Thieme

Eye Diseases: When the Solution Comes from Plant Alkaloids

Authors
Riccardo Lorrai[1], Dario Cavaterra[2], Sara Giammaria[3], Diego Sbardella[3], Grazia Raffaella Tundo[4], Alessandra Boccaccini[3]*

Affiliations
1 Dipartimento di Biologia e Biotecnologie "Charles Darwin", Sapienza Università di Roma, Rome, Italy.
2 Dipartimento di Scienze e Tecnologie Chimiche, Università di Tor Vergata, Rome, Italy
3 IRCCS – Fondazione Bietti, Rome, Italy
4 Dipartimento di Scienze Cliniche e Medicina Traslazionale, Università di Roma Tor Vergata, Rome, Italy

Keywords
plant alkaloids, retinal disease, plant metabolites, myopia, diabetic retinopathy

received October 10, 2023
accepted after revision March 6, 2024

Bibliography
Planta Med 2024
DOI 10.1055/a-2283-2350
ISSN 0032-0943

Georg Thieme Verlag KG, Rüdigerstraße 14,
70469 Stuttgart, Germany

Correspondence
Alessandra Boccaccini, PhD
IRCSS Fondazione G B Bietti per lo Studio e la Ricerca in Oftalmologia ONLUS
Via Santo Stefano Rotondo, 6, 00184 Rome, Italy
Phone: + 39 (0)6 85 35 67 27, Fax: + 39(0)6 84 24 23 33
alessandra.boccaccini@fondazionebietti.it

ABSTRACT

Plants are an incredible source of metabolites showing a wide range of biological activities. Among these, there are the alkaloids, which have been exploited for medical purposes since ancient times. Nowadays, many plant-derived alkaloids are the main components of drugs used as therapy for different human diseases. This review deals with providing an overview of the alkaloids used to treat eye diseases, describing the historical outline, the plants from which they are extracted, and the clinical and molecular data supporting their therapeutic activity. Among the different alkaloids that have found application in medicine so far, atropine and pilocarpine are the most characterized ones. Conversely, caffeine and berberine have been proposed for the treatment of different eye disorders, but further studies are still necessary to fully understand their clinical value. Lastly, the alkaloid used for managing hypertension, reserpine, has been recently identified as a potential drug for ameliorating retinal disorders. Other important aspects discussed in this review are different solutions for alkaloid production. Given that the industrial production of many of the plant-derived alkaloids still relies on extraction from plants, and the chemical synthesis can be highly expensive and poorly efficient, alternative methods need to be found. Biotechnologies offer a multitude of possibilities to overcome these issues, spanning from genetic engineering to synthetic biology for microorganisms and bioreactors for plant cell cultures. However, further efforts are needed to completely satisfy the pharmaceutical demand.

Introduction

Plants are incredibly important for life on Earth. They absorb CO_2 to produce oxygen, provide habitat, and regulate water cycles, and they are the most important source of food for humans and animals. Since ancient times, plants have also been exploited to heal human diseases. For instance, Mesopotamian populations left written records of approximately 12 recipes for drug preparation [1]. More detailed usage in medicine of plants for medications comes from the Ebers Papyrus (1550 BC), which cites more than 800 medications involving many ingredients, such as pomegranate, willow, poppy, and mandrake [2]. These recipes represent the earliest documented records of medicines, even though the isolation of the first pharmacologically active substances from one of the putative ingredients listed in the ancient texts awaited approximately 5000 more years [3]. The isolated substance originally named "*principium somniferum*", today known as morphine, marked the beginning of the drug discovery era. Plant-derived drugs are specialized or secondary metabolites that are grouped by their chemical formula alkaloids, flavonoids, terpenoids, and phenolics. Secondary metabolites are small molecules that are produced by metabolism and are dispensable for plant growth. They play a key role in the plant–environment interaction, including plant–plant communication and defense against herbivores, fungi, viruses, and bacteria. But they are also involved in the response to abiotic stresses. It is widely believed that plant second-

ary metabolites are pivotal for environmental adaptation by supporting the growth of symbiotic organisms and providing protection toward biotic and abiotic stressors [4–6].

Among plant secondary metabolites, alkaloids are low-molecular-weight nitrogen-containing organic compounds, typically alkaline, produced by most organisms, including bacteria, fungi, plants, and animals. Alkaloids are a large group of secondary metabolites; more than 20,000 have been identified in 20% of known vascular plants [7]. They are biosynthesized by different precursors, mostly aminoacidic, and can be classified according to their biogenesis or their ring structure [8]. In plants, alkaloids are thought to enhance plant fitness through a broad range of functions such as protection from ultraviolet radiation [9], oxidative stress [10, 11], or pathogens [12], as well as allelopathy toward other plants. The most characterized function is the protection against the microorganisms, i.e., bacteria [13, 14] and viruses [15, 16], but also against insects due to their strong biological activities, that interfere with many cell processes.

By virtue of their biological activity, alkaloids are extensively used in medicine; therefore, this review provides a focus on the alkaloids that are commonly used in the ophthalmology field, such as atropine and pilocarpine, and on those showing potential in treating eye disorders such as berberine, caffeine, and reserpine. Eye disorders leading to vision impairment or blindness affect at least 2.2 billion people worldwide and have a negative impact on the quality of life and an enormous cost to national health systems and productivity. Many of these disorders can be treated and the vision impairment improved, but finding new drugs is never-ending research, and plant-derived compounds are an incredible resource. However, the huge exploitation of plant-based drugs requires high industrial production that cannot be fulfilled only by extraction from plants. On the other hand, chemical synthesis can be challenging and expensive; hence, biotechnology approaches have been developed to meet the pharmaceutical demand, and the last part of this review wants to offer an overview of this topic.

Exploitation of Plant Alkaloids in Ophthalmology

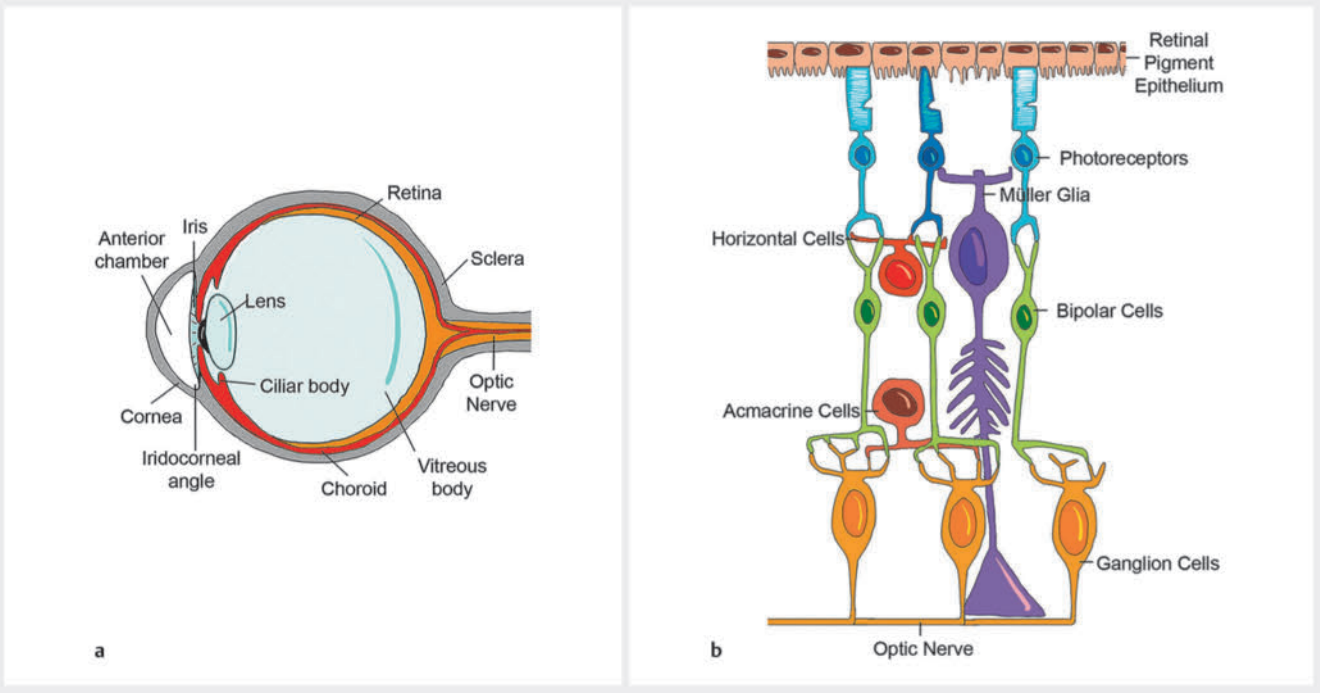
Atropine, a tropane alkaloid

Atropine belongs to the group of tropane alkaloids, characterized by a tropane ring in their structure. Tropane alkaloids are present in many plants belonging to the Solanaceae family, including *Datura Stramonium, Atropa mandragora, Atropa Belladonna*, and many others. All these plants were known to have potent effects on humans for millennia, and in fact, they were used as drugs, poisons, and hallucinogens. Atropine was isolated for the first time from the roots of *Atropa Belladonna* by the pharmacist Heinrich F. G. Mein in 1831 and consists of a racemic mixture of D- and L-hyoscyamine characterized by a tropane ring group [17] (▶ **Table 1**). Even though D- and L-hyoscyamine have distinct biological activities, atropine formulation is pharmacologically accepted because it is stable and reliable [18]. Atropine is known to be a non-specific reversible antagonist of muscarinic receptors, which respond to the neurotransmitter acetylcholine, and they are fundamental for the correct functioning of the central nervous system. In the eyes, muscarinic receptors have been found in all ocular tissues (▶ **Fig. 1**) where they regulate the activity of the conjunctival goblet cells, iris sphincter, and circular muscular fibers of the ciliary body, thus influencing tear production, pupil size, accommodation of the lens, eye development and growth, and retina function [19, 20]. The effects of atropine on the eyes have been known for a long time; Cleopatra and Italian women during the Renaissance used extracts of *Atropa belladonna* as eye drops to dilate pupils and look more attractive [21]. The pupil dilatation effect, or mydriasis, is due to the relaxation of the iris sphincteric muscle upon atropine binding to the muscarinic receptor M3 (▶ **Fig. 2**), antagonizing acetylcholine, combined with the unopposed action of the dilatator muscle. It routinely helps with the ophthalmoscopic evaluation of the posterior part of the eye, including the peripheral retina.

