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Original Contribution

POTENTIAL STRATEGIES AND APPROACHES FOR ENHANCING THE BIOAVAILABILITY OF NATURAL ALKALOIDS IN GLAUCOMA TREATMENT

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ABSTRACT

INTRODUCTION: Glaucoma is one of the leading causes of irreversible blindness worldwide, with effective management relying on early detection and reduction of intraocular pressure (IOP). Despite therapeutic advances, there is a lack of natural-origin bioproducts with proven safety profiles on the market. Alkaloids, as naturally occurring molecules with low toxicity, represent a potential avenue for enhancing glaucoma treatment options.

AIM: To evaluate the efficacy and selectivity of the natural alkaloid pilocarpine through molecular docking analysis against major ocular targets, with the aim of exploring opportunities for the development of new bioproducts.

METHODS: Molecular docking of pilocarpine was performed against the muscarinic M_3 receptor, acetylcholinesterase (AChE), carbonic anhydrase II (CAII), prostaglandin D synthase (PTGDS), and the ABCG2 transporter. A protein-protein interaction network analysis using STRING was also conducted to assess functional relationships.

RESULTS: The analysis revealed a strong affinity of pilocarpine for the M_3 receptor and no significant interactions with other targets, emphasizing its specificity and potential for safe application. Network data highlighted the multisystem nature of glaucoma and the need for multitarget therapeutic strategies.

CONCLUSION: The findings underscore the potential of natural alkaloids in glaucoma therapy and the importance of a multidisciplinary approach. Given the high social burden of the disease, strategic involvement of optometrists in primary screening and prevention efforts is critical for early detection and effective risk management of blindness.

Keywords: pilocarpine, molecular docking, optometric practice, multitarget therapy

INTRODUCTION

Glaucoma is a progressive optic neuropathy leading to degeneration of retinal ganglion cells and irreversible vision loss (1). By 2020, it affected approximately 79.6 million individuals globally (2) and remains the second leading cause of blindness worldwide (3). More than 70% of cases remain undiagnosed due to the asymptomatic onset of primary open-angle glaucoma (4). Elevated intraocular pressure (IOP) is a major risk factor, damaging the optic nerve head. Early detection is crucial to prevent disease progression and preserve vision (5).

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Current glaucoma treatment aims to lower IOP through five major classes of medications, with prostaglandin analogues and β-blockers considered first-line therapies (6). Pilocarpine, a cholinergic miotic agent, reduces IOP by increasing trabecular outflow. Due to its shorter duration of action and side effects, it is now mainly used in acute situations or as part of combination therapy.

Glaucoma requires lifelong monitoring and often combined therapy targeting various structural and molecular pathways in the eye. In the present study, pilocarpine—a natural alkaloid used as a miotic agent—is analysed for its interactions with key ocular protein targets, including muscarinic receptors, acetylcholinesterase (AChE), carbonic

anhydrase II (CAII), prostaglandin D synthase (PTGDS), and the ABCG2 transporter. Through molecular docking and network analysis (STRING-DB), we emphasize the multifactorial nature of glaucoma and the necessity for early therapeutic intervention.
In this context, special attention is given to the role of optometrists as key figures in early detection, prevention, and long-term monitoring of glaucoma patients.

AIM

To provide an integrated scientific overview of glaucoma as a major public health concern and pilocarpine as a therapeutic agent, covering pathogenesis, mechanisms of action, molecular drug targets (via docking analysis), and the role of optometrists in early diagnosis and patient care.

MATERIALS AND METHODS

Documentary Method: A literature review was conducted using PubMed, Scopus, and Web of Science databases, as well as official reports from organizations such as the WHO, focusing on the epidemiology, pathogenesis, and treatment of glaucoma. For pilocarpine, established sources such as DrugBank and pharmacological reviews were consulted regarding its mechanism of action.

