Scientific Bulletin. Series F. Biotechnologies, Vol. XXVII, No. 2, 2023
ISSN 2285-1364, CD-ROM ISSN 2285-5521, ISSN Online 2285-1372, ISSN-L 2285-1364

MECHANISM OF TOXICITY AND MICROSCOPICAL TESTS FOR *Datura* HERB

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Abstract

Datura sp. and related plants mandrake (Mandragora officinarum and M. autumnalis), black henbane or stinking nightshade (Hyoscyamus niger), belladonna (Atropa belladonna) and angel's trumpets (Brugmansia sp.) are considered some of the deadliest plant species. Datura stramonium (jimson weed, thorn apple or devil's snare) is a weed that sometimes is cultivated as ornamental for its large, white funnel-shaped flowers, but it is considered dangerous due to accidental or deliberate ingestion of any plant parts. Tropane alkaloids (atropine, hyoscyamine, scopolamine) with strong anticholinergic properties can cause a variety of adverse effects that can lead to death in humans and other animals. They are pharmaceutically active and their mind-altering properties have been known since ancient times. Characteristic toxic symptoms can also occur after skin penetration alone and neither drying nor boiling decrease the plant toxicity. Naturally occuring alkaloids (for example from Stramonii folium and Daturae innoxiae herba) or synthetic and semisynthetic substitutes have found use in modern medicine too. The present paper comprises a review on the mechanism of toxicity and photomicrographs of characteristic microscopic features of Datura leaf.

Key words*: anticholinergic effect; Datura sp.; light microscopy analysis; optical microscope; tropane alkaloids.*

INTRODUCTION

Datura sp., *Mandragora* sp. (mandrake), *Hyoscyamus niger* (black henbane or stinking nightshade), *Atropa belladonna* (belladonna or deadly nightshade), *Brugmansia* sp. (angel's trumpets) and other related species of the Solanaceae family are considered some of the deadliest species in the plant world. They are rich in tropane alkaloids with strong anticholinergic properties. The accidental or deliberate ingestion of fresh or dry plant parts can cause a variety of adverse effects that can lead to death in humans and other animals. Characteristic toxic symptoms can also occur after skin penetration alone and neither drying nor boiling decrease the plant toxicity.

The genus *Datura* has a confused taxonomy because of the variations that appear in different environmental conditions (Mahr, 2023). There are only 8 or 9 species, for example *D. stramonium, D. metel, D. inoxia*. According to Preda (1989), *Datura stramonium* is a herbaceous annual plant, 0.30-1.20 m tall, with unpleasant smell, that sometimes is cultivated as ornamental for its large, white funnel-shaped flowers, that appear throughout summer and autumn. The fruit of *Datura inoxia* and *D. stramonium* is round and spiky and contains black seeds (Figure 1). *Datura* plants tend to self-seed, are fast-growing and can become invasive (Mahr, 2023) (Figure 2).

It is believed the genus *Datura* is native to Central America and Mexico and to East India (Preda, 1989), but it has become naturalised in temperate and tropical regions world-wide (Mahr, 2023).

The genus *Datura* used to also include the semi-woody small trees or shrubs (~3-6 m height) such as *Datura arborea* and *Datura suaveolens* (angel's trumpets), which are now classified in the separate genus *Brugmansia* (Lockwood, 1973). *Brugmansia* are popular ornamental plants, highly appreciated for their large (20-30 cm long), pendulous, trumpet-shaped flowers. They are grown as ornamental in their native tropical regions of South and Central America, and also as container plants worldwide (Figure 3).

Figure 1. (a) *Datura* plant growing in Bucharest (summer 2018); (b) *Datura* fruit

Figure 2. Self-sown dense group of *Datura* plants in Bucharest (summer 2021)

Figure 3. (a)-(b) *Brugmansia* sp. growing in Mexico City (Photos used with permission from Ref. Mrs A.T.E., 2018)

Both *Datura* and *Brugmansia* species contain the same major toxic tropane alkaloids - scopolamine, hyoscyamine and atropine (Evans & Lampard, 1972) that are also pharmaceutically active and have mind-altering properties, known since ancient times in some cultures.

The naturally occuring alkaloids obtained from

dried *Datura stramonium* leaves, or from dried leaves and flowering tops of *D. metel* or *D. innoxia* (the latter two are still being cultivated for scopolamine extraction) or synthetic and semisynthetic substitutes have found use in modern medicine too (Gîrd, 2010; Gîrd et al., 2010; Wink, 2015).
The present paper presents a review on the mechanism of toxicity and photomicrographs of a light microscopy analysis of *Datura* sp. leaf.

MICROSCOPIC ANALYSIS

Materials and methods

The present study was carried out at the Laboratory of Biology of the Faculty of Biotechnologies, the University of Agronomic Sciences and Veterinary Medicine of Bucharest, using dry plant material. Wet mounts of hand-sections or surface preparations of plant material were examined whole or after clarification, using a Novex Holland optical microscope. To colour, toluidine blue was sometimes added (Figure 4).
Microscopic images have been photographed with a Sony Cyber-shot® digital camera (Carl Zeiss Vario-Tessar 5× zoom lens) or with an S-Eye 2.0 microscope digital camera, and were later compared to descriptions found in the literature (for example Pharmacognosy, 2023; for the pollen: Bombosi & Heigl, 2020 and Stebler, 2023).

