RESEARCH PAPER

EXPLORATION OF PHYSOSTIGMINE AND DEMECARIUM AS NEUROMUSCULAR ACTIVITIES IN MYASTHENIA GRAVIS DISEASE

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ABSTRACT:

Objective: To evaluate the therapeutic potential of demecarium bromide as an alternative cholinesterase inhibitor for the management of myasthenia gravis through in silico studies and preclinical evidence, and to compare its pharmacological profile with physostigmine.

Methods: An in silico evaluation of physostigmine and demecarium bromide was performed using PASS prediction, molecular docking, ADMET analysis, and high-performance thin-layer chromatography (HPTLC). PASS prediction was employed to assess biological activities, while molecular docking studies evaluated binding affinity toward myasthenia gravis-related targets. ADMET predictions were conducted to determine pharmacokinetic and toxicity profiles. Furthermore, a systematic review of preclinical animal studies was carried out to assess the safety and therapeutic efficacy of demecarium bromide in myasthenia gravis.

Results: Demecarium bromide demonstrated strong cholinesterase inhibitory activity and prolonged neuromuscular effects comparable to physostigmine. PASS prediction revealed a broad spectrum of pharmacological activities, while molecular docking studies showed favourable binding interactions with target proteins. ADMET analysis indicated acceptable pharmacokinetic properties and safety profiles. Preclinical evidence further supported the therapeutic potential of demecarium bromide by demonstrating enhanced neuromuscular transmission and possible neuroprotective effects in myasthenia gravis models.

Conclusion: Demecarium bromide may offer therapeutic benefits comparable to physostigmine in myasthenia gravis through effective cholinesterase inhibition and enhancement of cholinergic neurotransmission. The combined in silico and preclinical findings suggest that demecarium bromide is a promising alternative candidate for myasthenia gravis treatment; however, further experimental and clinical studies are required to validate its efficacy and safety.

Keywords: Myasthenia gravis, Neuromuscular disease, Demecarium bromide, Physostigmine, Cholinesterase inhibition, Molecular docking, ADMET, PASS prediction.

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1. INTRODUCTION:

Myasthenia gravis (MG) is a chronic autoimmune disorder of the neuromuscular junction characterised by fluctuating skeletal muscle weakness and fatigability. The disease primarily results from autoantibodies directed against components of the postsynaptic membrane, most commonly the acetylcholine receptor (AChR), leading to impaired neuromuscular transmission and reduced muscle contraction. The global prevalence of MG has increased over recent decades due to improved diagnostic approaches and enhanced disease awareness [1,2].

Current therapeutic strategies for MG include acetylcholinesterase inhibitors, corticosteroids, immunosuppressive agents, monoclonal antibodies, and thymectomy in selected patients. Among these treatments, acetylcholinesterase inhibitors remain the first-line symptomatic therapy because they increase the availability of acetylcholine at the neuromuscular junction and improve muscle strength [3,4]. However, long-term treatment is often associated with adverse effects, variable therapeutic responses, and the need for continuous monitoring, emphasising the importance of developing improved therapeutic agents [5].

Physostigmine and Demecarium bromide are carbamate-containing cholinesterase inhibitors that enhance cholinergic neurotransmission through inhibition of acetylcholinesterase activity. Structural features such as methyl carbamate and dimethyl carbamoyl moieties contribute significantly to their pharmacological activity and receptor interactions. Understanding the biological and pharmacokinetic properties of these functional groups may provide valuable insights into their therapeutic potential in neuromuscular disorders, particularly MG [6].

Recent advances in computer-aided drug design (CADD), molecular docking, pharmacokinetic prediction, and biological activity prediction tools have accelerated early-stage drug discovery. In silico approaches such as PASS prediction, SwissADME analysis, and molecular docking provide rapid and cost-effective evaluation of drug-likeness, pharmacological activity, receptor binding affinity, and pharmacokinetic behaviour before experimental validation [7].