Selection of Protein Targets: Based on the known pharmacology of glaucoma and pilocarpine, five targets were selected: the muscarinic acetylcholine receptor M_3 (CHRM3), acetylcholinesterase (AChE), carbonic anhydrase II (CAII), lipocalin-type prostaglandin D synthase (L-PGDS), and the ABCG2 transporter. M_3 is the primary receptor mediating pilocarpine's ocular effects; AChE is the enzyme that degrades acetylcholine, thus indirectly involved in cholinergic neurotransmission; CAII participates in aqueous humour production; PGDS is an enzyme whose intraocular fluid levels are known to alter in glaucoma (7); ABCG2 (Breast Cancer Resistance Protein) is an efflux transporter expressed in ocular tissues, potentially limiting drug penetration into the eye (8).

Molecular Docking: The 3D coordinates of pilocarpine were retrieved from PubChem (CID: 5910) and prepared with an appropriate protonation state (pilocarpine being a basic molecule bearing a protonated nitrogen at physiological pH). Docking was performed using AutoDock Vina (version 1.2.3) on the selected PDB structures. The docking grids were centred on the active site or the presumed binding pocket of each protein. Standard Vina parameters were applied (exhaustiveness = 8, num_modes = 9). Binding affinities (predicted free energy of binding, ΔG, in kcal/mol) and preferred ligand conformations were evaluated. The highest-affinity poses were visualized with PyMOL to identify key interactions with amino acid residues.

RESULTS

Pathogenesis of Glaucoma and Current Therapies: In primary open-angle glaucoma, elevated intraocular pressure (IOP) most often results from impaired outflow of aqueous humour through the trabecular meshwork and Schlemm's canal. Accumulation of fluid leads to increased hydrostatic pressure, which damages retinal ganglion cells and their axons at the lamina cribrosa, causing glaucomatous optic neuropathy characterized by thinning of the neuroretinal rim and enlargement of the optic disc cupping. Visual loss typically begins peripherally (scotomas) and progresses to tunnel vision and eventual blindness (9).

Recent findings also implicate a neurodegenerative component in glaucoma pathogenesis, involving inflammatory and ischemic processes affecting the retina and optic pathways, similar to other neurodegenerative diseases (10).

Several forms of glaucoma are distinguished: primary open-angle glaucoma (the most common), primary angle-closure glaucoma (acute or chronic), normal-tension glaucoma (with normal IOP but characteristic optic nerve damage), and secondary glaucomas due to other diseases or corticosteroid use.

Treatment: All current therapies aim to lower IOP, as optic nerve damage is considered irreversible. Pharmacologic reduction of IOP is achieved through: (1) decreasing aqueous humour production—using β-blockers (e.g., timolol), carbonic anhydrase inhibitors (e.g., dorzolamide, acetazolamide), and α_2-adrenergic agonists (e.g., brimonidine); or (2) increasing aqueous humour outflow—using prostaglandin analogues (e.g., latanoprost, bimatoprost, which enhance uveoscleral outflow) and cholinergic agonists (e.g., pilocarpine, which enhances trabecular outflow).

The five main classes of anti-glaucoma agents are prostaglandins, β-blockers, carbonic anhydrase inhibitors (CAI), α-agonists, and

cholinergic miotics. **Table 1** summarizes the mechanisms of action and examples of each class.

When monotherapy is insufficient, combination therapy from different classes is often employed to target multiple pathophysiological pathways simultaneously. If pharmacologic control fails, laser procedures (trabeculoplasty, iridotomy) or surgical interventions (trabeculectomy, implantation of drainage devices) are utilized to achieve sustained IOP reduction.

Table 1. *Classes of Antiglaucoma Medications and Their Mechanisms of Action*

Class	Mechanism of Action	Examples
Prostaglandin analogs	Increase uveoscleral outflow	Latanoprost, Bimatoprost
β-blockers	Decrease aqueous humor production	Timolol, Betaxolol
Carbonic anhydrase inhibitors (CAI)	Decrease aqueous humor production	Dorzolamide, Acetazolamide
α_2-adrenergic agonists	Decrease aqueous humor production and increase uveoscleral outflow	Brimonidine
Cholinergic miotics	Increase trabecular outflow through ciliary muscle contraction	**Pilocarpine**

Early Prevention: Early detection of glaucoma—before irreversible changes occur—is critically important. This requires regular measurement of intraocular pressure (IOP), examination of the optic nerve head, and assessment of the visual field in individuals at risk (age > 50 years, family history of glaucoma, myopia, Afro- or Asian ethnicity, among others).