Figure 4. *Datura* sp.: (a) dry plant material and (b) wet mount of clarified leaf used in the present study

Results and discussions

Several microscopic features cited for *Datura* sp. were seen in the leaves:

- epidermis in surface view showing anisocytic stomata (Figures 5-7);
- cluster crystals of calcium oxalate, sometimes prismatic calcium oxalate crystals are also present in cells of the crystal layer (Figures 8-9);
- covering multicellular, uniseriate, warty trichomes with a large base (Figure 10);
- vessels with spiral thickenings (Figure 11);
- pollen grains were also found caught on the leaf (Figures 12-13).

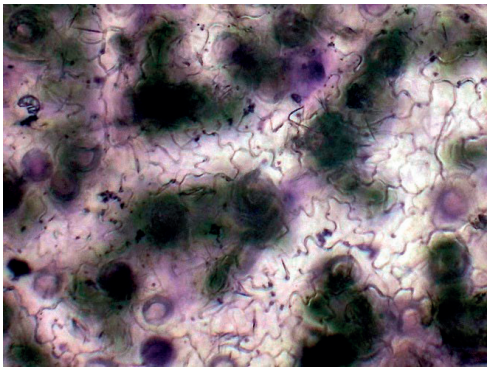

Figure 5. Lower epidermis in surface view, cells have wavy walls

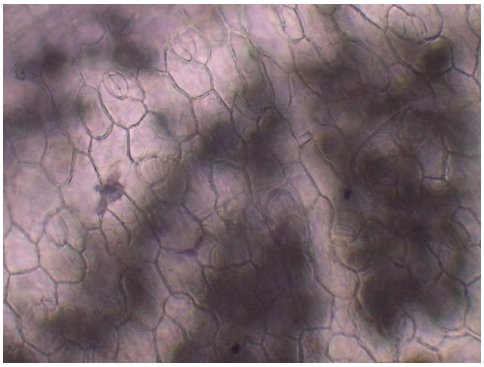

Figure 6. Upper epidermis in surface view

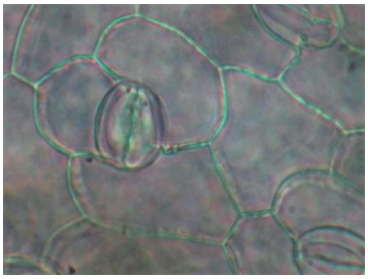

Figure 7. Anisocytic stomata in upper epidermis

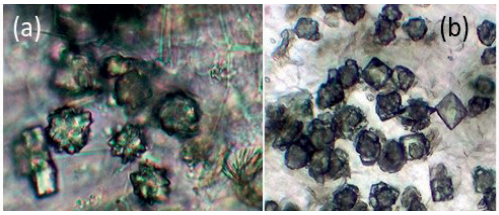

Figure 8. (a) Cluster crystals of calcium oxalate, (b) sometimes also calcium oxalate prisms are present in *Datura stramonium* leaves