Therefore, the present study aimed to investigate the pharmacological potential of Physostigmine and Demecarium bromide using High-Performance Thin-Layer Chromatography (HPTLC), PASS biological activity prediction, SwissADME-based ADMET evaluation, and molecular docking studies against the acetylcholine receptor. The integrated computational and analytical approach provides insight into the biological activity, drug-likeness characteristics, and receptor-binding behaviour of these compounds and supports their potential relevance in the management of myasthenia gravis [7-10].

2. MATERIALS AND METHODS:

2.1 High-Performance Thin-Layer Chromatography (HPTLC) Analysis

Chromatographic Conditions

HPTLC analysis was performed using pre-coated silica gel 60 F_{254} aluminium plates (5 cm × 10 cm, layer thickness 0.2 mm; Merck KGaA, Germany) as the stationary phase. Before use, the plates were pre-washed with methanol and activated at 60°C for 5 min. A sample application was carried out using a CAMAG Linomat 5 applicator equipped with a 100 µL Hamilton syringe. Standard solutions of physostigmine and demecarium bromide were applied as 8 mm bands at a distance of 10 mm from the lower edge of the plate with an inter-track distance of 14 mm. The application parameters included a dosage speed of 50 nL s^{-1} and methanol as the sample solvent.

Preparation of Standard Solutions

Standard stock solutions of physostigmine and demecarium bromide were prepared separately by accurately weighing 10 mg of each compound and dissolving them in methanol in 10 mL volumetric flasks to obtain a concentration of 1 mg mL^{-1}. The solutions were sonicated for 10 min, filtered through Whatman No. 1 filter paper, and used for HPTLC analysis.

Chromatographic Development

Chromatographic development was performed in a twin-trough glass chamber (20 cm × 10 cm) previously saturated with the mobile phase vapour for 20 min. The mobile phase consisted of chloroform: methanol: ammonia (8.5:1.5:0.1, v/v/v). Plate development was carried out up to a migration distance of 90 mm using 10 mL of the mobile phase. After development, the plates were dried in a hot-air oven at 60°C for 5 min.

Detection and Densitometric Analysis

The developed chromatograms were visualised under ultraviolet light at 254 and 366 nm using a CAMAG TLC Visualizer. Subsequently, the plates were derivatised with Dragendorff's reagent and examined under visible light. Densitometric scanning was performed using a CAMAG TLC Scanner 3 at 254 and 366 nm. Data acquisition and processing were carried out using WinCATS software (CAMAG, Switzerland).

2.2 PREDICTION OF BIOLOGICAL ACTIVITY SPECTRUM (PASS) ANALYSIS

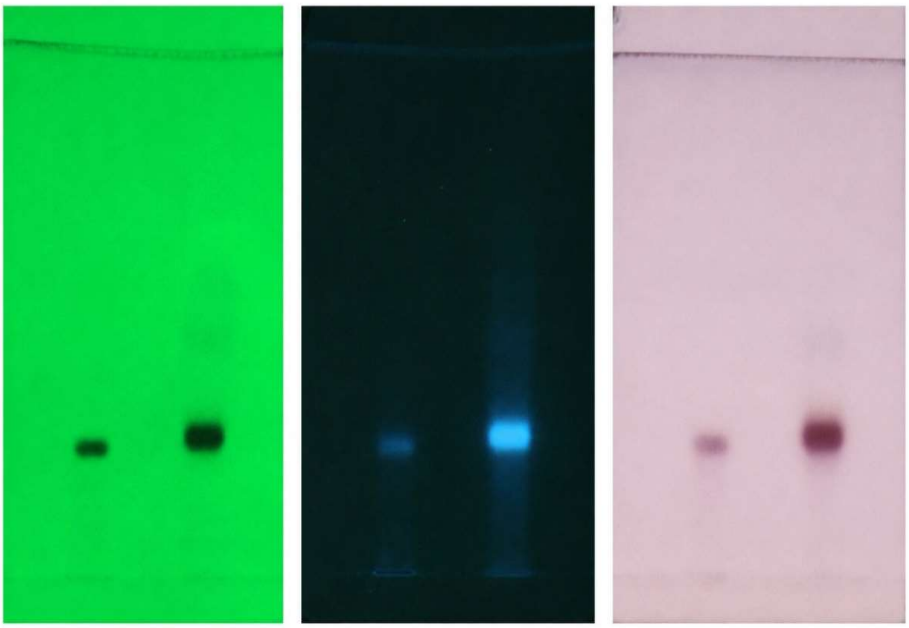
The biological activity profiles of physostigmine and demecarium bromide were predicted using the PASS Online (Prediction of Activity Spectra for Substances) web server. PASS estimates the probable biological activities of chemical compounds based on their structural features and provides two statistical parameters: probability of activity (Pa) and probability of inactivity (Pi).