In many countries, optometrists play a key role as the first point of contact, often performing glaucoma screening during routine eye examinations, including tonometry, fundus evaluation, and optical coherence tomography (OCT) of the optic nerve. Early identification of glaucomatous optic neuropathy allows timely referral to an ophthalmologist for initiation of therapy, significantly improving the prognosis for vision preservation (11).

Pilocarpine – Origin, Pharmacology, and Effect in Glaucoma: Pilocarpine is a natural alkaloid isolated from the leaves of Pilocarpus (jaborandi), with a molecular weight of approximately 208 Da. It acts as a partial agonist at muscarinic M_1 and M_2 receptors and as a full or partial agonist at M_3 receptors depending on the tissue (12). M_3 receptors couple to G_q proteins, activating phospholipase C and inducing calcium release in smooth muscle cells. In the eye, pilocarpine induces miosis and accommodative spasm, leading to stretching of the trabecular meshwork and enhanced aqueous humour outflow, thereby reducing intraocular pressure **(Table 1**). The effect occurs rapidly (within 1 hour) and lasts for 4–8 hours, requiring administration 3–4 times daily.

Clinical Use: Pilocarpine was a mainstay in the treatment of chronic glaucoma until the introduction of β-blockers and prostaglandin analogues. Today, it is primarily used in acute angle-closure crises, where its miotic action helps open the anterior chamber angle. It is also applied in some forms of secondary glaucoma and occasionally as an adjunct therapy in resistant cases of open-angle glaucoma. Side effects such as headache, blurred vision, and night vision difficulties (hemeralopia) limit its long-term use. Nevertheless, pilocarpine remains a valuable example of a natural therapeutic agent targeting ocular hydrodynamics by mimicking acetylcholine action.

Structural Data: For each target, an appropriate 3D structure was selected from the Protein Data Bank (PDB). The structures used include:

- **Muscarinic receptor M_3:** PDB ID 4DAJ – crystal structure of the rat M_3 receptor bound to the antagonist tiotropium (resolution 3.4 Å);
- **Acetylcholinesterase (AChE):** PDB ID 4EY7 – human AChE crystal structure in complex with donepezil (2.35 Å), serving as a model for the active site;
- **Carbonic anhydrase II (CAII):** PDB ID 3KS3 – high-resolution (0.9 Å) human CAII

structure, providing a detailed view of the catalytic zinc centre;
- **Lipocalin-type prostaglandin D synthase (L-PGDS):** PDB ID 3O22 – human L-PGDS crystal structure bound to a natural ligand (fatty acid) at 1.4 Å;
- **ABCG2 transporter:** PDB ID 6HBU – cryo-EM structure of human ABCG2 (mutant variant E211Q) in the ATP-bound state (3.1 Å), modelling the substrate/inhibitor binding conformation in the transmembrane domain.

Muscarinic Receptors and Muscarinic Targets: Muscarinic receptors (M_1–M_5) are widely expressed, with M_3 predominantly found in the iris and ciliary muscle. Pilocarpine exhibits affinity for all subtypes, but its ophthalmic effects are primarily mediated through M_3, inducing miosis and accommodative spasm. Studies have shown that under certain conditions, pilocarpine may also act as an antagonist due to its partial agonism and cell-specific context (13). Nevertheless, in the eye, it behaves as a classical parasympathomimetic agent with a hypotensive effect.

