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Study of the Bioecology, and Composition of Venom of Tords (Amphibia: Anura)

By

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Abstract: Toads belong to the group of two anurans, an order of amphibians characterized by the absence of a tail in the adult phase. The toxins produced by toad hairs, usually not harmful to humans, can be dangerous for pet animals, such as cats and dogs. When rapidly absorbed by the mucous membranes, these substances can trigger symptoms ranging from increased saliva to more serious problems, such as seizures. Study of the bioecology, and composition of venom of toard (Amphibia: Anura). Extensive research was carried out in the available literature, focusing on poisonous toads. The investigation opened various databases, including UpToDate, MEDLINE, Scielo, Google Scholar, and PubMed, covering the period from 1991 to the beginning of 2024.

Keywords: Bufotoxin, Glands, Parotoid, Poison, Skin

1. Introduction

All types of toads have toxins in their skin to protect themselves from predators. However, not all species are equally lethal, which means that some toads are more poisonous than others. The toxins of some toads are simply psychoactive, causing hallucinations and other similar symptoms but not death, while the venom of some species can be lethal. In general, most types of toads are not dangerous to humans, but some can be dangerous to other species (Figure 1) (Caratozzolo, 2017).

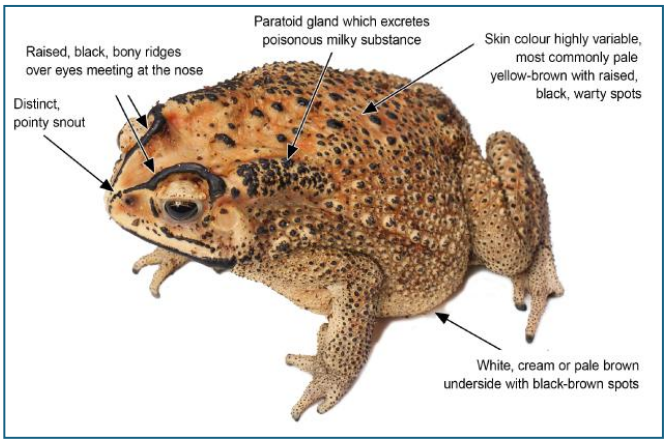

Figure 1: A cane toad can kill your dog
Source: https://blog.thepanamaadventure.com/2017/10/13/a-cane-toad-can-kill-your-dog/

Toads, also called Bufonids (Bufonidae), are amphibians of the anuran order. They inhabit humid, vegetated areas all over the world, except for the Arctic, where the cold climate makes it difficult for them to survive. Among the curiosities of toads, we can mention their lack of teeth, despite being carnivorous animals. How do they feed without teeth? Once they have their prey in their mouths, the toad presses its head down their throat to force the victim through without having to chew it, thus swallowing it alive (Figures 2-3) (Caratozzolo, 2017).

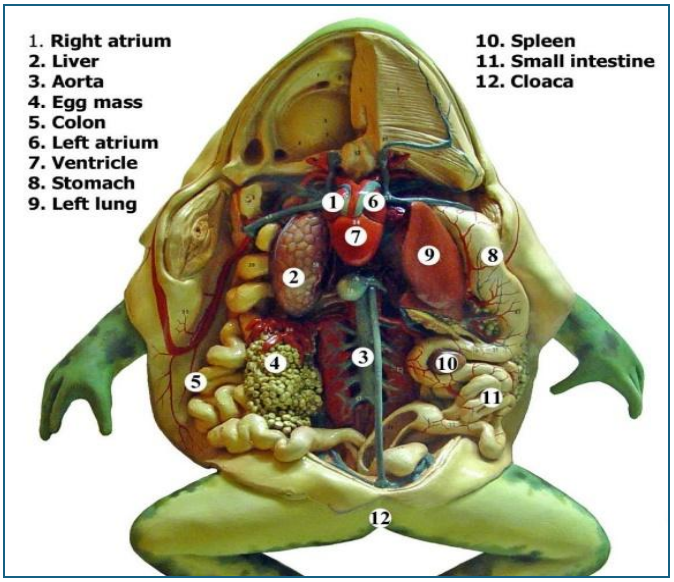

Figure 2: Internal morphology of a toard
Source: Jonathan McIntosh / Wiki; cc by 2.0