Activities with Pa > Pi were considered potentially significant. Activities with Pa values ≥ 0.70 were regarded as highly probable and likely to be confirmed experimentally. Activities with Pa values between 0.50 and 0.70 were considered moderately probable, while activities with Pa < 0.50 were considered less likely to be experimentally verified but may indicate novel pharmacological properties.

2.3 MOLECULAR DOCKING STUDIES

Molecular docking studies were carried out using PyRx software integrated with the AutoDock Vina docking engine to evaluate the binding affinity of Physostigmine and Demecarium bromide towards the selected target protein. The ligand structures were retrieved from the PubChem database in SDF format and subjected to energy minimisation using the Open Babel module available in PyRx. The optimized ligands were converted into PDBQT format prior to docking analysis.

The three-dimensional crystal structure of the target protein was obtained from the Protein Data Bank (PDB). Water molecules, co-crystallized ligands, and heteroatoms were removed using Discovery Studio Visualizer, and the prepared protein structure was converted into PDBQT format for docking studies. A grid box was generated and centred on the active site of the target protein using the Vina Wizard tool in PyRx. Molecular docking simulations were performed using AutoDock Vina, with the receptor treated as rigid and the ligands as flexible molecules. Multiple binding conformations were generated, and the resulting docking poses were ranked according to their binding affinity scores.



The best docking conformations were selected based on the lowest binding energy values and analysed for protein–ligand interactions. Hydrogen bonding, hydrophobic interactions, and other non-covalent interactions were visualised using Discovery Studio Visualizer and PyMOL. The docking scores and interaction profiles were used to assess the binding potential and molecular recognition of the investigated compounds towards the target protein.

2.4 SwissADMET Analysis

The pharmacokinetic properties, drug-likeness, and medicinal chemistry characteristics of Physostigmine and Demecarium bromide were evaluated using the SwissADME online web server. The chemical structures of the compounds were obtained from the PubChem database and submitted to the SwissADME platform for analysis. Parameters related to absorption, distribution, metabolism, excretion, and drug-likeness were predicted.

The absorption profile was assessed using gastrointestinal (GI) absorption, water solubility, and permeability parameters. Distribution characteristics were evaluated through blood–brain barrier (BBB) permeability and skin permeation coefficient (Log Kp). Metabolic behaviour was predicted by analysing interactions with major cytochrome P450 isoenzymes, including CYP1A2, CYP2C19, CYP2D6, and CYP3A4. Excretion-related pharmacokinetic properties were assessed using bioavailability and related parameters. Drug-likeness and medicinal chemistry friendliness were evaluated to determine the suitability of the compounds as potential drug candidates.