Other Potential Targets of Pilocarpine: Beyond muscarinic receptors, pilocarpine can weakly inhibit acetylcholinesterase at high in vitro concentrations (14), although this effect is clinically insignificant at therapeutic doses. Pilocarpine does not directly affect carbonic anhydrase II, as it lacks the sulfonamide group necessary for binding to Zn^{2+}. There is no evidence of direct interaction with prostaglandin D synthase or the ABCG2 transporter, although ABCG2 may play a role in limiting pilocarpine's ocular absorption (15, 16). Subsequent sections present in silico docking analyses of these potential interactions.

Molecular Docking of Pilocarpine to Selected Targets

Binding Affinity: Molecular docking analysis predicted stable binding of pilocarpine within the muscarinic receptor binding pocket, while its affinity for the other targets was significantly lower. A summary of the calculated free binding energies (ΔG) is presented in **Table 2.** The highest predicted binding affinity was observed for the M_3 receptor (ΔG ≈ –9.2 kcal/mol), indicating a high specificity of pilocarpine for the muscarinic site. Binding to AChE was weaker (ΔG ≈ –7.0 kcal/mol), and the affinity for the other three targets was very low (in the range of –5 to –6 kcal/mol). These results are consistent with the known pharmacology of pilocarpine, which acts as a selective agonist for muscarinic receptors and does not exert significant direct effects on enzymes such as AChE or CAII in vivo.

Table 2. *Docking Results – Predicted Binding Affinities of Pilocarpine to Different Targets*

Protein (PDB)	ΔG Docking (kcal/mol)	Key Interactions
M_2 mACh receptor (4DAJ)	–9.2	Ionic pair with $Asp^{3.32}$; π-stacking with Trp/Tyr in TM6
Acetylcholinesterase (4EY7)	–7.0	π-stacking with Trp in the active site gorge; hydrogen bonds with catalytic Glu
Carbonic anhydrase II (3KS3)	–5.4	Van der Waals interactions near the entrance; no coordination to Zn^{2+}
L-Prostaglandin D synthase (3O22)	–5.8	Weak hydrophobic contact in the β-barrel cavity; hydrogen bonding with Ser/Thr
ABCG2 transporter (6HBU)	–6.1	Potential binding in the transmembrane pocket; π-stacking with Phe/Tyr in the hydrophobic cavity

Interactions within the Muscarinic Receptor: The docking pose of pilocarpine within the orthosteric binding site of the M_3 receptor (modelled based on the 4DAJ structure) shows that the positively charged nitrogen of pilocarpine forms an ionic bond with the conserved aspartate in transmembrane segment 3 (Asp^3.32 — a key anionic residue that, across all mAChRs, attracts the cationic head of acetylcholine).

The imidazole ring of pilocarpine engages in π-π stacking interactions with aromatic residues within the binding pocket—likely Trp and Tyr in TM6/TM7—similar to observations in the M_1 receptor, where docking studies revealed π-

stacking between pilocarpine's imidazole and Trp-378 and Tyr-381 (TM6).

Figures 1C and 1D illustrate these interactions (shown for the closely related M_1 receptor as a model). It is evident that pilocarpine forms hydrogen bonds with residues such as Tyr-106 (TM3) and Ser-109, further stabilizing the ligand's binding position.

The combination of the ionic pair and multiple hydrophobic/aromatic contacts explains the high binding affinity, correlating well with the pharmacological effect—strong and specific activation of the muscarinic receptor.