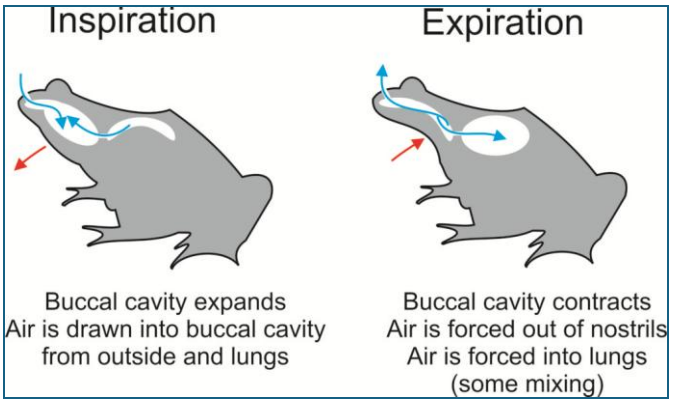

Figure 3: When inhaling, the lungs increase the size of the toard's body to scare away predators (increase body size)
Source: https://blog.thepanamaadventure.com/2017/10/13/a-cane-toad-can-kill-your-dog/

1.1. Characteristics of Toads

A. Contrary to other small animals, toads do not possess a well-organized venom system, but rather specific anatomical structures responsible for the secretion of various poisonous substances or zootoxins.

B. They present mucous glands of various sizes, located mainly in the skin of the head and in the dorsal regions of the body of the toads,

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responsible for the secretion of poisonous substances.

C. There are watery and branched secretions that toads secrete to scare away predators, since, whether larger or smaller, these substances are irritating. The number and distribution of glands vary from one species of toad to another, with the parotid gland being responsible for the secretion of zootoxins in toads considered the most toxic.

D. Poisonous toads tend to cause more cases of poisoning and mortality in domestic animals and people during the summer and spring months after their hibernation period ends, as poisoning generally occurs at night, so it is easier to contact these species because they cannot be seen clearly.

E. The effects of poisoning by toxins from poisonous toads are mainly due to contact of these toxins with the mucous membranes of people and/or infected animals, which suffer skin irritation, vomiting, respiratory failure, muscle paralysis, and cardiovascular failure within a few hours. Medications to try to stop or poison are sometimes ineffective.

F. The toads have some unique characteristics, such as the fact that their natural habitat is always in water, and they are made by having suction cups on their legs that allow them to climb trees. Also, these are poison glands, behind two eyes, which they use to defend themselves in case of a bite.

G. Toads are also notable for their smooth, soft skin and their ability to propel themselves from one side to the other very quickly. Toads reproduce in water and deposit their eggs in humid areas. When these explode, we give birth to the spins. Thus, during the first weeks, the toad's offspring only manage to survive by living underwater. To stimulate reproduction, the males of both species emit sounds to attract females and mark territory. These are produced from areas with water, such as rivers, swamps, or pools.

H. They release poison if they are attacked voluntarily or involuntarily by other types of animals.

I. Bright color: Many species of poisonous toads display bright and vivid colors, such as yellow, orange, or red. This vibrant color is a warning signal for potential predators.

J. Defensive posture: When threatened, poisonous toads generally adopt a defensive posture, erecting their body and exposing their parotid glands.

K. Nighttime habits: Many poisonous toads are most active at night when they are feeding and reproducing (Mueses-Cisneros *et al.*, 2013; Antonelli and Sanmartín, 2011; Albornoz-Espinel *et al.*, 2017; Hutter *et al.*, 2017).

1.2. Objective

Study of bioecology, and composition of venom of toard (Amphibia: Anura).

2.0. Methods

Extensive research was carried out in the available literature, focusing on poisonous toads. The investigation opened various databases, including UpToDate, MEDLINE, Scielo, Google Scholar, and PubMed, covering the period from 1991 to the beginning of 2024. The search strategy used a series of keywords, both individually and in combinations, such as Biotechnology, medications, therapy, toxins, and poisons, The objective was to identify studies relevant to the objectives of this review. It includes peer-reviewed publications in English, Portuguese, and Spanish. Devido à natureza two achados, optou-se pela syntese narrative two results two selected articles.