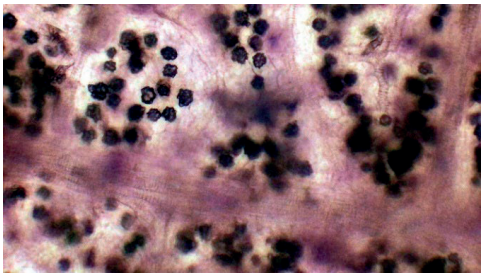

Figure 9. Surface view of the crystal layer in *Datura* leaf

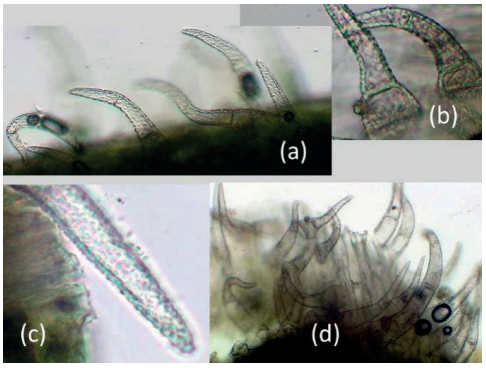

Figure 10. (a)-(d) Covering trichomes in *Datura* leaves

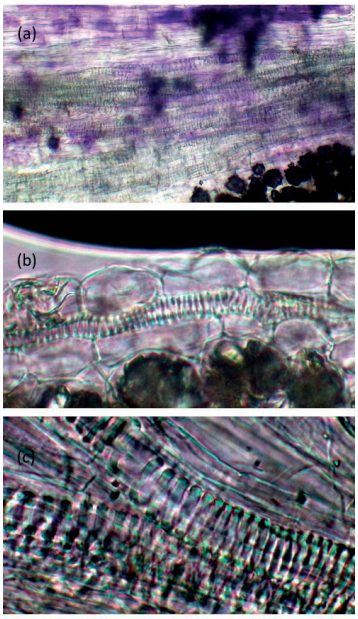

Figure 11. (a)-(c) Vessels with spiral thickenings in *Datura* leaf

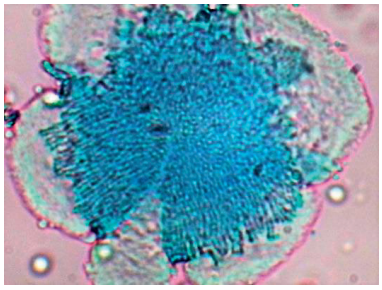

Figure 13. *Datura* sp. pollen grain rupture – striate ornamentation

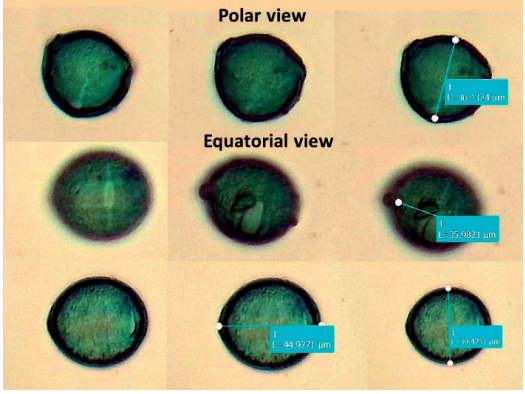

Figure 12. Medium size, tricolporate *Datura* sp. pollen grains

MECHANISM OF TOXICITY

The tropane alkaloids present in *Datura* species have strong anticholinergic properties and are present in all plant parts, but highest concentration is found in seeds and roots (Wink, 2010).

The cholinergic synapses are using acetylcholine (ACh) as a neurotransmitter. Acetylcholine was the first neurotransmitter discovered and it is found in the brain, neuromuscular junctions and the autonomic ganglia (Table 1).

There is a vast literature on the cell biology of the cholinergic synapse (a review can be found in Whittaker, 1990, for example), but relevant information is presented below from Waymire (2023) and Auf Der Heide (2007):

Firstly, acetylcholine is synthesized in the presynaptic (cholinergic) neuron and transported to the end of the axon where it is taken up into secretory vesicles by active transport. Ca^{2+} signaling controls the fusion of the secretory vesicle membrane with the neuron membrane and the acetylcholine secretion in the synaptic space. Then, acetylcholine binds to the cholinergic receptors of the postsynaptic cells, namely the nicotinic (nAChR) and muscarinic receptors (mAChR). ACh binds only briefly to the receptors. After dissociation from the receptor, ACh is rapidly hydrolyzed by the enzyme acetylcholinesterase (AChE). AChE is synthesized in the cell body of cholinergic and non-cholinergic neurons and distributed throughout the neuron. AChE is present in the extracellular matrix.

Drugs that inhibit ACh breakdown are effective in altering cholinergic neurotransmission. This inhibition is produced because ACh molecules accumulate in the synaptic space and keep the receptors occupied. Cholinesterase inhibitors also include certain insecticides and chemical warfare nerve agents.

Datura toxic tropane alkaloids are naturally occurring cholinergic blocking agents that act directly by blocking ACh from binding to muscarinic receptors, because they are competitive antagonists to acetylcholine at muscarinic receptors.

Since these substances bind to the muscarinic cholinergic receptors postsynaptically without activating them, they are muscarinic antagonist or antimuscarinic agents.

The muscarinic receptors are G-protein-coupled receptors that activates a heterotrimeric G-protein when bound to extracellular ACh. Muscarinic receptors do not influence skeletal muscles, but they influence the activity of smooth muscle, exocrine glands and the cardiac conduction system (Table 1).

In the case of plant ingestion the symptoms of toxicity appear after 1-4 hours and include tachycardia, disturbances of vision, dilated pupils due to mydriatic effect, dry skin, dry mouth, inhibition of motility of the gastrointestinal tract, urinary retention, restlessness, delirium, disorientation, hallucinations, convulsions and could lead to coma and death (Dodd-Butera & Broderick, 2005; Serrano, 2018; Wink, 2015).

Table 1. Location of cholinergic synapses in the central and peripheral nervous system (Auf Der Heide, 2007; Waymire, 2023)

CNS	Between neurons in the brain and spinal cord (nAChR, mAChR)		
PNS	Somatic nervous systems	At the neuromuscular junction between the motor nerve and skeletal muscle (nAChR)	
	Autonomic nervous systems	Sympathetic nervous systems	In the preganglionic neurons (nAChR)
			In the postganglionic neurons that inervate the sweat glands (mAChR)
		Parasympathetic nervous systems	In the preganglionic neurons (nAChR)
			In the postganglionic neurons that inervate the heart, exocrine glands and smooth muscle (mAChR)

CONCLUSIONS

Datura species are plants associated with poisoning, but they have applications in the pharmaceutical industry. Recent reports of intoxications from *Datura* use as a recreational drug show the need for a more increased awareness of its harmful and deadly consequences.

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