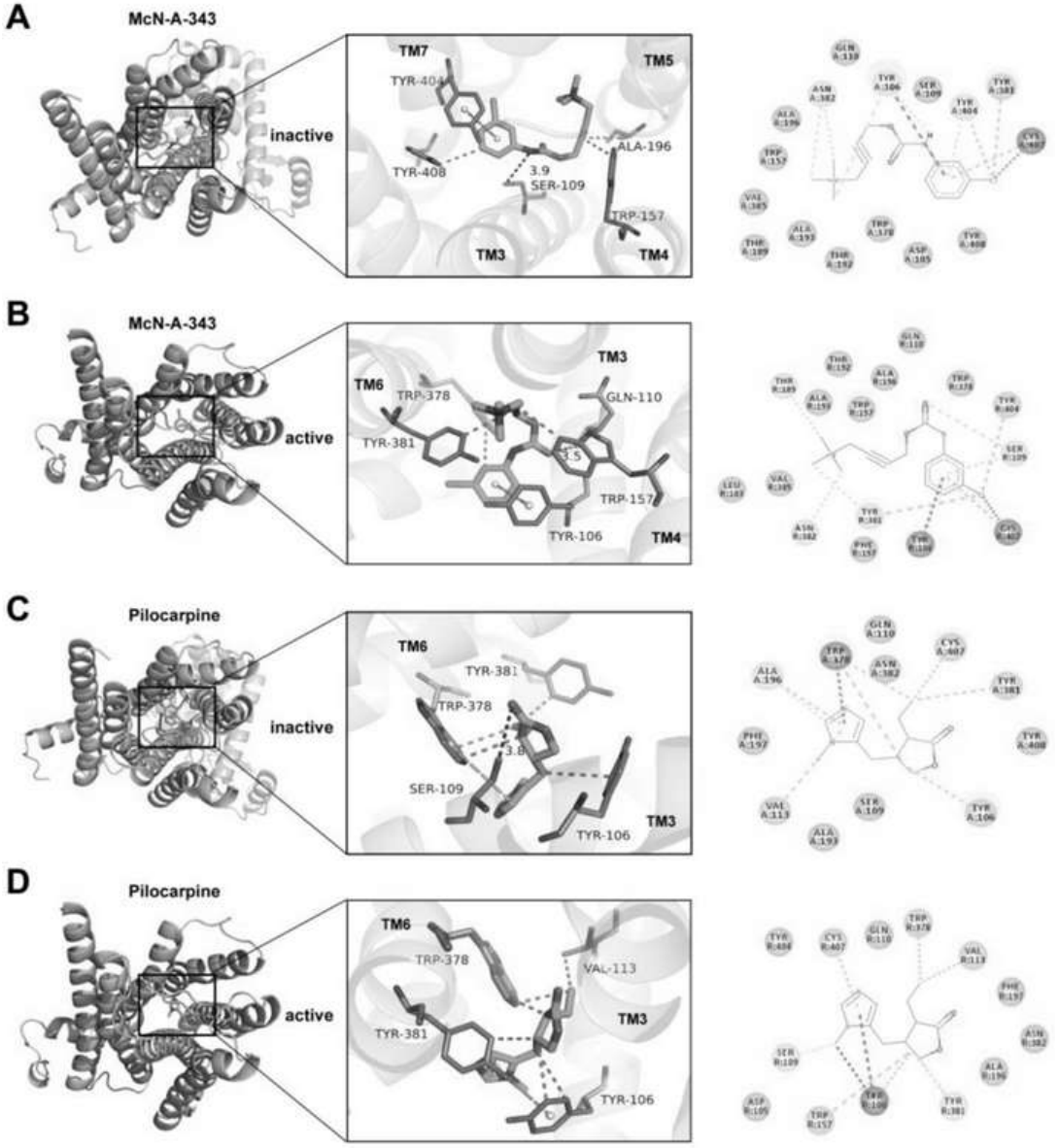

Figure 1. Docking of agonists into the muscarinic receptor (M_1) – comparison between pilocarpine and a reference ligand. *Source: modified based on data from Docking, IJMS 2023.*

Binding to Acetylcholinesterase: The active site of acetylcholinesterase (AChE) is a deep hydrophobic gorge, at the bottom of which lie the catalytic triad (Ser, His, Glu) and the anionic subsite that accommodates the quaternary nitrogen of acetylcholine.