3.0. Selected Studies

3.1. Skin And Ectothermic Animals

Amphibians are cold-blooded ectothermic animals that are not capable of generating a constant body temperature, which means that it varies depending on the external temperature. One of the major problems of these animals is the poor insulation of their skin, which must be constantly moist, which creates a dependence on water that is accentuated during the reproductive phase. This physiological characteristic determines their worldwide distribution. From the Equator, they inhabit the north and south as long as the temperatures allow. In winter, they hibernate, which also serves as a stimulus for the reproductive phase (Figure 4) (Barrio-Amorós, 2004; Garcia-Porta *et al.*, 2012; Flechas *et al.*, 2015; Frost, 2017).

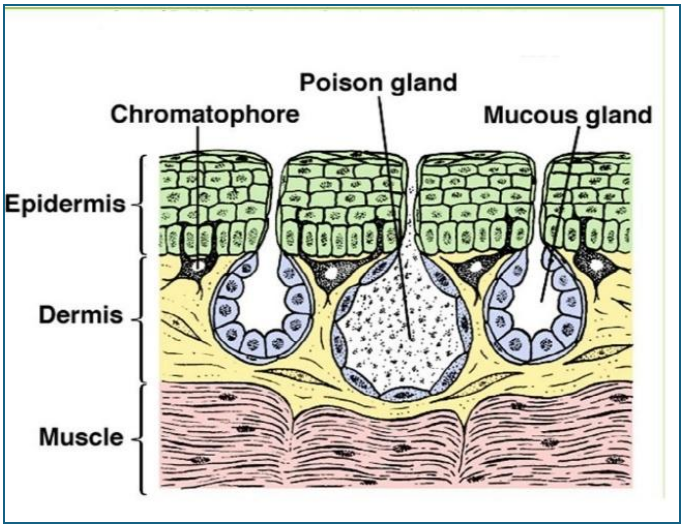

Figure 4: Chromatophore, poison gland, and mucous gland
Source: https://quizlet.com/393024690/anatomy-stude-guide-diagram/

3.2. Distribution

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They are not found in Antarctica or the northern regions of Europe, America, and Asia. Within their areas of distribution, there are suitable places, which are humid environments: rivers, ponds, forests, or jungles. Most species do not depend directly on water, but suitable areas nearby are necessary for reproduction (Flechas *et al.*, 2015; Frost, 2017).

3.3. Reproduction

Normally, males tend to be smaller and mate by climbing on the female and embracing her. Tailless anuran amphibians with short, very wide bodies and hind legs adapted for jumping, such as frogs and toads, are called amplexus. This embrace can be done by the armpits (thoracic amplexus) or by the front of the hind legs (abdominal amplexus). Fertilization is external, the eggs are covered by a protective gelatinous mass and form a string. The eggs can be laid alternately, in pairs, or the form of round masses. The moist skin allows the skin to breathe, even in the juvenile stages, and also helps the gills. Metamorphosis transforms them into adults (Figure 5) (Reading *et al.*,1991; Simpson, 2007; Recuero, 2012; Acevedo *et al.*, 2016; Albornoz-Espinel *et al.*, 2017).

Figure 5: Three stages in a frog's life: Egg, tadpole, and adult
Source: Life cycle Orin Zebes /Flickr /orig. artist unk; cc by 2.0

Amphibians are always associated with wetlands, although there are some surprising exceptions. In Spain, for example, there is a very curious species of toad: the shovel toad. This animal digs holes in arid areas, thanks to a type of spur developed on its hind legs, and lives buried waiting for favorable conditions at night, although it depends on vernal pools for reproduction. Midwife toads, of which there are two species in Spain, are an exception in terms of the egg maturation process. They are carried by the male between his legs until they hatch, which can take 30 to 60 days when they are deposited in a humid area where the larval forms, or tadpoles, develop (Lozoya, 1994; Zweifel, 1998; Bruna, 2000; Osunoğlua and Taskavak, 2001; Brown *et al.*, 2002; Moyano *et al.* 2009).

3.4. Venom/Constitution and Therapeutics

A. Painkillers: Compounds in toad venom are being studied as potential painkillers for treating chronic pain.

B. Antibacterials: Some compounds in toad venom have demonstrated antibacterial activity, which could lead to the development of new antibiotics. This is especially encouraging considering that antibiotic resistance is a growing concern in the medical community.