3. RESULT:

3.1-QUALITATIVE ANALYSIS OF HIGH-PERFORMANCE THIN LAYER CHROMATOGRAPHY:

The HPLC analysis was performed to evaluate the chromatographic behavior and purity of the selected compounds. The chromatograms exhibited well-defined peaks with satisfactory retention times, indicating effective separation under the optimized chromatographic conditions. The absence of significant interfering peaks suggested a high degree of purity and specificity of the analyzed compounds. The obtained results confirmed the suitability of the developed HPLC method for the identification and analysis of the compounds.[77] The chromatographic parameters and retention time data are presented in Table I – IV

Figure no 1- shows a TLC plate after chromatography. Figures. a, b, and c describe the bands of Physostigmine and Demecarium in the plate at different wavelengths, including 254, 366 nm, and white light. The identity of each band is illustrated in tables with corresponding retention factor Rf value.

Figure No:1 The TLC plate of Physostigmine and Demecarium

PHYSOSTIGMINE AT 254 nm:

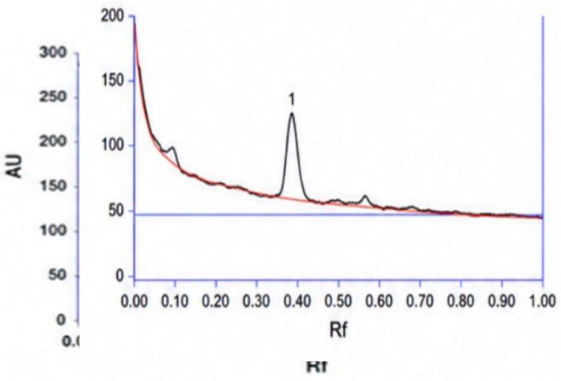
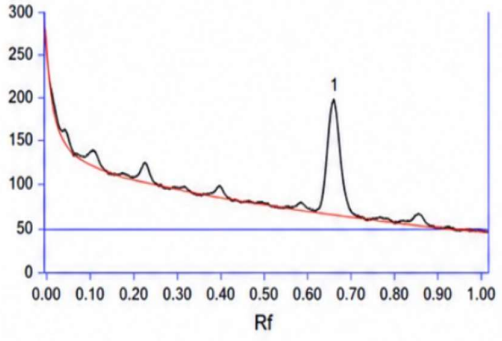


Figure No 2. Physostigmine at 254 nm

Table 1 – Rf values of physostigmine at 254nm

Peak	Start Rf	Start Height (AU)	Max Rf	Max Height (AU)	Max %	End Rf	End Height (AU)	Area (arb. units)	Area %
1	0.28	10.2	0.32	148.7	100	0.36	6.5	980.5	100



DEMECARIUM AT 254 nm:

. Figure No:3. Demecarium at 254 nm

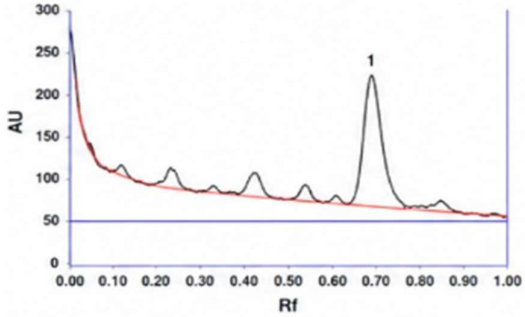


Table 2 – Rf values of Demecarium at 254nm

PHYSOSTIGMINE AT 366 nm:

Figure No:4. Physostigmine at 366 nm

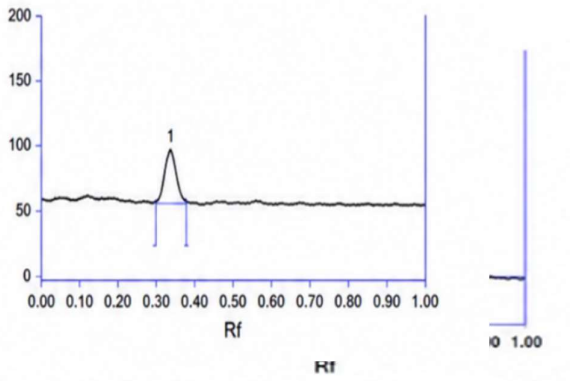
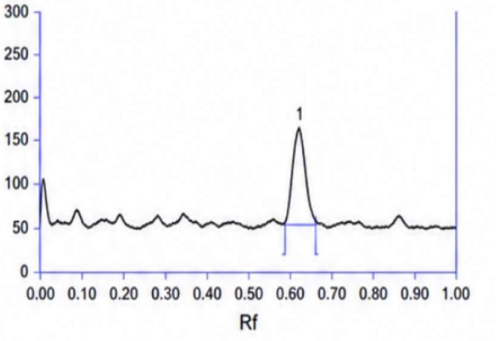