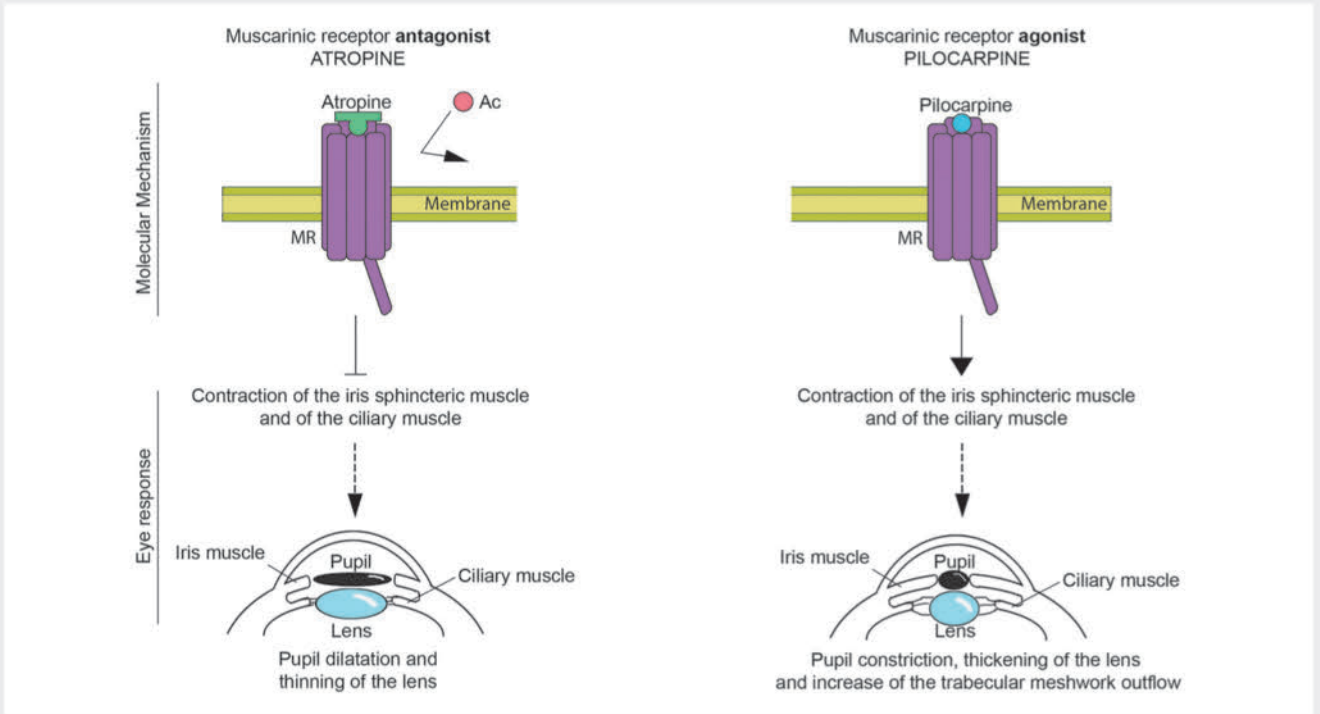
The inhibition of the M3 receptors of the ciliary muscle, which causes its relaxation, induces cycloplegia (paralysis of the accommodation of the lens) (▶ **Fig. 2**), which is useful for the correct evaluation of refractive errors, especially in children, who may frequently show an excess of accommodation. The cycloplegic property of the atropine is also applied for relieving pain caused by keratitis and anterior uveitis by blocking the ciliary spasm. In the case of uveitis, the concomitant mydriatic effect prevents the formation of synechiae between the iris and the cornea (anterior synechiae) or the lens (posterior synechiae), avoiding complications such as an increase in intraocular pressure (IOP).

The cycloplegia induced by atropine also showed beneficial effects in the treatment of myopia. Myopia is characterized by blurred vision of distant objects, which can be due to the increase in the axial length of the eye that causes the light to focus in front of the retina instead of on the retina [22]. Despite lenses and laser surgery being largely used in the management of myopia, atropine seems to be a very effective pharmacological therapy, at least for children [23]. In fact, it is largely used in Asia, while it is less diffused in Europe because of possible side effects such as photophobia and poor visual acuity [24]. However, many pieces of evidence suggest that atropine may additionally prevent myopia via a non-accommodative mechanism (revised in [25]). Although the exact mechanism by which atropine reduces myopic defect has not been clarified yet [26, 27], many studies showed how this alkaloid affects different eye tissues.

Atropine treatment seems to revert the remodeling of the sclera occurring in myopic eyes, consequently improving visual acuity [28–32] (▶ **Fig. 1**). In the myopic eyes, the composition of the extracellular matrix of the sclera is altered, leading to a thinning of the tissue as the axial length of the eye increases and the myopia progresses [33]. In the retina, atropine administration causes the release of dopamine [34], which is a well-known neuromodulator for myopia. However, recent data show that the positive effect of atropine on form-deprivation myopia in chicks is independent of the atropine-mediated release of dopamine, given that the co-administration of atropine with a dopaminergic antagonist does not block the protection provided by atropine [35]. This data should be validated in other model organisms and other forms of myopia, but they point out the importance of further dissecting the role of retinal tissue in the atropine-mediated correc-

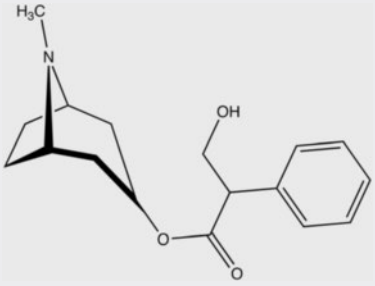
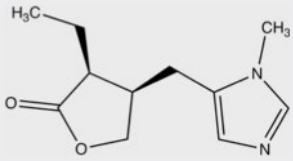
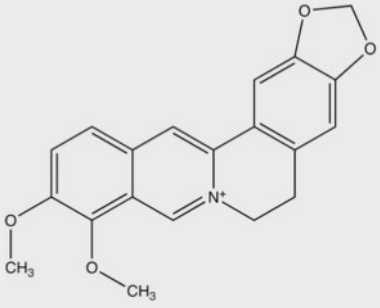
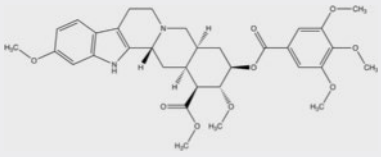
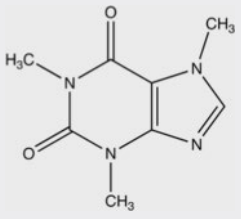


▶ **Fig. 1** Schematic representation of eye tissues (**a**) and cell types in the retina (**b**).



▶ **Fig. 2 Representation of atropine and pilocarpine mechanism of action.** Both atropine and pilocarpine bind muscarinic receptors (MR), which are seven transmembrane proteins responding to the neurotransmitter acetylcholine (Ac) and controlling the contraction of the iris and ciliary muscles. Atropine acts as an antagonist of the receptor; hence, it avoids the binding of Ac, inhibiting the activation of the receptor, and the eye muscles become relaxed. This leads to pupil dilatation and thinning of the lens that focuses on distant objects. In contrast, pilocarpine acts as an agonist enhancing the function of muscarinic receptors leading to eye muscle contraction. Hence, the pupil constricts, and the lens thickens, increasing the refractive power and the focus on close objects. Moreover, pilocarpine facilitates trabecular meshwork outflow, contributing to the reduction in IOP.

▶ **Table 1** Summary of the alkaloids presented in this review, with their structure and biological activity. The list of the plants producing the alkaloids and their applications in the ophthalmology field are also reported.

Drug	Alkaloid Type	Biological Activity	Structure	Plants	Eye Disease
Atropine	Tropane alkaloid	Antagonist of muscarinic receptors		Family of Solanaceae	Myopia
Pilocarpine	Imidazole alkaloid	Agonist of muscarinic receptors		Genus *Pilocarpus*	Acute close-angle glaucoma
Berberine	Isoquinoline alkaloid	Antioxidant and anti-inflammatory		Families of Berberidaceae, Annonaceae, Papaveraceae, Ranunculaceae, Rutaceae	Diabetic retinopathy
Reserpine	Indole alkaloid	Interactor of vesicular monoamine transporters and competitive inhibitor of endogenous monoamines		Genus *Rauwolfia*	Retinal ciliopathies
Caffeine	Methylxanthine alkaloid	Antagonist of adenosine receptors		*C. arabica, C. sinensis, Theobroma plants, P. cupana, I. paraguariensis*	Glaucoma, cataract

tion of myopia. Zhu et al., 2022, carried out a proteomics experiment in guinea pigs to identify new proteins and pathways of retinal cells that could be affected by atropine administration [36]. The analysis revealed that eukaryotic initiation factor 2 (EIF2) signaling and glycolysis are significantly affected by atropine. Some pieces of evidence suggested that these two pathways could be misregulated in myopia, but they have never been associated with atropine treatment; hence, they could be the subjects of future investigations to deeply understand the atropine-mediated effects on the retina.

Lastly, atropine has an effect also on the choroid, which is positioned between the sclera and retina (▶ **Fig. 1 a**). It is highly vascularized, and its main function is to supply oxygen and nourishment to the retina. Choroid is involved in myopia development because its thickness determines the position of the retina and it produces factors for eye growth and sclera remodeling [37]. Atropine administration at low concentrations (between 0.01% and 0.05%) increased choroid thickness in children [38, 39] and adults [40], contributing to the recovery of the myopic defect.

In conclusion, atropine is an important drug in ophthalmology that deserves to be further investigated, and it could be used as a tool to define the molecular mechanisms underlying myopia.

Pilocarpine, an imidazole alkaloid

Pilocarpine is an imidazole alkaloid, synthesized from L-histidine [11]. It is extracted from the leaves of South American plants belonging to the *Pilocarpus* genus (▶ **Table 1**), which are still used for the commercial production of this alkaloid. The medical properties of jaborandi (the common name of *Pilocarpus* plants) extracts had already been known by South Americans for a long time and in the 19th century were introduced into Europe where, in 1875, changes in eye accommodation and pupil contraction were observed for the first time in patients treated with an ophthalmic solution made of jaborandi extracts and glycerin [41]. Pilocarpine interacts with M3 receptors, but unlike atropine, pilocarpine is an agonist, hence causing the activation of these receptors (▶ **Fig. 2**). It is worth pointing out that studies on atropine and pilocarpine helped to formulate the receptor theory of drug action. In fact, John Newport Langley, one of the founders of this theory, showed that the administration of only a certain quantity of atropine could counteract the effect of a determined quantity of pilocarpine on the heartbeat and salivary secretion [42]. These experiments led him to formulate the hypothesis that the activity of these alkaloids depends on the binding to specific molecules in the cells, which were afterward discovered to be the muscarinic receptors, and no less important, it was learned that the effect is dose-dependent. At the ocular level, the opposite activity of these two alkaloids can be explained by several observations, such as pilocarpine-induced myopia in both rabbit and guinea pigs [43, 44], while atropine reduces myopia defect. Moreover, choroidal thickness is reduced by pilocarpine [45] and increased by atropine, and lastly, the pupil constricts upon pilocarpine administration [41], while atropine dilatates it.