C. Anticancer agents: Peptides found in toad venom have demonstrated antitumor properties in in vitro studies and in animal models. Although more research is still needed, these findings open the door to the possibility of using components of toad venom in the fight against cancer.

D. Neurological treatments: Some compounds in toad venom, especially that of the Colorado toad *Bufo alvarius* Girard in Baird, 1859, contain alkaloids that affect neurotransmitter systems, which could have applications in the treatment of neurological diseases such as depression, anxiety, and perhaps even more complex disorders such as Alzheimer's and Parkinson's.

E. From treating chronic pain to fighting cancer and neurological diseases: These amphibian toxins are proving to have unexpected and valuable applications. In addition to their medical applications, understanding the toxicity of tords is crucial to human safety and biodiversity conservation. So, far from being creatures to be exterminated, poisonous frogs deserve our attention both for our health and their preservation (Birstein and Mazin, 1982; Zweifel, 1998; Dubois and Bour, 2010; Frost, 2011, Garcia-Porta *et al.*, 2012).

These compounds are absorbed through the skin or mucous membranes and are non-toxic if ingested, as they are neutralized by the acidity of gastric juices. Poisoning ranges from the mildest form, involving excessive salivation, to the most severe, which can lead to death in very rare cases. Ancient Chinese physicians used and still use the crushed and dried skin of the spotted toad *Bufo agrarians* Cantor, 1842 (Amphibia: Anura: *Bufo*nidae), to **(a) treat toothaches and sinusitis.** New medicines now use it to **(b) treat dropsy, carbuncles, and Alzheimer's.** They are also useful for certain heart conditions, as the toad's venom has a digitalis effect that **(c) stimulates the heart and is also the venom used to treat herpes** (Garcia-Porta *et al.*, 2012; Flechas *et al.*, 2015; Frost, 2017).

Toad venom is not only popular for its ability to act as an effective defense mechanism in the animal kingdom but also for its potential therapeutic applications in human medicine. It may surprise you, but **(a) poisonous toads have contributed or are contributing to different areas of pharmacology).** Toads feed mainly on insects, such as crickets, grasshoppers, mosquitoes, flies, larvae, ants, termites, and small rodents, ensuring

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the **(b) balance of the species and the ecosystem** (Acevedo *et al.*, 2016; Albornoz-Espinel *et al.*, 2017).

Controlling some of these insects also prevents diseases transmitted by them, such as dengue fever (c). The toad is not capable of producing its venom. Instead, it extracts toxic substances called gephyrotoxin and solenopsin alkaloids from the insects it feeds on, such as ants and termites. The diet of these animals consists of fungi that contain alkaloids, and when the toad ingests them, it ends up absorbing these toxins and depositing them in special tissues in its skin (Figure 6) (Dubois and Bour, 2010; Frost, 2011, Garcia-Porta *et al.*, 2012; Hutter *et al.*, 2017).

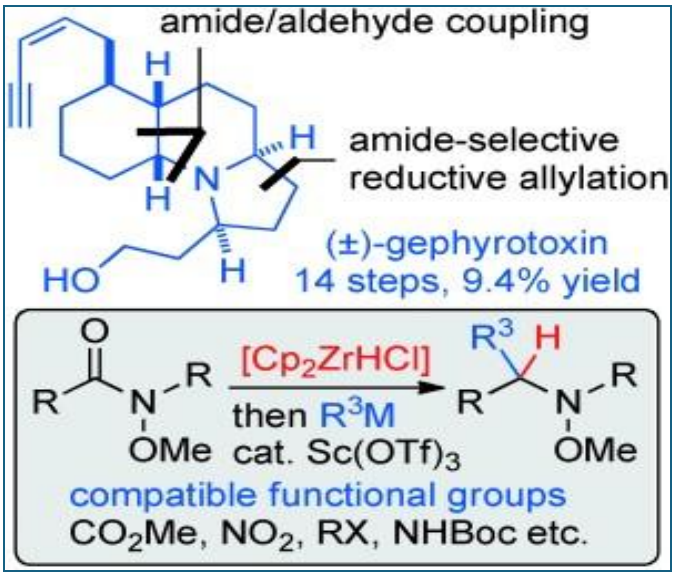

Figure 6: Total synthesis of gephyrotoxin by amide-selective reductive nucleophilic addition
Source: https://doi.org/10.1002/anie.201308905