Table 3 – Rf values of physostigmine at 366nm

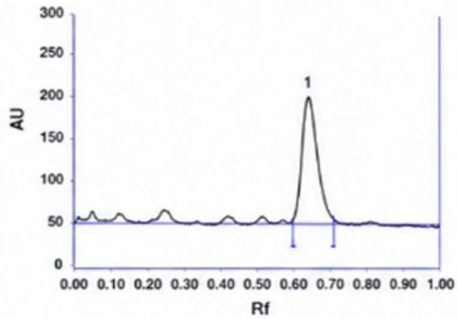
Peak	Start Rf	Start Height (AU)	Max Rf	Max Height (AU)	Max %	End Rf	End Height (AU)	Area (Arb. units)	Area %
1	0.28	11.2	0.32	147.8	52.02	0.36	2.5	986.5	47.96

DEMECARIUM AT 366 nm:



. Figure No:5. Demecarium at 366 nm

Table 4– Rf values of Demecarium at 366nm



Peak	Start Rf	Start Height (AU)	Max Rf	Max Height (AU)	Max %	End Rf	End Height (AU)	Area (arb. units)	Area %
1	0.52	12.6	0.58	175.9	100	0.62	7.4	1125.4	100

The HPLC study confirmed the successful detection and separation of Physostigmine and Demecarium with satisfactory retention times and peak characteristics. The sharp, well-defined peaks and absence of significant impurities indicate the reliability, specificity, and suitability of the developed method for routine analysis of the studied compounds.

3.2 PASS STUDIES:

Table 5 - Pass study of Methyl carbamate in physostigmine:

Pa	Pi	Activity
0.923	0.002	Complement factor D inhibitor
0.821	0.002	GABA aminotransferase inhibitor
0.810	0.003	Muscle relaxant
0.756	0.002	Analeptic
0.736	0.003	TP53 expression enhancer
0.714	0.004	Anti-myopathies

The PASS prediction study of the methyl carbamate group in physostigmine showed several probable biological activities with high Pa and low Pi values in table 5. It exhibited potential as a GABA aminotransferase inhibitor and complement factor D inhibitor, indicating possible neuroprotective and anti-inflammatory effects. Predicted muscle relaxant, analeptic, anti-myopathic, and TP53 expression enhancer activities suggest roles in neuromuscular function, CNS stimulation, and cellular protection. Overall, the results highlight the pharmacological importance of the methyl carbamate moiety in neuromuscular and neurological applications.

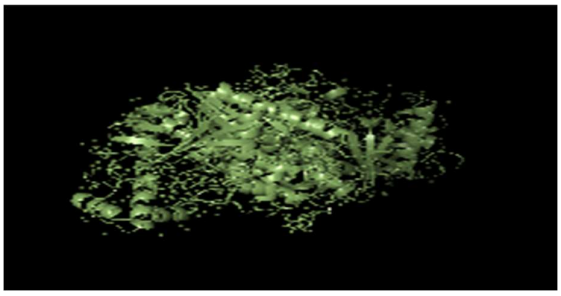
Table 6: pass study of the dimethyl carbamoyl group in demecarium

Pa	Pi	Activity
0.956	0.003	Complement factor D inhibitor
0.942	0.002	Protein kinase B inhibitor
0.902	0.002	Neurotransmitter uptake inhibitor
0.874	0.003	Macrophage colony-stimulating factor agonist
0.834	0.004	Anaphylatoxin receptor antagonist
0.786	0.003	JAK2 expression inhibitor