In 1877, Adolf Weber introduced the cholinergic agent pilocarpine as a treatment for glaucoma [46]. The term glaucoma includes a variety of forms that differ in cause, risk factors, and symptoms, but they all have in common the reduction in the visual field caused by the degeneration of the retinal ganglion cells (RGCs), the thinning of the retinal nerve fiber layer composed of the axons of the RGCs, and a consequently increased cupping of the optic disc [47]. Actually, the only modifiable risk factor for glaucoma is lowering IOP, which can be obtained by medical treatment, surgery, or laser therapy [48]. Pilocarpine has been used in glaucoma to lower the IOP because of its effects on the trabecular meshwork. The trabecular meshwork, located in the iridocorneal angle, allows the drainage of the aqueous humor into the Schlemm canal, which is connected to the blood system. The dysfunction or the blockage of this system causes the accumulation of aqueous humor in the eye and the elevation of the IOP, which, if not treated, causes damage to the optic nerve typical of glaucoma.

In the type of glaucoma known as open-angle glaucoma, pilocarpine lowers the IOP because it increases outflow through the trabecular meshwork, induced by the contraction of the ciliary muscle and the expansion of the anterior chamber of the eye. In angle closure glaucoma, in which the iridocorneal angle is closed and the trabecular meshwork is obstructed, the IOP can rise dramatically and in a short time. In this case, pilocarpine opens the trabecular meshwork and the Schlemm canal by reducing pupil size and contributing to the decrease in IOP [49–51] (▶ **Fig. 2**). Pilocarpine can be also used before a laser iridotomy is performed or in patients in whom the laser iridotomy is not resolutive. However, to date, pilocarpine is not used in the chronic treatment of glaucoma because it has several ocular and systemic side effects (including induced myopia, cataract development, retinal detachment, bradycardia, vomiting, salivation, and bronchial spasm) [52], and more effective drugs exist. Furthermore, in patients with glaucoma linked to lens abnormalities (e.g., spherophakia, phacomorphic glaucoma, and pseudoexfoliative syndrome), pilocarpine may cause a paradoxical IOP increase because of the mycotic effect that determines an anterior shift of the lens–iris diaphragm and the consequent closure of the iridocorneal angle [53]. Lastly, more recently, many clinical trials have shown the benefit of low-concentrated pilocarpine administration on patients with presbyopia [54–57], which is the progressive inability to focus on close objects associated with the elderly.

In addition to the ophthalmology field, pilocarpine is used for the treatment of several other diseases, pointing out the valuable potential of this alkaloid.

Berberine, an isoquinoline alkaloid

Berberine belongs to the group of isoquinoline alkaloids that are biosynthesized from tyrosine in many plants of the families Berberidaceae, Annonaceae, Papaveraceae, Ranunculaceae, and Rutaceae (for a detailed review check [58]) (▶ **Table 1**). *B. vulgaris* (barberry), belonging to the Berberidaceae family, is the most diffused source of berberine, where it is present in bark and roots. *B. vulgaris* was used in traditional medicine both in Asia and Europe, and traditional Iranian textbooks reported Berberis as a valid remedy for treating gastrointestinal diseases [59]. *Coptis chinensis*, belonging to the Ranunculaceae family, was used more than 2000 years ago in China for the same purpose [60]. In the last few years, berberine has found some applications in ophthalmology because of its anti-diabetic properties. Berberine in combination with a

correct lifestyle helps to lower blood glucose [61], making this alkaloid a good candidate for the treatment and prevention of diabetes. Diabetic retinopathy (DR) is a microvascular complication of diabetes and represents one of the leading causes of irreversible blindness in the world. At clinical levels, DR is characterized by typical neuronal and microvascular disfunction, which consists of microaneurysms, microhemorrhages, and exudates from the damaged retinal vessels (non-proliferative DR). In a more advanced stage (proliferative DR), the development of areas of retinal ischemia leads to the release of vascular endothelial growth factor (VEGF) and consequent formation of neo-vessels. Alterations of the vessels' microarchitecture may cause vitreous hemorrhages and scar tissue development that may cause predisposition to retinal detachment and vision loss [62]. At cellular levels, persistent hyperglycemia causes the activation of inflammatory pathways and oxidative stress that, together with vascularization, leads to degeneration of RGCs [63]. The berberine effects on DR have been extensively studied both at cellular and molecular levels. In the DR rat model, the retinal ganglion cells show high levels of ROS (reactive oxygen species) and malondialdehyde, two markers of oxidative stress, which were reduced after administration of berberine for 8 weeks. The reduction in these two markers was accompanied by an increase in SOD (superoxide dismutase) activity and GSH (glutathione) levels, two enzymes known to act as antioxidants and to contrast oxidative stress [64]. Berberine effects on RGCs were also studied on the GABA (gamma-aminobutyric acid) receptors. In fact, diabetic RGCs showed a reduction in the activity and levels of these receptors. It was shown that feeding diabetic rats with berberine increased the levels of GABA receptors, decreasing RGC apoptosis [65]. In addition to RGCs, Müller cells have a prominent role in the pathogenesis of DR. Müller cells are the main glial cells of the retina, and they provide nutritional and regulatory support to the retinal neurons (▶ **Fig. 1 b**). In the presence of high glucose, Müller cells show increased signaling through the nuclear factor-kappa B (NF-kB) pathway, which is the most important pro-inflammatory pathway (a thorough description of the pathway is provided elsewhere [66]). It has been shown, both in animal and in cell culture, that berberine mitigates the high-glucose-induced death of Müller cells, reducing NF-kB levels and the negative effect of chronic inflammation [64]. Berberine seems to protect Müller cells from high-glucose-induced apoptosis also modulating the AMPK/mTOR pathway [67], which is a network that integrates intracellular and extracellular stimuli to adapt intracellular metabolism. Among the downstream pathways regulated by this network, macroautophagy, often referred to as autophagy (microautophagy and chaperone-mediated autophagy are currently considered more targeted pathways) has a prominent role in cell homeostasis. Macroautophagy is a major proteolytic pathway degrading damaged or unwanted organelles (e.g., mitochondria) by lysosome [68]. Basal activity of autophagy is maintained by almost all cell types for general homeostasis, but it can be triggered in response to intra- and extracellular stresses, which finely tune the Akt/mTOR axis. However, depending on type, duration and amplitude of the stress cells can undergo autophagy or apoptosis, and in some cases, treatments inducing autophagy avoid cell apoptosis and vice versa [69]. In fact, although autophagy activation is commonly viewed as a protective pathway, excessive autophagy activation can be followed by apoptotic death, also called autophagic death. In this framework, rat primary Müller cells showed reduced autophagy after high glucose treatment, which was linked to reduced levels of phosphorylated AMPK and high levels of phosphorylated mTOR. However, 48 hours of berberine treatment restored the rate of phosphorylated AMPK and mTOR, inducing autophagy and inhibiting apoptosis [67]. Nevertheless, the evidence that high glucose levels (typically > 25 mM) may impact the autophagy signaling cascade, but also the ubiquitin-proteasome system (UPS), has been envisaged by studies carried out in rMC1 cells, an immortalized strain of rat Müller cells [70, 71]. In 2021, Wang and co-authors proposed that berberine improves the response of retinal endothelial cells to insulin treatment [72]. Insulin is the main treatment for the control of glycemia in type I diabetes but also in some cases of type II diabetes. Even though the tight control of glycemia is critical for avoiding sight loss, some studies suggested that insulin could cause an early but reversible worsening of DR [73]. The link between insulin and retinopathy is controversial, but it could be explained by molecular experiments showing that insulin activates hypoxia inducible factor 1 (HIF1), which in turn induces the expression of *VEGF* [74, 75]. High levels of *VEGF* cause vascular endothelial cell proliferation, contributing to the neovascularization typical of the proliferative stage of DR. Given the importance of VEGF in DR, many therapies aim to inhibit VEGF by different strategies [76]. In this regard, berberine could be a valid remedy given that it was shown to inhibit the insulin-mediated induction of the HIF1/VEGF-pathway in endothelial cell lines and to improve the endothelial dysfunction in the retina of type I and II diabetic mice [72].

In addition to high glucose, high blood lipid levels are also a risk factor for DR, and berberine reduces apoptosis caused by highly oxidized and glycated LDL, a modified form of low-density lipoprotein [77]. Taken together, these data suggest that berberine has a protective role against stresses that occur during DR pathogenesis, but despite all these promising results, berberine is still suggested as a diet supplement rather than an effective therapy. Future research should be directed to better understand berberine metabolism and to find formulations for improving its low bioavailability [78].

Reserpine, an indole alkaloid

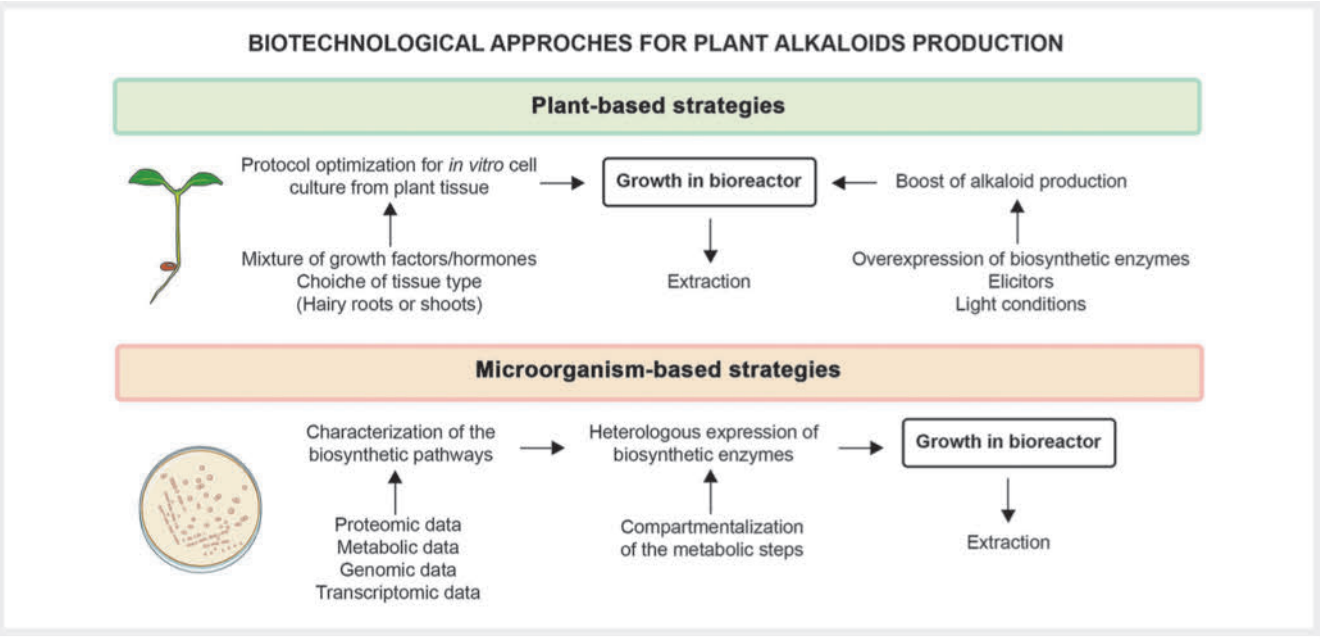
Reserpine is an indole alkaloid present in some plants of the genus *Rauwolfia* [79] (▶ **Table 1**), consisting of evergreen plants typical of tropical regions. This alkaloid is synthesized starting from the tryptophane. The first report of reserpine used for medical purposes dates back to the 16th century when it was extracted from the roots of *R. serpentine*; then it was brought to the attention of Western medicine in the 1950 s for its effect on blood pressure and mental condition [80]. In fact, reserpine successfully lowers blood pressure in patients with hypertension in combination with diuretics and vasodilators [81–83]. Even though this alkaloid reduces mortality linked to hypertension, in the last years, it has been substituted by other classes of drugs. The clinical use of reserpine has been questioned because its assumption was linked to a depression state in treated patients [84], but a recent revision of the literature has questioned this link [85]. The pharmacological