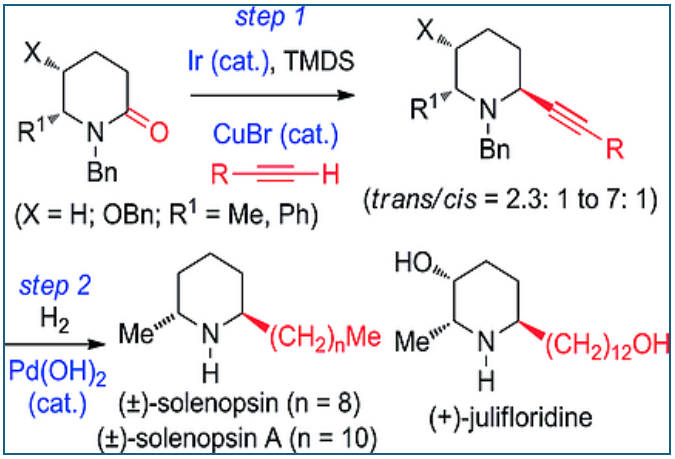

Figure 6: Two-step catalytic transformation of n-benzyllactams to alkaloids (±)-solenopsin, (±)-solenopsin a, and (+)-julifloridine
Source: https://doi.org/10.1002/ejoc.201901752

What is known is that some compounds are psychoactive and, when they come into contact with the mucous membrane of the mouth, eyes, or nose, can cause hallucinations and convulsions in predators, as well as respiratory and cardiac problems. There are about 300 of these substances described depending on the species, although the most common is the one produced by typical toads, Bufotanine (Figure 7) (Dubois and Bour, 2010; Frost, 2011, Garcia-Porta *et al.*, 2012; Hutter *et al.*, 2013).

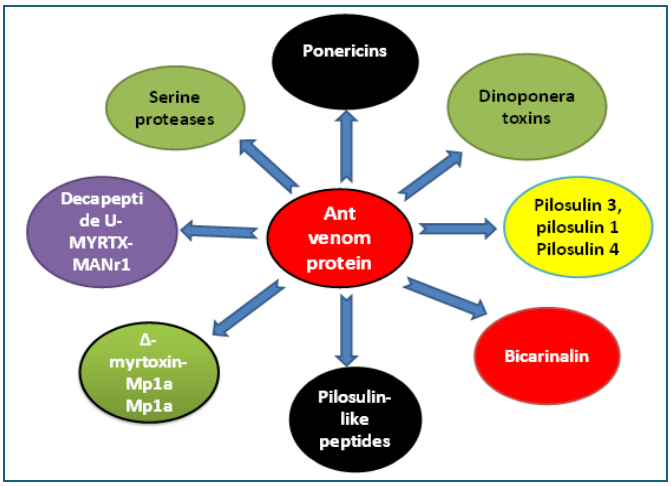

Figure 7: The venom of imported fire ants is produced in the poison glands. It contains two major components: alkaloids and proteins. The oily aliphatic alkaloids, the piperidine alkaloids, and solenopsin are toxic to cells. piperidine is a relative of piperine, the main chemical ingredient in black pepper
Source: Fire ant project fact sheet, medical problems, and treatment considerations for red imported fire ant stings

The Bufonidae Family is made up of 70 species and is known as two true toads or popularly the cururu toad. This family presents as its main morphological characteristics, the presence of a large parotoid gland on the side of the head and tubercles on the skin on the dorsal part of the body *Rhinella marine* (Linnaeus, 1758) (Amphibia: Anura: Bufonidae) is a typical representative of the Bufonidae family, and like most of the *Rhinella* Fitzinger, 1826, species, it is terrestrial and has a foraging agent. A diversified diet and wide geographical distribution can be found in the southern center of Brazil, in Argentina, Bolivia, Paraguay, and Uruguay (Figure 8) (Acevedo *et al.*, 2016; Albornoz-Espinel *et al.*, 2017; Frost, 2017; Hutter *et al.*, 2017).

Figure 8: *Rhinella marine* (Linnaeus, 1758) (Amphibia: Anura: *Bufo*nidae)
Source: https://www.fishipedia.com/amphibians/rhinella-marina

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3.5. Some Species:

Bufo spinosus Daudin, 1803, and B. *bufo* are both known for their ability to inhabit different types of environments, from forests to agricultural areas, and their presence indicates the good health of the ecosystem. In all of these toads, the paratoid glands play a key role in their protection (Figure 9) (Garcia-Porta *et al.*, 2012; Meza-Joya and Torres, 2016).