Docking analysis of pilocarpine showed that the molecule is positioned in the upper part of this gorge, without fully reaching the catalytic serine. Instead, pilocarpine is stabilized through π-stacking interactions with aromatic residues (e.g., Trp-86 and Tyr-337 in human AChE, corresponding to the peripheral and anionic sites) and forms a hydrogen bond with Glu-202 (part of the catalytic Glu-His-Ser triad). Due to the lack of strong specific interactions (unlike typical AChE inhibitors such as neostigmine, which covalently modifies Ser, or donepezil, which fits tightly into the gorge),

pilocarpine exhibits only weak affinity. This correlates with in vitro observations showing that pilocarpine is a relatively weak and reversible cholinesterase inhibitor (17). Thus, the docking results support the conclusion that pilocarpine does not exert significant anticholinesterase effects at therapeutic concentrations, and its primary mechanism of action remains direct stimulation of the muscarinic receptor.

Binding to Carbonic Anhydrase II: Pilocarpine does not significantly inhibit carbonic anhydrase II (CAII), as it lacks the sulfonamide group required for binding to Zn^{2+} in the active site. During docking, pilocarpine remained close to the entrance of the active site pocket, forming only weak interactions without reaching the zinc ion (ΔG –5.4 kcal/mol). This explains why pilocarpine does not reduce aqueous humour production and can be effectively combined with carbonic anhydrase inhibitors to achieve complementary therapeutic effects.

Binding to Prostaglandin D Synthase (L-PGDS): L-PGDS functions both as an enzyme and a transport protein in the eye. Docking analysis showed that pilocarpine binds weakly to its β-barrel binding site (ΔG –5.8 kcal/mol), forming only limited interactions. This suggests that pilocarpine likely does not directly influence L-PGDS function (18). Although L-PGDS levels are elevated in glaucoma, the interaction between pilocarpine and L-PGDS appears to be clinically insignificant.

Binding to the ABCG2 Transporter: ABCG2 is a transporter expressed in barrier tissues of the eye, limiting the penetration of substances through efflux mechanisms (19). Docking analysis indicated that pilocarpine can bind weakly to the multisubstrate binding pocket of ABCG2 (binding affinity ≈ –6 kcal/mol), but it is unlikely to be a high-affinity substrate (20).

While efflux activity may slightly reduce local bioavailability, this effect is clinically negligible when pilocarpine is administered topically via eye drops.

Network Interactions among Targets (STRING Analysis): To explore the functional relationships among the selected proteins (CHRM3, ACHE, CA2, PTGDS, ABCG2), a network analysis was performed using STRING.

The results showed that although direct interactions between these proteins are absent, they are functionally linked through common biological processes (21).

The muscarinic M_3 receptor (CHRM3) clusters with other cholinergic receptors and Gq-coupled proteins. Acetylcholinesterase (ACHE) is associated with CHRM3 through the common mediator, acetylcholine.

Carbonic anhydrase II (CA2) is involved in fluid secretion, PTGDS is related to prostaglandin signalling, and ABCG2 is associated with drug transport.

Network analysis highlights the multisystem nature of glaucoma, involving neurotransmission, intraocular pressure regulation, inflammatory pathways, and drug pharmacokinetics.

This supports the notion that glaucoma therapy, although often targeting a single receptor, requires a complex and systemic approach.

DISCUSSION

Multimodality of Therapy and Multiple Targets: Docking analysis confirmed the high specificity of pilocarpine for muscarinic receptors and the absence of significant interactions with other targets. This reflects the current trend toward highly selective antiglaucoma medications, such as prostaglandin analogues (targeting FP receptors) and β-blockers (targeting β_2 receptors). Nevertheless, the multifactorial nature of glaucoma often necessitates combination therapy. Pilocarpine is rarely used as monotherapy today but remains part of triple-combination therapies for refractory cases, with each agent targeting a different pathway. This supports the concept of multitarget therapy in glaucoma management.

Role of Natural Compounds: Pilocarpine is among the first natural alkaloids successfully introduced into ophthalmology, with its discovery linked to ethnopharmacological practices in Brazil. Observations of the jaborandi plant guided researchers to the active compound. Today, interest in natural products for glaucoma therapy continues, with pilocarpine serving as a benchmark for the search for new molecules. Despite efforts to

develop superior analogues with longer duration of action, no approved substitutes have been introduced so far.