effect of reserpine is mainly the consequence of its binding to the vesicular monoamine transporters (VMATs), acting as a competitive inhibitor of endogenous monoamines [86]. VMATs ensure the storage, sorting, and release of the monoamine neurotransmitters into synaptic vesicles of the neurons, modulating the synaptic activity [87]. Hence, reserpine seems to affect blood pressure and mental state, modulating the levels of catecholamines such as dopamine and adrenaline. Recently, reserpine raised interest in the ophthalmology field because it seems to preserve photoreceptor integrity in retinal ciliopathies. Ciliopathies include a wide group of diseases (e.g., non-syndromic retinitis pigmentosa, cone-rod syndrome, Bardet-Biedl syndrome, and Usher syndrome) that are caused by mutations affecting cilia. A cilium is a cell organelle made by microtubules that works as a sensor for external stimuli. In the retina, cilia are very important for the activity of photoreceptor cells since they capture the light stimulus starting the phototransduction. Hence, defects in these organelles may lead to photoreceptor degeneration and severe visual impairment. Retinal ciliopathies [88] cause irreversible blindness from a young age; hence, many researchers are focused on finding new drugs and therapies. In 2023, Chen and co-authors [89] published the results of a screening of over 6000 existing drugs for their ability to improve photoreceptor survival in the retinal organoids derived from a mice model for ciliopathy [90]. Among the drugs tested, reserpine showed the best output in terms of photoreceptor survival, which was also confirmed in organoids derived from affected patients and *in vivo* animal models. The authors also found that reserpine modulates two pathways that are critical for protein homeostasis, which is mainly regulated through the degradative activity of autophagy and the ubiquitin-proteasome system, cited previously [89]. Clinical trials will be necessary to evaluate the real efficacy of reserpine in retinal diseases.

Caffeine, a methylxanthine alkaloid

Caffeine is a methylxanthine alkaloid synthesized from purine nucleotides [91]. Caffeine is widely diffused in the plant kingdom, but high concentrations of this alkaloid can be found in the seeds of Coffea plants, with *Coffea arabica* being the most famous source of caffeine, in the leaves of *Camellia sinensis* (tea plant), and also in the seeds of cacao plants and those related (*Theobroma cacao, Theobroma grandiflorum*, and *Herrania* sp.) (▶ **Table 1**). In South America, *Paullinia cupana* (guarana) and leaves of *Ilex paraguariensis* (mate) are also well-known sources of caffeine [92]. Caffeine was first isolated in 1819, and then synthetized and characterized by Hermann Emil Fischer in 1895 [93]. However, the stimulating properties of caffeine-based beverages have been known since ancient times. Beverages from coffee plants date back to 1000 BC in Ethiopia and Yemen, and in the 14th century, Arabs discovered the process of roasting coffee seeds, spreading this beverage in Europe and the rest of the world. Tea was used in China in 1000 BC, while cocoa beans were used as a source of caffeine by the Mayans [92]. In addition to the social use of caffeine as a stimulating beverage, the interest in using this alkaloid in medicine started in the 19th century, when it was used for the treatment of asthma [92]. The confirmed molecular targets of caffeine include acetylcholinesterase, adenosine receptor, glycogen phosphorylase, Notum, phosphodiesterase, and ryanodine receptor (for an exhaustive review check [94]). However, the most interesting pharmacological effect of caffeine on the nervous system is its activity as an antagonist of adenosine receptors. Adenosine receptors are G-protein-coupled receptors that respond to adenosine endogenous levels and act as modulators of neurotransmitter release [95]. Four adenosine receptors have been identified (A_1, A_{2A}, A_{2B}, and A_3) and all of them are confirmed targets of caffeine, making caffeine a nonselective adenosine receptor antagonist. Despite excessive consumption of caffeine being considered dangerous for health, especially for the cardiovascular system, this molecule has some positive effects–for example, in pain management [96]; it is in fact often present in over-the-counter medications for headaches. To highlight the value of caffeine in medicine, it is noteworthy to mention that caffeine citrate is listed by the WHO as an essential drug for treating apnea in preterm infants [97]. In addition, caffeine seems to have a neuroprotective role in neurodegenerative diseases, such as Parkinson's and Alzheimer's disease [98]. Hence, given these potential neuroprotective roles, it has been proposed that this alkaloid could be used to preserve RGCs from degeneration, which is, as already mentioned, a typical signature of glaucoma. In support of this hypothesis, Sprague–Dawley rats fed with caffeine in drinking water showed reduced degeneration of RGCs when ocular hypertension (OHT) is induced by laser photocoagulation of the limbal veins to mimic human glaucoma [99]. This benefit was associated with reduced inflammation, determined as low levels of soluble mediators of inflammation (e.g., IL1β and TNFα) and microglia reactivity, with respect to animals fed without caffeine, suggesting that the alkaloid has anti-inflammatory properties. The anti-inflammatory properties of caffeine in the retina were also confirmed by Conti and colleagues, who showed how caffeine counteracts retinal degeneration in mice retina damaged by ischemia/reperfusion treatment [100]. The idea that caffeine, acting on the adenosine receptors, could work as a neuroprotector was also supported by data showing that chemically synthetized selective inhibitors of the A2A receptor protect the injured retina from degeneration, decreasing inflammation [101, 102]. Moreover, epidemiological data suggest that daily consumption of caffeine does not elevate IOP, at least in people without a genetic predisposition for glaucoma [103].

Further data sustaining the benefit of caffeine come from experiments done in animal models of cataracts. Kronschläger and co-authors showed that caffeine administration as eye drops before exposition to UV-B radiation reduces the formation of cataracts, measured as lens light scattering, in a rat model [104]. These results are in agreement with *in vitro* data showing that caffeine supplementation in the culture media protected cultured mouse lenses from photodamage induced by oxidant agents [105, 106]. The protective role of caffein eye drops was further examined in a rat model where cataracts were induced by oral administration of galactose, an experimental model that recapitulates cataract formation in diabetic patients [107]. These results showed that animals treated with caffeine eye drops five times a day developed fewer galactose-induced cataracts, both in short- (5 days) and long-term (25 days) experiments [107], than those in the placebo group.



▶ **Fig. 3 Summary of biotechnology approaches for plant alkaloid production based on microorganisms and plant cell culture.** Potentially, all plant alkaloids can be produced by genetically engineered microorganisms, such as yeast and cell cultures. The advantage of using these approaches is that both microorganisms and plant cells can be grown in bioreactors that provide controlled growth conditions. In the figure, the different steps needed for the improvement of alkaloid production have been mentioned.

Taken together, these results point out that the right amount of daily caffeine uptake can help in preserving eye health, but further investigation will be needed to understand if caffeine-based eye drops could be used as a proper therapy for eye diseases.

Biotechnology Approaches for the Production of Medicinal Plant Alkaloids

Both atropine and pilocarpine are present in the WHO list of essential drugs, which are enlisted as the most efficacious and cost-effective medicines to treat severe and life-threatening diseases. This means that a shortage of these compounds could endanger the healthcare system with serious repercussions on people's lives worldwide. Chemical synthesis can be very challenging for plant alkaloids and also expensive. Hence, the demand of the pharmaceutical market is satisfied mainly by the extraction and isolation of alkaloids directly from plants. Unfortunately, this method of production is burdened by several drawbacks. First of all, alkaloid synthesis in plants is often restricted to specific tissues, limiting the tissue available for alkaloids isolation. Moreover, extraction methods are often not specific and provide a mixture of metabolites with a low biological function and not pure alkaloid. Then, temperature fluctuations, floodings, drought periods, and pathogen attacks not only affect plant fitness, altering the harvestable biomass, but also influence secondary metabolite synthesis, determining inconsistencies in the final yield of alkaloids. Lastly, alkaloids are highly species- and genus-specific, meaning that only a few plant species can be used for the pharmaceutical market. This means that the heavy exploitation of these plants endangers biodiversity and their survival in their native habitats. Hence, many lines of research looked into biotechnologies based on the reconstitution of alkaloid biosynthetic pathways in microorganisms and *in vitro* culture of plant cells (▶ **Fig. 3**).

The main advantage of using microorganisms for alkaloid synthesis is the possibility of propagation in industrial bioreactors that provide controlled growth conditions, eliminating the problem of environmental fluctuations and yield instability. Among the microorganisms, baker yeast *S. cerevisiae* showed the best potential because many genetic tools are available, and it allows a reliable expression of many plant genes, making possible the reconstitution of several biosynthetic pathways. Even though there are no technical restrictions for yeast engineering, the following aspects should be considered. First of all, in plants, the enzymes for secondary metabolites are often distributed in different cellular compartments and they are cellular/tissue-specific, meaning that their activity and/or expression could require a specific regulatory context lacking in yeast. Second, many biosynthetic pathways have not been fully elucidated yet. Hence, transcriptomic, genomic, and proteomic data need to be analyzed and compared to identify the missing enzymes. Third, it is not always possible to obtain the quantity required for industrial production, but yeast can still be a valuable platform to produce high-quality intermediates that can be used in the semisynthetic process to obtain the final drug. One milestone in tropane alkaloid production was recently achieved by Srinivasan and Smolke, who optimized the biosynthesis of hyoscyamine and scopolamine in yeast. Their system expressed 26 genes, encoding not only metabolic enzymes but also transporters to ensure the compartmentalization of the metabolic steps in different cellular locations [108]. Afterward, the

▶ **Table 2** Experimental evidence showing the effects of plant alkaloids on cellular pathways affected in eye diseases.