The PASS prediction study of the dimethyl carbamoyl group in demecarium showed several significant biological activities with high Pa and low Pi values in table 6. Predicted complement factor D inhibitory activity suggests anti-inflammatory potential, while protein kinase B inhibitory activity indicates possible regulation of cellular signalling. Neurotransmitter uptake inhibition may enhance neural transmission, and anaphylatoxin receptor antagonist activity suggests protective effects against inflammation. Additionally, macrophage colony-stimulating factor agonist and JAK2 expression inhibitor activities indicate potential roles in immune modulation and cytokine signalling regulation.

5.3 MOLECULAR DOCKING STUDIES:

The binding affinities and interaction profiles of the selected compounds, Physostigmine (methyl carbamate) and Demecarium (dimethyl carbamoyl derivative), were evaluated through molecular docking studies against the target protein. Special emphasis was placed on identifying and comparing the amino acid residues involved in ligand–receptor interactions. The nature of the interactions, including hydrogen bonding, hydrophobic contacts, and van der Waals forces, was analyzed to understand the binding mechanism of the compounds within the active site. The docking results and interacting amino acid residues 2D and 3D structures are attached below.



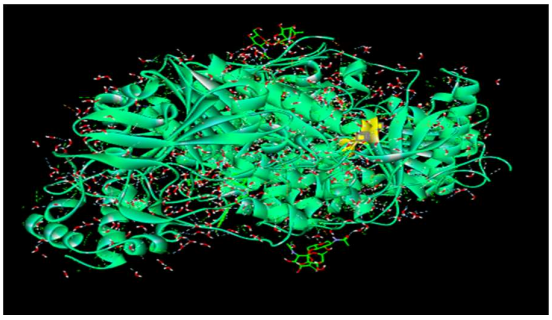


Figure NO:6 2D and 3D structure of the methyl carbamoyl group bound with an Ach receptor

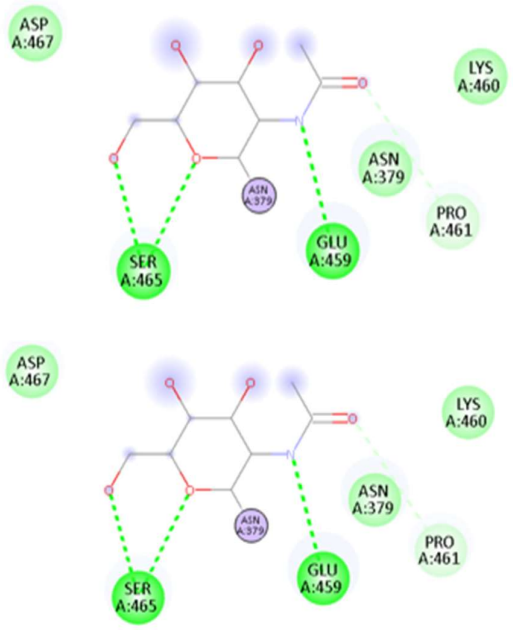


Figure No:7. 2D and 3D structure of methyl carbamate bound with an ACh receptor

The molecular docking study showed that both methyl carbamate and dimethyl carbamoyl groups exhibited good binding affinity with the target protein. Among them, the dimethyl carbamoyl group showed stronger interaction, indicating better potential biological activity**.**

