025.
100. Cook D, Welch KD, Green BT, Gardner DR, Pfister JA, Constantino JR, et al. The relative toxicity of *Delphinium stachydeum* in mice and cattle. Toxicon. 2015;99:36-43. doi: 10.1016/j.toxicon.2015.03.005.
101. Welch KD, Green BT, Gardner DR, Stonecipher CA, Panter KE, Pfister JA, et al. The effect of low larkspur (*Delphinium* spp.) co-administration on the acute toxicity of death camas (*Zigadenus* spp.) in sheep. Toxicon. 2013;76:50-8. doi: 10.1016/j.toxicon.2013.09.007.
102. Welch KD, Gardner DR, Green BT, Stonecipher CA, Cook D, Pfister JA. Comparison of the serum toxicokinetics of larkspur toxins in cattle, sheep and goats. Toxicon. 2016;119:270-3. doi: 10.1016/j.toxicon.2016.06.020.
103. Welch KD, Gardner DR, Stonecipher CA, Green BT, Pfister JA. Serum toxicokinetics after intravenous and oral dosing of larkspur toxins in goats. Toxicon. 2017;133:91-4. doi: 10.1016/j.toxicon.2017.05.008.
104. Tomassoni AJ, Snook CP, McConville BJ, Siegel EG. Recreational use of *Delphinium*–an ancient poison revisited. J Toxicol Clin Toxicol. 1996;34(5):598.
105. El Jaoudi R, Yahia C, Satté A, Mounach J, Ouhabi H, Elomri N, et al. Unusual human poisoning with *Delphinium peregrinum*. Clin Toxicol (Phila). 2011;49(10):949-50. doi: 10.3109/15563650.2011.630321.