Alkaloid	Experimental model	Alkaloid effect	References
Atropine	BJ mice; White Leghorn chicks; FDM guinea pigs; *In vitro* Scleral fibroblasts	Sclera remodeling	[28–32]
	Male White Leghorn chickens	Release of dopamine from retina	[34]
	Studies in children and young adults	Increase in choroid thickness	[39, 40, 117–121]
Pilocarpine	Study in healthy adults	Decrease in choroid thickness	[45]
	Study in healthy and glaucoma patients; Mice model	Dilatation of Schlemm's canal	[50, 51]
Berberine	RGC of DR rat model	Reduction in oxidative stress	[64]
	RGC of DR rat model	Increase in RGC survival	[64, 65]
	Primary rat retinal Müller cells	Reduction in high-glucose-mediated activation of NF-kB pathway	[64]
	Primary rat retinal Müller cells	Modulation of autophagy through AMP/mTOR pathway	[67]
	HREC cell line	Inhibition of the insulin-mediated induction of the HIF1/VEGF-pathway	[72]
	Cultured human Müller cells	Reduction in cell apoptosis mediated by highly oxidized and glycated LDL	[77]
Reserpine	iPSC-derived retinal organoids; retina of rd16 mouse model	Modulation of UPS and autophagy pathway to preserve photoreceptor integrity	[89]
Caffeine	Sprague–Dawley rats, Male C57BL/6 J mice	Reduction in inflammation and RGC apoptosis	[99, 100]

Abbreviations: BJ–Balb/CJ; FDM–form deprivation myopia; RGC–retinal ganglion cells; DR–diabetic retinopathy; HREC–human retinal endothelial cells; iPSC–induced pluripotent stem cells; UPS–ubiquitin proteasome pathway; OHT–ocular hypertension.

same authors were able to scale up to 172 µgr/L and 480 µgr/L of scopolamine and hyoscyamine, respectively, solving the problem of the vacuolar compartmentalization of the littorine intermediate by the identification and expression in yeast of functional vacuolar alkaloid transporters [109].

Also, berberine was successfully produced in yeast [110, 111] after many years of attempts to produce intermediates. Han and Li were able to improve the initial yield of 1.68 µg/L, obtained from glucose and by the expression of 15 enzymes, to the final yield of 1.08 mg/L by fermentation and the engineering of rate-limiting enzymes [111]. Moreover, the authors suggest yeast also as a platform to overcome the limit of low bioactivity and bioavailability of berberine.

On the other hand, plant cells can also be grown in bioreactors providing renewable materials for metabolite production. Even though this system is used for the industrial production of some plant metabolites, such as the alkaloids berberine and scopolamine [112], it shows some weaknesses and limitations. First, cell cultures growing for a long time are susceptible to mold and bacteria contaminations; hence, cultures should be kept in highly controlled environments. Second, to provide enough oxygen for plant growth, bioreactors are equipped with systems, such as rotation, stirring, and air suppliers, that ensure the correct air circulation [113]. However, these systems create mechanical stresses for the cell culture, which can be easily damaged, slowing proliferation and reducing production. Third, while microorganisms release metabolites into the media, plants tend to store them in the cells, making the recovery of metabolites more difficult. Fourth, the alkaloid synthesis in plants is often tissue-specific; for example, many biosynthetic enzymes for tropane alkaloid biosynthesis are expressed in roots [114]. To overcome this issue, it is possible to use differentiated cultures, such as hairy roots. Hairy root cultures can be generated, adjusting the mixture of hormones to favorite roots development (e.g., more auxin than cytokines), but the most efficient procedure is infecting wounded plants with *Agrobacterium rhizogenes*. During the infection, this bacteria transfers the *Rol* genes into the plant genome, wherein the product of this gene stimulates root development, which can be then grown in liquid media for large-scale production. Similarly to hairy root cells, cultures of the aerial parts of plants can be made to produce metabolites that usually accumulate in the shoots [112]. However, hairy root cultures are more diffused than

the shoot ones because they do not need particular light conditions for the proliferation in bioreactors, making their growth less demanding. The last limitation of plant cell culture is that not all plant species can be easily cultivated *in vitro* and used for tissue regeneration, and this can be a problem, considering that many alkaloids are highly genus-specific. Hence, many researchers constantly develop new species-specific protocols for plant regeneration that consist of growing shoots and roots at different concentrations of plant growth regulators such as auxin and cytokines.

Despite the advantages of cell cultures, the final alkaloid yield is often far away from the industrial demand. To improve this aspect, one strategy can be the overexpression of key enzymes of the alkaloid synthesis pathway, which has been successfully used for tropane alkaloids, for which the biosynthetic pathway has been extensively studied. The review written by Gong and colleagues offers a complete overview of this topic [115].

Another interesting method to improve alkaloid accumulation is through elicitors. Elicitors are commonly defined as biotic and abiotic agents that trigger stress responses in plants [116]. Given that many secondary metabolites are produced in response to stresses, elicitors can be added to the media of cell culture to stimulate metabolite production. Some microorganisms, such as fungi and bacteria, act as biotic elicitors of plant alkaloids, but molecules that work as signaling modulators such as the hormone jasmonic acid (JA) can also be used.

Conclusions

Vision impairment and loss are medical conditions that affect millions of people all around the world, numbers that are anticipated to be doubled by 2050, at least in the USA, for the four main eye diseases, which are glaucoma, DR, cataract, and macular degeneration.

Blindness, but also mild visual impairment, has an incredible cost for society, not only considering the direct medical care but also as loss of productivity. Moreover, eye disorders highly affect quality of life, and they contribute to other conditions such as psychiatric problems (such as depression and anxiety) and cognitive dysfunctions, especially in the elderly. As described in this review, plant-based drugs are commonly used to treat eye conditions, with alkaloids playing an important role, given their plethora of biological activities (▶ **Table 2**). Even though plant alkaloids have been largely exploited for pharmaceutical purposes, it is important to continue the efforts to identify effective drug treatments and to develop new formulations. This can be achieved only by an intense and active collaboration between different areas of research. First, plant biologists are necessary for the identification of the plant species exploitable for the purpose of deeply studying their physiology. Second, biochemists need to work on effective extraction methods for obtaining pure metabolites. Alkaloids show an incredible diversity in the structures, consisting in basic nitrogen atoms and rings; hence, specific extraction methods from raw materials need to be optimized depending on the alkaloid of interest. Once the molecule has been identified, researchers in the cell biology field have to test these compounds in proper cell lines and animal models to evaluate the cytotoxicity and the effects on the molecular pathways that are relevant to the ophthalmology field. Even though pharmaceutical research still relies on animal models, progress has been made to identify *in vitro* cell cultures that can be used in pre-clinical studies. For example, the organoids obtained from retinal iPSC derived from patients [89] were shown to be a valid system for drug screening, and it allowed the identification of reserpine as a possible treatment for retinal disease.

Another important aspect to consider in drug development in ophthalmology is the route of administration, which can vary according to the disease to treat and its localization (e.g., corneal vs. retinal diseases). The most frequently used is topical administration, which has the advantage of being noninvasive but cannot ensure the effective concentration of the drug in the posterior chamber of the eye. Less used are systemic administration and intravitreal injection. The former has the disadvantage of presenting systemic side effects and poor bioavailability in ocular tissues, while the latter, despite the local high availability of drugs directly injected in the posterior chamber of the eye, is a very invasive procedure for the patients. For these reasons, the establishment of new formulations that optimize retention time and posology (e.g., slow-release formulations), absorption, and drug delivery will be necessary.

Lastly, a full exploitation of plant alkaloids in medicine can be obtained only through a deep characterization of the different biosynthetic pathways that allow the identification of the rate-limiting plant enzymes and important intermediates of reaction. This is critical for the creation of synthetic toolboxes that can be used for the engineering of metabolic pathways in industrial production. In recent years, microorganisms and plant cell cultures have been successfully used as a platform for this purpose, but further optimization will be necessary to fulfill the pharmaceutical market. This process includes the maximization of the enzyme activity and the capacity to obtain a reproducible yield of metabolites over time.

In conclusion, the finding of sustainable and reliable methods for alkaloid production will increase their diffusion in medical practice with great benefit for human and animal health.

Literature research method

Pubmed was used as main browser for searching the literature; the research was focused mainly over the last 10 years (from 2013 to September 2023), but older publications were further considered if they were particularly relevant or when the research retrieved no significant results. The terms [eye disease] AND [plant alkaloid] were used to identify the main alkaloids used in ophthalmology. Then, the following terms were used for the role of the alkaloids presented in ophthalmology: [atropine] AND [myopia]; [atropine] AND [sclera]; [atropine] AND [choroid]; [pilocarpine] AND [glaucoma]; [pilocarpine] AND [myopia]; [diabetic retinopathy] AND [berberine]; [reserpine] AND [retinal disease]; [caffeine] AND [retinal disease]. For the part regarding alkaloid production, the following terms were used: [yeast] AND [atropine]; [yeast] AND [reserpine]; [yeast] AND [pilocarpine]; [yeast] AND [berberine]; [plant culture] AND [atropine]; [plant culture] AND [reserpine]; [plant culture] AND [berberine]; [plant culture] AND [pilocarpine].

For historical background, Google was used, and the following terms were used: “atropine history”, “caffeine history”, “berberine history, “pilocarpine history”, and “reserpine history”.

Contributors’ Statement

Conception and design of the work: A. Boccaccini and R. Lorrai; data collection: A. Boccaccini, R. Lorrai, S. Giammaria, D. Cavaterra, D. Sbardella, G. R. Tundo; analysis and interpretation of the data: A. Boccaccini, R. Lorrai, S. Giammaria, D. Cavaterra, D. Sbardella, G. R. Tundo; drafting the manuscript: A. Boccaccini, R. Lorrai, S. Giammaria, D. Cavaterra, D. Sbardella, G. R. Tundo; critical revision of the manuscript: A. Boccaccini, R. Lorrai, S. Giammaria, D. Cavaterra, D. Sbardella, G. R. Tundo.

Acknowledgements

Authors acknowledge Fondazione Roma and the Ministry of Health for their support.

Conflict of Interest

The authors declare that they have no conflict of interest.