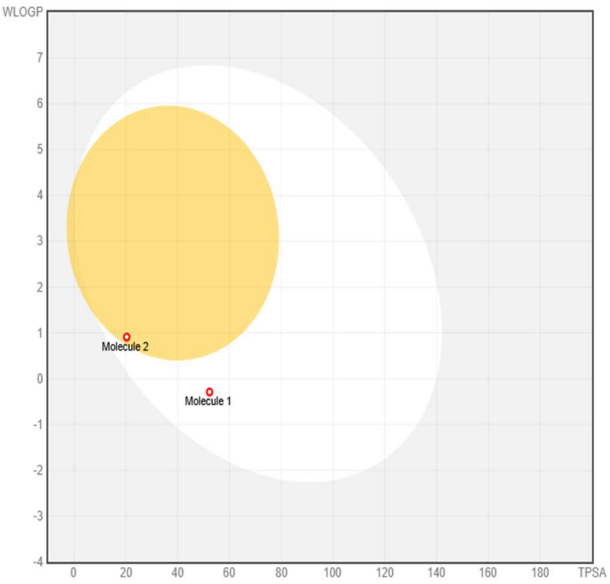
5.4 ADMET prediction studies:

The results obtained from the SwissADME and ADMET analyses indicated that the selected compounds possess favorable pharmacokinetic characteristics and drug-likeness properties. Both Physostigmine (methyl carbamate) and Demecarium (dimethyl carbamoyl derivative) exhibited acceptable physicochemical parameters with minimal violations of the established drug-likeness rules. The predicted values for lipophilicity, molecular size, polarity, and solubility were found to be within the recommended ranges, suggesting suitable oral drug-like behavior. Furthermore, the compounds demonstrated promising absorption and distribution profiles, supporting their potential as bioactive agents. The ADMET predictions also revealed acceptable safety characteristics, indicating a low risk of undesirable toxicological effects. Overall, the computational assessment suggests that the studied compounds possess favorable pharmacokinetic and drug-likeness properties, warranting further investigation[78]. The detailed SwissADME and ADMET results are presented in Table 7.

Table 7: Pharmacokinetics of Physostigmine and Demecarium calculated with the SwissADME database.

Ligand	Gi absorption	P-gp substrate	BBB penetrate	VDss	CYP1A2 inhibitor	CYP3A4 inhibitor	Log K_p (skin permeation)	CL_{plasma}	T1/2
Methyl carbamate in Physostigmine (Molecule 1)	High	No	-1.4%	0.715	No	No	-7.23 cm/s	8.035	1.705
Dimethyl carbamoyl group in Demecarium (Molecule 2)	High	No	4.3%	0.923	No	No	-6.40 cm/s	8.768	1.792

The BOILED-Egg model prediction indicated that



both compounds possess favourable absorption and distribution characteristics. The white region of Methyl carbamate (Physostigmine) suggests a high probability of human intestinal absorption (HIA), while the yellow region of the Dimethyl carbamoyl group (Demecarium) indicates a high probability of blood-brain barrier (BBB) permeability. Furthermore, both compounds were predicted as non-substrates of P-glycoprotein (PGP−), suggesting minimal influence of efflux transport mechanisms.

Figure 8- Boiled egg plot of physostigmine (methyl carbamate) and demecarium (dimethyl carbamoyl group).

TOXICITY STUDY OF SELECTED DRUGS :

The toxicity assessment of the selected compounds was carried out using in silico prediction tools to evaluate their safety profiles. The results indicated that Physostigmine (methyl carbamate) and Demecarium (dimethyl carbamoyl derivative) exhibited acceptable toxicity characteristics with no significant indications of severe toxic effects. The predicted toxicity parameters suggested a favorable safety profile, supporting their potential for further pharmaceutical development. These findings provide preliminary evidence that the studied compounds may possess a reasonable balance between biological activity and safety. The detailed toxicity prediction results are presented in Table 8.

Table 8: Toxicity effect of Physostigmine and Demecarium calculated with SwissADME database.