Figure 9*: Bufo spinosus* Daudin, 1803

Source: https://commons.wikimedia.org/wiki/File:*Bufo_Bufo*_spinosus_2.png

Epidalea calamita Laurenti, 1768, is prominent in several regions of the Iberian Peninsula, being a particularly agile animal that moves easily in sandy areas. Its ability to survive in drier areas differentiates it from other species that require humid environments. During the breeding season, the natterjack toad stands out for its distinctive call, which is essential for attracting mates (Figure 10) (Flechas *et al.*, 2015; Frost, 2017).

Figure 10: *Epidalea calamita* Laurenti, 1768

Source: Martin Šandera

The cane toad *R. marina*, sometimes known as the "*Bufo*", giant or marine toad, is native to the southern tip of Texas, Mexico, Central America, and tropical South America, but is established in Florida. Cane toads were initially introduced to Florida as a method of biological pest control. The toads were supposed to eat beetles that threatened the sugarcane crop, but the introduced population did not survive (Flechas *et al.*, 2015; Meza-Joya and Torres, 2016).

The mucous membranes are acinar, formed by a single layer of secretory cells, presenting their clear lumen. The secretion of mucous glands is mainly composed of glycosaminoglycans and proteoglycans. On the other hand, the granular glands secrete a variety of substances, generally belonging to four categories of composts: biogenic amines, alkaloids, steroids, and proteins (Figures 11-13) (Clarke, 1997; Terrena *et al.*, 2003; Ribeiro *et al.*, 2005; Sousa *et al.*, 2015).

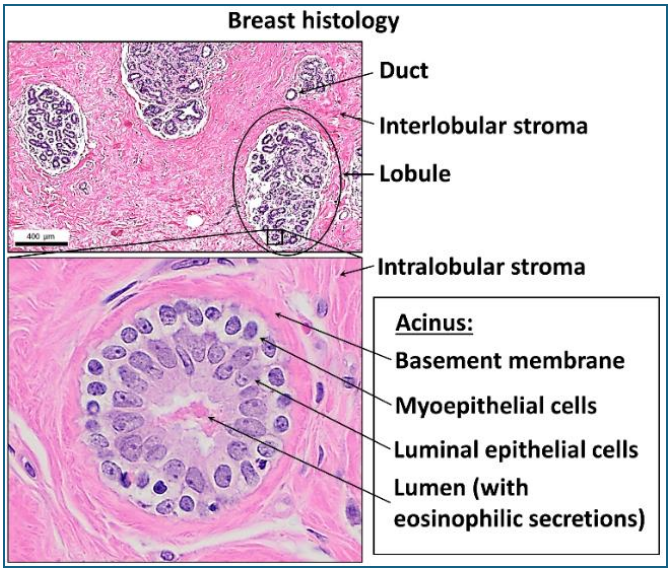

Figure 11: Normal histology of the breast, including an acinus in the lower image. The terminal duct connected to the magnified acinus is not within this microsection