References

[1] Thompson RC. The Assyrian Herbal. Luzac and Company; 1924

[2] “The Papyrus Ebers”/translated from the German version by Cyril P. Bryan; with an introduction by G. Elliot Smith. London; 1930

[3] Serturner F. Darstellung der reinen Mohnsäure (Opiumsäure) nebst einer Untersuchung des Opiums mit vorzüglicher Hinsicht auf einen darin neu entdeckten Stoff und die dahin gehörigen Bemerkungen. Vom Herrn Serturner in Paderborn. Journal der Pharmacie 1806; 14: 47–93

[4] Li J, Liu A, Najeeb U, Zhou W, Liu H, Yan G, Gill RA, Yun X, Bai Q, Xu L. Genome-wide investigation and expression analysis of membrane-bound fatty acid desaturase genes under different biotic and abiotic stresses in sunflower (Helianthus annuus L.). Int J Biol Macromol 2021; 175: 188–198. doi: doi:10.1016/j.ijbiomac.2021.02.013

[5] Xu L, Cao M, Wang Q, Xu J, Liu C, Ullah N, Li J, Hou Z, Liang Z, Zhou W, Liu A. Insights into the plateau adaptation of Salvia castanea by comparative genomic and WGCNA analyses. J Advanc Res 2022; 42: 221–235. doi: doi:10.1016/j.jare.2022.02.004

[6] Makhumbila P, Rauwane ME, Muedi HH, Madala NE, Figlan S. Metabolome profile variations in common bean (Phaseolus vulgaris L.) resistant and susceptible genotypes incited by rust (Uromyces appendiculatus). Frontiers in Genetics 2023; 14

[7] Yang L, Stöckigt J. Trends for diverse production strategies of plant medicinal alkaloids. Nat Prod Rep 2010; 27: 1469–1479. doi: doi:10.1039/C005378C

[8] Dey P, Kundu A, Kumar A, Gupta M, Lee BM, Bhakta T, Dash S, Kim HS. Chapter 15 – Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). In: Sanches Silva A, Nabavi SF, Saeedi M, et al., Hrsg. Recent Advances in Natural Products Analysis. Elsevier; 2020: 505–567

[9] do Nascimento NC, Menguer PK, Sperotto RA, de Almeida MR, Fett-Neto AG. The alkaloid brachycerine contributes to protection against acute UV-B damage in Psychotria. Ind Crops Prod 2020; 147: 112216. doi: doi:10.1016/j.indcrop.2020.112216

[10] Steppuhn A, Gase K, Krock B, Halitschke R, Baldwin IT. Nicotine’s defensive function in nature. PLoS Biol 2004; 2: E217. doi: doi:10.1371/journal.pbio.0020217

[11] Santos AP, Moreno PRH. Alkaloids Derived from Histidine: Imidazole (Pilocarpine, Pilosine). In: Ramawat KG, Mérillon J–M, Hrsg. Natural Products: Phytochemistry, Botany and Metabolism of Alkaloids, Phenolics and Terpenes. Berlin, Heidelberg: Springer; 2013: 861–882

[12] Marciniak P, Kolińska A, Spochacz M et al. Differentiated Effects of Secondary Metabolites from Solanaceae and Brassicaceae Plant Families on the Heartbeat of Tenebrio molitor Pupae. Toxins (Basel) 2019; 11: 287. doi: doi:10.3390/toxins11050287

[13] González-Lamothe R, Mitchell G, Gattuso M, Diarra MS, Malouin F, Bouarab K. Plant Antimicrobial Agents and Their Effects on Plant and Human Pathogens. Int J Mol Sci 2009; 10: 3400–3419. doi: doi:10.3390/ijms10083400

[14] Ettefagh KA, Burns JT, Junio HA, Kaatz GW, Cech NB. Goldenseal (Hydrastis canadensis L.) extracts synergistically enhance the antibacterial activity of berberine via efflux pump inhibition. Planta Med 2011; 77: 835–840. doi: doi:10.1055/s-0030–1250606

[15] An T, Huang RQ, Yang Z, Zhang DK, Li GR, Yao YC, Gao J. Alkaloids from Cynanchum komarovii with inhibitory activity against the tobacco mosaic virus. Phytochemistry 2001; 58: 1267–1269. doi: doi:10.1016/s0031-9422(01)00382-x

[16] Chen J, Yan XH, Dong JH, Sang P, Fang X, Di YT, Zhang ZK, Hao XJ. Tobacco mosaic virus (TMV) inhibitors from Picrasma quassioides Benn. J Agric Food Chem 2009; 57: 6590–6595. doi: doi:10.1021/jf901632j

[17] Kohnen-Johannsen KL, Kayser O. Tropane Alkaloids: Chemistry, Pharmacology, Biosynthesis and Production. Molecules 2019; 24: 796. doi: doi:10.3390/molecules24040796

[18] Grynkiewicz G, Gadzikowska M. Tropane alkaloids as medicinally useful natural products and their synthetic derivatives as new drugs. Pharmacological Reports 2008;

[19] Nietgen GW, Schmidt J, Hesse L, Hönemann CW, Durieux ME. Muscarinic receptor functioning and distribution in the eye: Molecular basis and implications for clinical diagnosis and therapy. Eye (Lond) 1999; 13: 285–300. doi: doi:10.1038/eye.1999.78

[20] Ruan Y, Patzak A, Pfeiffer N, Gericke A. Muscarinic Acetylcholine Receptors in the Retina-Therapeutic Implications. Int J Mol Sci 2021; 22: 4989. doi: doi:10.3390/ijms22094989

[21] Smulyan H. The Beat Goes On: The Story of Five Ageless Cardiac Drugs. Am J Med Sci 2018; 356: 441–450. doi: doi:10.1016/j.amjms.2018.04.011

[22] Morgan IG, Ohno-Matsui K, Saw SM. Myopia. Lancet 2012; 379: 1739–1748. doi: doi:10.1016/S0140–6736(12)60272–4

[23] Chierigo A, Ferro Desideri L, Traverso CE, Vagge A. The Role of Atropine in Preventing Myopia Progression: An Update. Pharmaceutics 2022; 14: 900. doi: doi:10.3390/pharmaceutics14050900

[24] Gong Q, Janowski M, Luo M, Wei H, Chen B, Yang G, Liu L. Efficacy and Adverse Effects of Atropine in Childhood Myopia. JAMA Ophthalmol 2017; 135: 624–630. doi: doi:10.1001/jamaophthalmol.2017.1091

[25] McBrien NA, Moghaddam HO, Reeder AP. Atropine reduces experimental myopia and eye enlargement via a nonaccommodative mechanism. Invest Ophthalmol Vis Sci 1993; 34: 205–215

[26] McBrien NA, Stell WK, Carr B. How does atropine exert its anti-myopia effects? Ophthalmic Physiol Opt 2013; 33: 373–378. doi: doi:10.1111/opo.12052

[27] Upadhyay A, Beuerman RW. Biological Mechanisms of Atropine Control of Myopia. Eye Contact Lens 2020; 46: 129–135. doi: doi:10.1097/ICL.0000000000000677

[28] Barathi VA, Beuerman RW. Molecular mechanisms of muscarinic receptors in mouse scleral fibroblasts: Prior to and after induction of experimental myopia with atropine treatment. Mol Vis 2011; 17: 680–692

[29] Gallego P, Martínez-García C, Pérez-Merino P, Ibares-Frías L, Mayo-Iscar A, Merayo-Lloves J. Scleral changes induced by atropine in chicks as an experimental model of myopia. Ophthalmic Physiol Opt 2012; 32: 478–484. doi: doi:10.1111/j.1475-1313.2012.00940.x

[30] Zou L, Liu R, Zhang X, Chu R, Dai J, Zhou H, Liu H. Upregulation of regulator of G-protein signaling 2 in the sclera of a form deprivation myopic animal model. Mol Vis 2014; 20: 977–987

[31] Hsiao YT, Chang WA, Kuo MT, Lo J, Lin HC, Yen MC, Jian SF, Chen YJ, Kuo PL. Systematic Analysis of Transcriptomic Profile of the Effects of Low Dose Atropine Treatment on Scleral Fibroblasts using Next-Generation Sequencing and Bioinformatics. Int J Med Sci 2019; 16: 1652–1667. doi: doi:10.7150/ijms.38571

[32] Cristaldi M, Olivieri M, Pezzino S, Spampinato G, Lupo G, Anfuso CD, Rusciano D. Atropine Differentially Modulates ECM Production by Ocular Fibroblasts, and Its Ocular Surface Toxicity Is Blunted by Colostrum. Biomedicines 2020; 8: 78. doi: doi:10.3390/biomedicines8040078

[33] Boote C, Sigal IA, Grytz R, Hua Y, Nguyen TD, Girard MJA. Scleral structure and biomechanics. Prog Retin Eye Res 2020; 74: 100773. doi: doi:10.1016/j.preteyeres.2019.100773

[34] Schwahn HN, Kaymak H, Schaeffel F. Effects of atropine on refractive development, dopamine release, and slow retinal potentials in the chick. Vis Neurosci 2000; 17: 165–176. doi: doi:10.1017/S0952523800171184

[35] Thomson K, Kelly T, Karouta C, Morgan I, Ashby R. Insights into the mechanism by which atropine inhibits myopia: evidence against cholinergic hyperactivity and modulation of dopamine release. Br J Pharmacol 2021; 178: 4501–4517. doi: doi:10.1111/bph.15629

[36] Zhu Y, Bian JF, Lu DQ, To CH, Lam CS, Li KK, Yu FJ, Gong BT, Wang Q, Ji XW, Zhang HM, Nian H, Lam TC, Wei RH. Alteration of EIF2 Signaling, Glycolysis, and Dopamine Secretion in Form-Deprived Myopia in Response to 1% Atropine Treatment: Evidence From Interactive iTRAQ-MS and SWATH-MS Proteomics Using a Guinea Pig Model. Front Pharmacol 2022; 13: 814814. doi: doi:10.3389/fphar.2022.814814

[37] Liu Y, Wang L, Xu Y, Pang Z, Mu G. The influence of the choroid on the onset and development of myopia: from perspectives of choroidal thickness and blood flow. Acta Ophthalmol 2021; 99: 730–738. doi: doi:10.1111/aos.14773

[38] Wang Y, Zhu X, Xuan Y, Wang M, Zhou X, Qu X. Short-Term Effects of Atropine 0.01% on the Structure and Vasculature of the Choroid and Retina in Myopic Chinese Children. Ophthalmol Ther 2022; 11: 833–856. doi: doi:10.1007/s40123-022–00476-0

[39] Yam JC, Jiang Y, Lee J, Li S, Zhang Y, Sun W, Yuan N, Wang YM, Yip BHK, Kam KW, Chan HN, Zhang XJ, Young AL, Tham CC, Cheung CY, Chu WK, Pang CP, Chen LJ. The Association of Choroidal Thickening by Atropine With Treatment Effects for Myopia: Two-Year Clinical Trial of the Low-concentration Atropine for Myopia Progression (LAMP) Study. Am J Ophthalmol 2022; 237: 130–138. doi: doi:10.1016/j.ajo.2021.12.014

[40] Sander BP, Collins MJ, Read SA. The effect of topical adrenergic and anticholinergic agents on the choroidal thickness of young healthy adults. Exp Eye Res 2014; 128: 181–189. doi: doi:10.1016/j.exer.2014.10.003

[41] Ringer S, Gould A. ON JABORANDI. The Lancet 1875; 105: 157–159. doi: doi:10.1016/S0140–6736(02)47201–7

[42] Maehle AH. "Receptive Substances": John Newport Langley (1852–1925) and his Path to a Receptor Theory of Drug Action. Med Hist 2004; 48: 153–174. doi: doi:10.1017/S0025727300000090