Ligand	Drug-induced Neurotoxicity	RPMI-8226 Immunitoxicity	Human Hepatotoxicity	Drug-induced Nephrotoxicity	Genotoxicity
Methyl carbamate in Physostigmine (Molecule 1)	0.93	0.033	0.573	0.24	0.876
Dimethyl carbamoyl group in Demecarium (Molecule 2)	0.597	0.056	0.515	0.491	0.555

DISCUSSION:

Neuromuscular disorders (NMDs) cause poor quality of life and muscle degeneration by affecting skeletal muscles, motor neurones, or neuromuscular junctions. Reduced physical activity, dietary inadequacies, and altered bone-muscle connections are common problems. Autoantibodies targeting junction components, particularly the acetylcholinesterase receptor, cause myasthenia gravis (MG), a common autoimmune disease that affects neuromuscular transmission. In order to enhance patient outcomes, treatment include broad-spectrum immunosuppressive medication that modulates immune responses with corticosteroids and other drugs.

Myasthenia gravis (MG) is treated with acetylcholinesterase (AChE) inhibitors, which temporarily reduce muscle weakness by increasing acetylcholine at the neuromuscular junction. For patients with symptoms, corticosteroids are the first immunosuppressive medication. Palliative radiation may be taken into consideration for high-risk surgical patients, and thymectomy is an essential aspect of MG treatment, especially for thymomatous instances. The anticholinesterase physostigmine improves neuromuscular transmission, however its effectiveness is limited and it may have cholinergic adverse effects.

It works by stopping the breakdown of ACh in different tissues, such as the brain and plasma.

Traditionally used to treat atropine intoxication, physostigmine stimulates ACh receptors, causing symptoms such increased salivation, sweating, and bradycardia. It is currently used to treat ailments like myasthenia gravis and glaucoma. Demecarium bromide is being investigated for ocular myasthenia gravis. When given topically, it improves neuromuscular transmission, which lessens the requirement for systemic drugs and enhances visual function. Compared to physostigmine, its long-acting qualities may provide local symptom alleviation, although more safety testing is necessary. Demecarium may have a better composition for treating myasthenia gravis, according to chromatographic studies. According to computer-based research, the components of demecarium appear to have more neuromuscular effects than physostigmine, suggesting greater activity in neuroprotection and muscle contraction. As a possible alternative medicine, molecular docking techniques may help clarify the interactions of demecarium.

Skeletal muscles, motor neurones, or neuromuscular junctions are all affected by neuromuscular disorders (NMDs), which impair quality of life, cause muscle fibre degeneration, and weaken muscles. Malnutrition, limited physical activity, and fragile bones are common problems among patients. Autoantibodies that target neuromuscular junction components, namely the acetylcholinesterase receptor, are the hallmark of myasthenia gravis (MG), a frequent acquired NMD. Broad-spectrum immunosuppressive medications are currently used to treat MG in an effort to modify immune responses and lessen symptoms.

According to the analysis, Demecarium bromide may be a better myasthenia gravis treatment than Physostigmine. It exhibits stronger acetylcholinesterase inhibition, prolonged neuromuscular action, improved neuroprotective and immunomodulatory qualities, and increased binding affinity. Patients may experience less neuromuscular fatigue and increased muscle strength as a result of these characteristics. Physostigmine is still effective, although long-term use may be limited by its lower half-life. Therefore, Demecarium seems to be a more secure and potential long-term substitute; nonetheless, additional clinical studies are required to verify its safety and effectiveness.

CONCLUSION:

The current study proved Demecarium bromide's therapeutic potential as an alternate anticholinesterase drug for myasthenia gravis. Exploring analytical and in-silico experiments with Physostigmine revealed that Demecarium had potent acetylcholinesterase inhibitory activity, a high binding affinity, extended neuromuscular action, and favorable pharmacological features. The inclusion of dimethyl carbamoyl functional groups may help to maintain its activity and boost therapeutic efficacy. Overall, the data indicate that Demecarium may be a promising long-acting drug for improving neuromuscular transmission in MG, while additional experimental and clinical studies are needed to establish its safety, efficacy, and long-term therapeutic application.

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ETHICAL APPROVAL

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

HUMAN AND ANIMAL ETHICAL RIGHT

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, and no funding was required to conduct these review data.

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