Source: https://en.wikipedia.org/wiki/Acinus

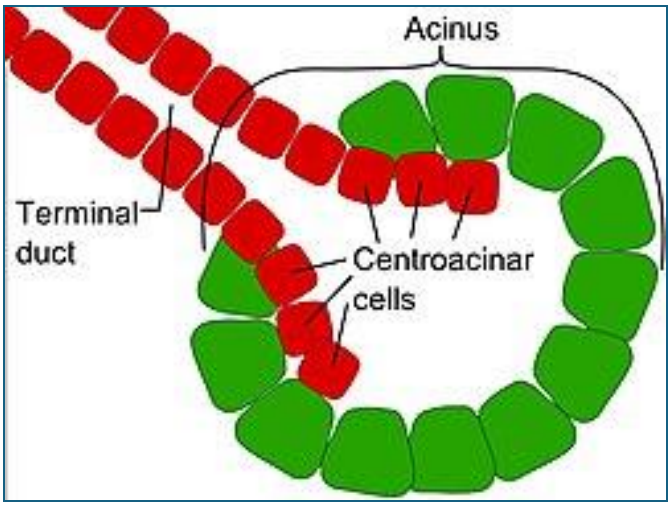

Figure 12: Centroacinar cells

Source: https://en.wikipedia.org/wiki/Acinus

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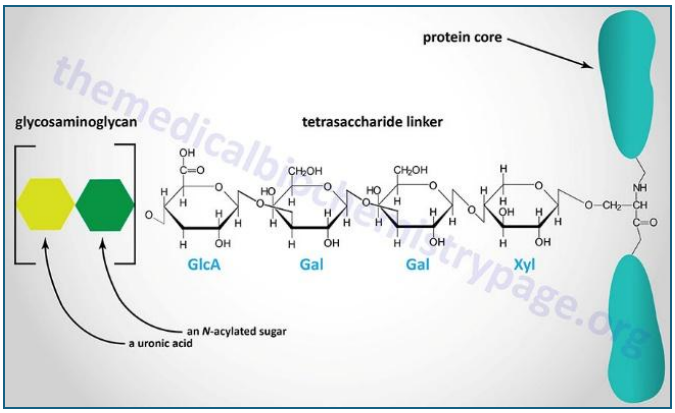

Figure 13: Structure of the GAG linkage to protein in proteoglycans

Source:
https://themedicalbiochemistrypage.org/glycosaminoglycans-and-proteoglycans/

*Bufo*toxins are a family of toxic substances found in the parotoid glands, skin, and venom of many toads of the genus *Bufo* Laurenti, 1768, other amphibians, and other plants and cogumels. The exact composition varies according to the source of the toxin. It may contain 5-MeO-DMT, bufagins, bufotaline, bufotenin, bufothionine, adrenaline, norepinephrine, and serotonin. Thermo bufotoxin can also be used specifically to remove the combination of bufagins and suberilargin (Figures 14-15) (Clarke, 1997; Terrena *et al.*, 2003; Ribeiro *et al.*, 2005; Sousa *et al.*, 2015; Grant *et. al.*, 2024).

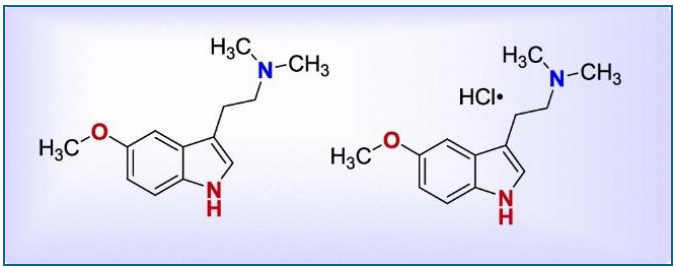

Figure 14: 5-Methoxy-N,N-Dimethyltryptamine (5-MeO-DMT) for use in treating mental disorders

Source:
https://doi.org/10.1021/acschemneuro.4c00513

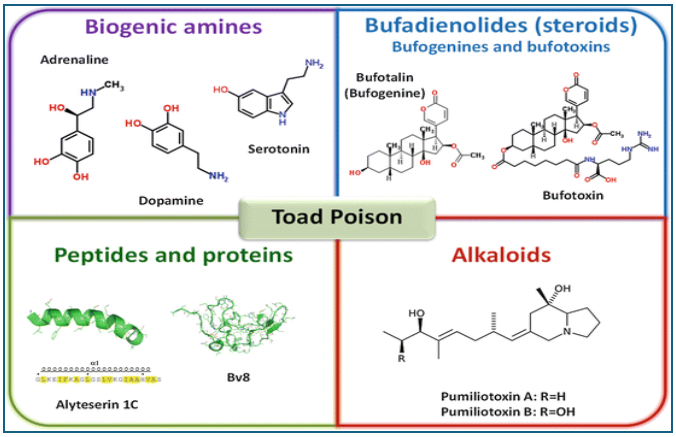

Figure 15: Toad poison and drug discovery
Source: doi.org/10.1007/978-94-007-6452-1_16

4. Conclusion

In the context of rituals and popular beliefs, some toad toxins have been used for their hallucinogenic effects. However, this use carries significant risks to human health and is considered an act of animal abuse. Bufotenine, one of the most recognized toxins, can induce severe effects such as vomiting, diarrhea, and seizures. Therefore, it is important to demystify the use of these substances and promote an approach based on respect and conservation of these amphibians.

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