[43] Fang F, Huang F, Xie R, Li C, Liu Y, Zhu Y, Qu J, Zhou X. Effects of muscarinic receptor modulators on ocular biometry of guinea pigs. Ophthalmic Physiol Opt 2015; 35: 60–69. doi: doi:10.1111/opo.12166

[44] Tong L, Cui D, Zeng J. Effects of topical pilocarpine on ocular growth and refractive development in rabbits. Eur J Ophthalmol 2021; 31: 2107–2115. doi: doi:10.1177/1120672120934962

[45] Maltsev DS, Kulikov AN, Vasiliev AS. Effect of Topical Pilocarpine on Choroidal Thickness in Healthy Subjects. Optom Vis Sci 2020; 97: 457. doi: doi:10.1097/OPX.0000000000001521

[46] Packer M, Brandt JD. Ophthalmology's botanical heritage. Surv Ophthalmol 1992; 36: 357–365. doi: doi:10.1016/0039–6257(92)90113–8

[47] Jonas JB, Aung T, Bourne RR, Bron AM, Ritch R, Panda-Jonas S. Glaucoma. The Lancet 2017; 390: 2183–2193. doi: doi:10.1016/S0140–6736(17)31469-1

[48] Coleman AL, Miglior S. Risk factors for glaucoma onset and progression. Surv Ophthalmol 2008; 53 Suppl 1: S3–10. doi: doi:10.1016/j.survophthal.2008.08.006

[49] Lütjen-Drecoll E, Wiendl H, Kaufman PL. Acute and chronic structural effects of pilocarpine on monkey outflow tissues. Trans Am Ophthalmol Soc 1998; 96: 171–191; discussion 192–195

[50] Li G, Farsiu S, Chiu SJ, Gonzalez P, Lütjen-Drecoll E, Overby DR, Stamer WD. Pilocarpine-Induced Dilation of Schlemm's Canal and Prevention of Lumen Collapse at Elevated Intraocular Pressures in Living Mice Visualized by OCT. Invest Ophthalmol Vis Sci 2014; 55: 3737–3746. doi: doi:10.1167/iovs.13–13700

[51] Skaat A, Rosman MS, Chien JL, Mogil RS, Ren R, Liebmann JM, Ritch R, Park SC. Effect of Pilocarpine Hydrochloride on the Schlemm Canal in Healthy Eyes and Eyes With Open-Angle Glaucoma. JAMA Ophthalmol 2016; 134: 976–981. doi: doi:10.1001/jamaophthalmol.2016.1881

[52] Zimmerman TJ, Wheeler TM. Miotics: side effects and ways to avoid them. Ophthalmology 1982; 89: 76–80. doi: doi:10.1016/s0161-6420(82)34866-6

[53] Ritch R. The pilocarpine paradox. J Glaucoma 1996; 5: 225–227

[54] Price FW Jr, Hom M, Moshirfar M, Evans D, Liu H, Penzner J, Robinson MR, Lee S, Wirta DL. Combinations of Pilocarpine and Oxymetazoline for the Pharmacological Treatment of Presbyopia: Two Randomized Phase 2 Studies. Ophthalmology Science 2021; 1. doi: doi:10.1016/j.xops.2021.100065

[55] Waring GO 4th, Price FW Jr, Wirta D, McCabe C, Moshirfar M, Guo Q, Gore A, Liu H, Safyan E, Robinson MR. Safety and Efficacy of AGN-190584 in Individuals With Presbyopia: The GEMINI 1 Phase 3 Randomized Clinical Trial. JAMA Ophthalmol 2022; 140: 363–371. doi: doi:10.1001/jamaophthalmol.2022.0059

[56] Westheimer G. Topical Review: Pilocarpine-induced Miosis as Help for Early Presbyopes? Optom Vis Sci 2022; 99: 632. doi: doi:10.1097/OPX.0000000000001924

[57] Tucker T, Early J. Pilocarpine 1.25% Ophthalmic Solution (Vuity) for the Treatment of Presbyopia. Am Fam Physician 2023; 107: 659–660

[58] Neag MA, Mocan A, Echeverría J, Pop RM, Bocsan CI, Crişan G, Buzoianu AD. Berberine: Botanical Occurrence, Traditional Uses, Extraction Methods, and Relevance in Cardiovascular, Metabolic, Hepatic, and Renal Disorders. Frontiers in Pharmacology 2018; 9

[59] Sobhani Z, Akaberi M, Amiri MS, Ramezani M, Emami SA, Sahebkar A. Medicinal Species of the Genus Berberis: A Review of Their Traditional and Ethnomedicinal Uses, Phytochemistry and Pharmacology. In: Barreto GE, Sahebkar A, Hrsg. Pharmacological Properties of Plant-Derived Natural Products and Implications for Human Health. Cham: Springer International Publishing; 2021: 547–577

[60] Song D, Hao J, Fan D. Biological properties and clinical applications of berberine. Front Med 2020; 14: 564–582. doi: doi:10.1007/s11684-019–0724-6

[61] Lan J, Zhao Y, Dong F, Yan Z, Zheng W, Fan J, Sun G. Meta-analysis of the effect and safety of berberine in the treatment of type 2 diabetes mellitus, hyperlipemia and hypertension. J Ethnopharmacol 2015; 161: 69–81. doi: doi:10.1016/j.jep.2014.09.049

[62] Cheung N, Mitchell P, Wong TY. Diabetic retinopathy. The Lancet 2010; 376: 124–136. doi: doi:10.1016/S0140–6736(09)62124-3

[63] Madsen-Bouterse SA, Kowluru RA. Oxidative stress and diabetic retinopathy: Pathophysiological mechanisms and treatment perspectives. Rev Endocr Metab Disord 2008; 9: 315–327. doi: doi:10.1007/s11154-008–9090-4

[64] Zhai J, Li Z, Zhang H, Ma L, Ma Z, Zhang Y, Zou J, Li M, Ma L, Wang X, Li X. Berberine protects against diabetic retinopathy by inhibiting cell apo-

ptosis via deactivation of the NF-κB signaling pathway. Mol Med Rep 2020; doi: doi:10.3892/mmr.2020.11505

[65] Fang W, Huang X, Wu K, Zong Y, Yu J, Xu H, Shi J, Wei J, Zhou X, Jiang C. Activation of the GABA-alpha receptor by berberine rescues retinal ganglion cells to attenuate experimental diabetic retinopathy. Front Mol Neurosci 2022; 15: 930599. doi: doi:10.3389/fnmol.2022.930599

[66] Liu T, Zhang L, Joo D, Sun SC. NF-κB signaling in inflammation. Sig Transduct Target Ther 2017; 2: 17023. doi: doi:10.1038/sigtrans.2017.23

[67] Chen H, Ji Y, Yan X, Su G, Chen L, Xiao J. Berberine attenuates apoptosis in rat retinal Müller cells stimulated with high glucose via enhancing autophagy and the AMPK/mTOR signaling. Biomed Pharmacother 2018; 108: 1201–1207. doi: doi:10.1016/j.biopha.2018.09.140

[68] Dikic I, Elazar Z. Mechanism and medical implications of mammalian autophagy. Nat Rev Mol Cell Biol 2018; 19: 349–364. doi: doi:10.1038/s41580-018-0003-4

[69] Maiuri MC, Zalckvar E, Kimchi A, Kroemer G. Self-eating and self-killing: crosstalk between autophagy and apoptosis. Nat Rev Mol Cell Biol 2007; 8: 741–752. doi: doi:10.1038/nrm2239

[70] Mecchia A, Palumbo C, De Luca A, Sbardella D, Boccaccini A, Rossi L, Parravano M, Varano M, Caccuri AM. High glucose induces an early and transient cytoprotective autophagy in retinal Müller cells. Endocrine 2022; 77: 221–230. doi: doi:10.1007/s12020-022–03079-8

[71] Sbardella D, Tundo GR, Mecchia A, Palumbo C, Atzori MG, Levati L, Boccaccini A, Caccuri AM, Cascio P, Lacal PM, Graziani G, Varano M, Coletta M, Parravano M. A novel and atypical NF-KB pro-inflammatory program regulated by a CamKII-proteasome axis is involved in the early activation of Muller glia by high glucose. Cell Biosci 2022; 12: 108. doi: doi:10.1186/s13578-022–00839-x

[72] Wang N, Zhang C, Xu Y, Tan HY, Chen H, Feng Y. Berberine improves insulin-induced diabetic retinopathy through exclusively suppressing Akt/mTOR-mediated HIF-1α/VEGF activation in retina endothelial cells. Int J Biol Sci 2021; 17: 4316–4326. doi: doi:10.7150/ijbs.62868

[73] Jingi AM, Tankeu AT, Ateba NA, Noubiap JJ. Mechanism of worsening diabetic retinopathy with rapid lowering of blood glucose: the synergistic hypothesis. BMC Endocr Disord 2017; 17: 63. doi: doi:10.1186/s12902-017–0213-3

[74] Treins C, Giorgetti-Peraldi S, Murdaca J, Semenza GL, Van Obberghen E. Insulin Stimulates Hypoxia-inducible Factor 1 through a Phosphatidylinositol 3-Kinase/Target of Rapamycin-dependent Signaling Pathway. J Biol Chem 2002; 277: 27975–27981. doi: doi:10.1074/jbc.M204152200

[75] Meng D, Mei A, Liu J, Kang X, Shi X, Qian R, Chen S. NADPH Oxidase 4 Mediates Insulin-Stimulated HIF-1α and VEGF Expression, and Angiogenesis In Vitro. PLoS One 2012; 7: e48393. doi: doi:10.1371/journal.pone.0048393

[76] Arrigo A, Aragona E, Bandello F. VEGF-targeting drugs for the treatment of retinal neovascularization in diabetic retinopathy. Ann Med 2022; 54: 1089–1111. doi: doi:10.1080/07853890.2022.2064541

[77] Fu D, Yu JY, Connell AR, Yang S, Hookham MB, McLeese R, Lyons TJ. Beneficial Effects of Berberine on Oxidized LDL-Induced Cytotoxicity to Human Retinal Müller Cells. Invest Ophthalmol Vis Sci 2016; 57: 3369. doi: doi:10.1167/iovs.16–19291

[78] Imenshahidi M, Hosseinzadeh H. Berberine and barberry (Berberis vulgaris): A clinical review. Phytother Res 2019; 33: 504–523. doi: doi:10.1002/ptr.6252

[79] Bindu S, Rameshkumar KB, Kumar B, Awantika S, Anilkumar C. Distribution of reserpine in Rauvolfia species from India – HPTLC and LC–MS studies. Ind Crops Prod 2014; 62: 430–436. doi: doi:10.1016/j.indcrop.2014.09.018