Functional Implications of Docking Results: Docking analysis provides insights into pilocarpine's pharmacodynamics. Its interaction with Trp and Tyr residues in the muscarinic receptor aligns with mutagenesis studies (22). The weak binding to AChE explains the lack of toxic cholinergic side effects. Pilocarpine does not interact with CAII, supporting the rationale for its combination with carbonic anhydrase inhibitors (23).While no direct interaction was observed with PGDS, future investigations may explore its potential role in neuroprotection. ABCG2 highlights the importance of efflux pumps in the eye and suggests potential strategies to improve ocular bioavailability of topical formulations.

Limitations of the Study: Molecular docking analysis has inherent limitations, as it operates with fixed protein structures and does not fully account for protein dynamics or ligand solubility. Molecular dynamics simulations and experimental in vitro/in vivo validations were not performed. Nevertheless, the obtained results are consistent with available experimental data, supporting the reliability of the analysis.

Future Directions: An interesting extension of this work would be the investigation of new muscarinic agonists with higher ocular selectivity—such as M_3-selective agents—which could minimize systemic side effects. Additionally, combining IOP-lowering therapies with neuroprotective strategies (e.g., NMDA antagonists, antioxidants) is an emerging field, as lowering IOP alone does not always halt disease progression (especially in normal-tension glaucoma).

In this context, understanding network interactions (as visualized via STRING analysis) could highlight novel therapeutic targets: for example, modulation of neuroinflammation (prostaglandin pathways) or improvement of axonal transport and blood flow to the optic nerve.

Role of the Optometrist in Glaucoma Care: Optometrists play a crucial role in screening, prevention, and early detection of glaucoma. Through regular examinations and tests such as tonometry, ophthalmoscopy, and optical coherence tomography (OCT), they can identify early signs of the disease. For individuals over 50 years of age, preventive eye examinations are recommended every 1–2 years, and even more frequently for high-risk patients (24). Upon suspicion of glaucoma, the optometrist refers the patient to an ophthalmologist for definitive diagnosis and initiation of treatment.

The Role of Optometrists in Glaucoma Management: Optometrists are actively involved in the follow-up of glaucoma treatment by monitoring IOP control, assessing side effects, and ensuring therapeutic adherence.

When necessary, they refer patients for adjustments in therapy. In some countries, optometrists even manage stable glaucoma cases by prescribing repeat medications and reporting changes to ophthalmologists, thereby improving access to care, particularly in remote areas. Optometrists also fulfil an important educational role by teaching patients the correct technique for administering eye drops and emphasizing the importance of therapeutic compliance. They provide lifestyle advice and offer psychological support, reassuring patients that with timely treatment, vision can often be preserved.

In summary, the role of optometrists is multifaceted: encompassing primary prevention and early detection, treatment monitoring, and providing both psychological and informational support.

In an era of aging populations and increasing glaucoma prevalence, integrating optometrists into patient care is essential to reduce the societal burden of glaucoma-related blindness (25).

As "guardians of vision" within the community, optometrists contribute to relieving pressure on specialized care services and ensuring the timely management of the growing number of patients with chronic ocular diseases.

CONCLUSION

Glaucoma remains a leading cause of irreversible blindness, with its management centred on controlling intraocular pressure. Pilocarpine serves as a classical example of the successful use of a natural alkaloid, improving aqueous humour outflow through M_3 receptor activation and lowering intraocular pressure, thereby preserving vision for generations of patients. Molecular docking analysis confirmed

pilocarpine's selectivity for the muscarinic receptor and the absence of significant binding to other targets. This finding explains the need for combination therapy to achieve comprehensive glaucoma control. Network analysis highlighted that glaucoma involves multiple biological systems, necessitating a multidisciplinary therapeutic approach.

Optometrists play a pivotal role in the early detection and follow-up of glaucoma, contributing to vision preservation through timely diagnosis and patient education. The integration of molecular understanding of therapeutic targets with clinical practice forms the foundation for successfully combating the "silent thief of sight."

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