[80] Curzon G. How reserpine and chlorpromazine act: the impact of key discoveries on the history of psychopharmacology. Trends Pharmacol Sci 1990; 11: 61–63. doi: doi:10.1016/0165–6147(90)90318-3

[81] Shamon SD, Perez MI. Blood pressure-lowering efficacy of reserpine for primary hypertension. Cochrane Database Syst Rev 2016; 2016: CD007655. doi: doi:10.1002/14651858.CD007655.pub3

[82] Tsioufis C, Thomopoulos C. Combination drug treatment in hypertension. Pharmacol Res 2017; 125: 266–271. doi: doi:10.1016/j.phrs.2017.09.011

[83] Weir MR. Reserpine: A New Consideration of an Old Drug for Refractory Hypertension. Am J Hypertens 2020; 33: 708–710. doi: doi:10.1093/ajh/hpaa069

[84] Beers MH, Passman LJ. Antihypertensive Medications and Depression. Drugs 1990; 40: 792–799. doi: doi:10.2165/00003495–199040060-00003

[85] Strawbridge R, Javed RR, Cave J, Jauhar S, Young AH. The effects of reserpine on depression: A systematic review. J Psychopharmacol 2023; 37: 248–260. doi: doi:10.1177/02698811221115762

[86] Yaffe D, Forrest LR, Schuldiner S. The ins and outs of vesicular monoamine transporters. J Gen Physiol 2018; 150: 671–682. doi: doi:10.1085/jgp.201711980

[87] Wimalasena K. Vesicular monoamine transporters: Structure-function, pharmacology, and medicinal chemistry: VESICULAR MONOAMINE TRANSPORTERS. Med Res Rev 2011; 31: 483–519. doi: doi:10.1002/med.20187

[88] Adams NA, Awadein A, Toma HS. The retinal ciliopathies. Ophthalmic Genet 2007; 28: 113–125. doi: doi:10.1080/13816810701537424

[89] Chen HY, Swaroop M, Papal S, Mondal AK, Song HB, Campello L, Tawa GJ, Regent F, Shimada H, Nagashima K, de Val N, Jacobson SG, Zheng W, Swaroop A. Reserpine maintains photoreceptor survival in retinal ciliopathy by resolving proteostasis imbalance and ciliogenesis defects. Elife 2023; 12: e83205. doi: doi:10.7554/eLife.83205

[90] Rachel RA, Yamamoto EA, Dewanjee MK, May-Simera HL, Sergeev YV, Hackett AN, Pohida K, Munasinghe J, Gotoh N, Wickstead B, Fariss RN, Dong L, Li T, Swaroop A. CEP290 alleles in mice disrupt tissue-specific cilia biogenesis and recapitulate features of syndromic ciliopathies. Hum Mol Genet 2015; 24: 3775–3791. doi: doi:10.1093/hmg/ddv123

[91] Ashihara H, Sano H, Crozier A. Caffeine and related purine alkaloids: Biosynthesis, catabolism, function and genetic engineering. Phytochemistry 2008; 69: 841–856. doi: doi:10.1016/j.phytochem.2007.10.029

[92] Fredholm BB. Notes on the History of Caffeine Use. In: Fredholm BB, Hrsg. Methylxanthines. Berlin, Heidelberg: Springer; 2011: 1–9

[93] Fischer E, Ach L. Synthese des Caffeïns. In: Fischer E, Hrsg. Untersuchungen in der Puringruppe. Berlin, Heidelberg: Springer; 1907: 219–227

[94] Faudone G, Arifi S, Merk D. The Medicinal Chemistry of Caffeine. J Med Chem 2021; 64: 7156–7178. doi: doi:10.1021/acs.jmedchem.1c00261

[95] Dunwiddie TV, Masino SA. The Role and Regulation of Adenosine in the Central Nervous System. Annu Rev Neurosci 2001; 24: 31–55. doi: doi:10.1146/annurev.neuro.24.1.31

[96] Baratloo A, Rouhipour A, Forouzanfar MM, Safari S, Amiri M, Negida A. The Role of Caffeine in Pain Management: A Brief Literature Review. Anesth Pain Med 2016; 6: e33193. doi: doi:10.5812/aapm.33193

[97] World Health Organization. World Health Organization Model List of Essential Medicines, 21st List, 2019. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO. 2019;

[98] Kolahdouzan M, Hamadeh MJ. The neuroprotective effects of caffeine in neurodegenerative diseases. CNS Neurosci Ther 2017; 23: 272–290. doi: doi:10.1111/cns.12684

[99] Madeira MH, Ortin-Martinez A, Nadal-Nícolas F, Ambrósio AF, Vidal-Sanz M, Agudo-Barriuso M, Santiago AR. Caffeine administration prevents retinal neuroinflammation and loss of retinal ganglion cells in an animal model of glaucoma. Sci Rep 2016; 6: 27532. doi: doi:10.1038/srep27532

[100] Conti F, Lazzara F, Romano GL, Platania CBM, Drago F, Bucolo C. Caffeine Protects Against Retinal Inflammation. Frontiers in Pharmacology 2022; 12

[101] Madeira MH, Elvas F, Boia R, Gonçalves FQ, Cunha RA, Ambrósio AF, Santiago AR. Adenosine A2AR blockade prevents neuroinflammation-induced death of retinal ganglion cells caused by elevated pressure. J Neuroinflammation 2015; 12: 115. doi: doi:10.1186/s12974-015–0333-5

[102] Boia R, Elvas F, Madeira MH, Aires ID, Rodrigues-Neves AC, Tralhão P, Szabó EC, Baqi Y, Müller CE, Tomé ÂR, Cunha RA, Ambrósio AF, Santiago AR. Treatment with A2A receptor antagonist KW6002 and caffeine intake regulate microglia reactivity and protect retina against transient ischemic damage. Cell Death Dis 2017; 8: e3065. doi: doi:10.1038/cddis.2017.451

[103] Kim J, Aschard H, Kang JH, Lentjes MAH, Do R, Wiggs JL, Khawaja AP, Pasquale LR. Intraocular Pressure, Glaucoma, and Dietary Caffeine Consumption: A Gene–Diet Interaction Study from the UK Biobank. Ophthalmology 2021; 128: 866–876. doi: doi:10.1016/j.ophtha.2020.12.009

[104] Kronschläger M, Löfgren S, Yu Z, Talebizadeh N, Varma SD, Söderberg P. Caffeine eye drops protect against UV-B cataract. Exp Eye Res 2013; 113: 26–31. doi: doi:10.1016/j.exer.2013.04.015

[105] Varma SD, Hegde KR. Prevention of Oxidative Damage to Lens by Caffeine. J Ocul Pharmacol Ther 2010; 26: 73–78. doi: doi:10.1089/jop.2009.0097

[106] Varma SD, Hegde KR. Kynurenine-induced photo oxidative damage to lens in vitro: protective effect of caffeine. Mol Cell Biochem 2010; 340: 49–54. doi: doi:10.1007/s11010-010–0399-4

[107] Varma SD, Kovtun S, Hegde K. Effectiveness of topical caffeine in cataract prevention: Studies with galactose cataract. Mol Vis 2010; 16: 2626–2633

[108] Srinivasan P, Smolke CD. Biosynthesis of medicinal tropane alkaloids in yeast. Nature 2020; 585: 614–619. doi: doi:10.1038/s41586-020–2650-9

[109] Srinivasan P, Smolke CD. Engineering cellular metabolite transport for biosynthesis of computationally predicted tropane alkaloid derivatives in yeast. Proc Natl Acad Sci U S A 2021; 118: e2104460118. doi: doi:10.1073/pnas.2104460118

[110] Galanie S, Smolke CD. Optimization of yeast-based production of medicinal protoberberine alkaloids. Microb Cell Fact 2015; 14: 144. doi: doi:10.1186/s12934-015–0332-3

[111] Han J, Li S. De novo biosynthesis of berberine and halogenated benzylisoquinoline alkaloids in Saccharomyces cerevisiae. Commun Chem 2023; 6: 27. doi: doi:10.1038/s42004-023–00821-9

[112] McCoy E, O'Connor SE. Natural products from plant cell cultures. In: Petersen F, Amstutz R, Hrsg. Natural Compounds as Drugs Volume I. Basel: Birkhäuser; 2008: 329–370

[113] Furusaki S, Takeda T. 2.36 – Bioreactors for Plant Cell Culture☆. In: Moo-Young M, Hrsg. Comprehensive Biotechnology (Third Edition). Oxford: Pergamon; 2017: 519–530

[114] Zhang F, Qiu F, Zeng J, Xu Z, Tang Y, Zhao T, Gou Y, Su F, Wang S, Sun X, Xue Z, Wang W, Yang C, Zeng L, Lan X, Chen M, Zhou J, Liao Z. Revealing evolution of tropane alkaloid biosynthesis by analyzing two genomes in the Solanaceae family. Nat Commun 2023; 14: 1446. doi: doi:10.1038/s41467-023–37133-4

[115] Gong H, He P, Lan X, Zeng L, Liao Z. Biotechnological Approaches on Engineering Medicinal Tropane Alkaloid Production in Plants. Front Plant Sci 2022; 13: 924413. doi: doi:10.3389/fpls.2022.924413

[116] Humbal A, Pathak B. Influence of exogenous elicitors on the production of secondary metabolite in plants: A review ("VSI: secondary metabolites"). Plant Stress 2023; 8: 100166. doi: doi:10.1016/j.stress.2023.100166

[117] Li W, Jiang R, Zhu Y, Zhou J, Cui C. Effect of 0.01% atropine eye drops on choroidal thickness in myopic children. J Fr Ophtalmol 2020; 43: 862–868. doi: doi:10.1016/j.jfo.2020.04.023

[118] Ye L, Shi Y, Yin Y, Li S, He J, Zhu J, Xu X. Effects of Atropine Treatment on Choroidal Thickness in Myopic Children. Invest Ophthalmol Vis Sci 2020; 61: 15. doi: doi:10.1167/iovs.61.14.15

[119] Jiang Y, Zhang Z, Wu Z, Sun S, Fu Y, Ke B. Change and Recovery of Choroid Thickness after Short-term Application of 1% Atropine Gel and Its Influencing Factors in 6–7-year-old Children. Curr Eye Res 2021; 46: 1171–1177. doi: doi:10.1080/02713683.2020.1863431

[120] Mathis U, Feldkaemper MP, Schaeffel F. Effects of Single and Repeated Intravitreal Applications of Atropine on Choroidal Thickness in Alert Chickens. Ophthalmic Res 2021; 64: 664–674. doi: doi:10.1159/000515755

[121] Zhou Y, Zhu Y, Huang XB, Xiong YJ, Guo YL, Cai Q, Wang M, Gong YX, Cao X, Li JJ, Cai JR, Song Y, Sun ZM. Changes of Choroidal Thickness in Children after Short-erm Application of 1% Atropine Gel. Ophthalmic Res 2022; doi: doi:10.1159/000526448