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Concentration-Response Curve for Acetylcholine (ACh) in the Absence and Presence of Atropine and Measurement of the Equilibrium Dissociation Constant (KB) Using Schild Analysis

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ABSTRACT

Introduction: Acetylcholine (ACh) is a fundamental neurotransmitter in the parasympathetic nervous system, mediating smooth muscle contraction through muscarinic receptors. Understanding its interaction with antagonists such as atropine provides insights into pharmacological modulation of smooth muscle activity.

Objective: The study aimed to evaluate the competitive interaction between ACh and atropine on muscarinic receptors in guinea pig ileum and to characterize the receptor-binding dynamics, particularly focusing on atropine's inhibitory effects.

Methodology: The research utilized isolated guinea pig ileum tissues, which were exposed to increasing concentrations of ACh in the absence and presence of atropine. Concentration-response curves were generated to determine receptor affinity and inhibitory effects. Equilibrium dissociation constants for both ACh and atropine were calculated to analyze the binding properties and competitive nature of the interaction.

Results: Atropine was confirmed as a competitive antagonist of muscarinic receptors, effectively reducing the contractile responses induced by ACh. The data demonstrated that atropine prevents ACh binding, thereby inhibiting smooth muscle contraction, as evidenced by significant shifts in concentration-response curves. These findings reaffirm atropine's role as a potent therapeutic agent for conditions involving cholinergic overactivity.

Keywords: Muscarinic receptor antagonists, acetylcholine, atropine, smooth muscle contraction, ileum.

INTRODUCTION

Acetylcholine (ACh) is an essential neurotransmitter responsible for several physiological processes, including smooth muscle contraction, heart rate modulation, and glandular secretion. These effects are mediated by

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muscarinic acetylcholine receptors (mAChRs), which are G protein-coupled receptors classified into five subtypes (M1-M5). Among these, the M3 subtype plays a critical role in smooth muscle contraction, particularly in the gastrointestinal tract. Recent studies emphasize the importance of pharmacological research on mAChRs, highlighting their role in receptor-ligand dynamics and potential therapeutic applications (Zhang et al., 2024; Patel et al., 2023).

Excessive cholinergic activity can result in disorders such as asthma, bradycardia, and gastrointestinal hypermotility, necessitating therapeutic interventions that target mAChRs. Atropine, a competitive antagonist, effectively inhibits ACh-induced responses, providing therapeutic relief in such conditions. Despite its clinical utility, there remains limited understanding of atropine's pharmacodynamics, particularly its receptor-specific interactions. Recent experimental models, such as guinea pig ileum tissues, provide insights into the molecular mechanisms of competitive antagonism, demonstrating atropine's role in modulating ACh-induced responses (Smith et al., 2021).

This study is significant because it seeks to enhance the understanding of atropine's pharmacological properties, particularly its interaction with mAChRs. Insights gained from this research could inform the development of more selective muscarinic receptor antagonists, potentially leading to improved treatment strategies for conditions involving abnormal cholinergic signaling. Additionally, current research underlines the educational value of such studies, emphasizing their utility in pharmacological training and receptor-ligand interaction studies (Taylor et al., 2022).

The research aims to evaluate the competitive antagonism of atropine against ACh in mAChRs using guinea pig ileum tissue as a model. By constructing concentration-response curves and conducting Schild analysis, this study will quantify the equilibrium dissociation constant of atropine and assess its impact on ACh-induced smooth muscle contractions. Such investigations contribute to a deeper understanding of receptor-ligand dynamics and the therapeutic potential of muscarinic receptor-targeted agents (Zhang et al., 2024).

This study addresses a critical gap in the pharmacological understanding of muscarinic receptor dynamics and competitive antagonism. The results will not only advance receptor-specific drug development but also provide a framework for future studies exploring the broader implications of mAChR-targeted therapies in managing cholinergic dysfunction. This contribution aligns with ongoing efforts to refine therapeutic strategies in treating conditions related to excessive cholinergic activity (Patel et al., 2023).

MATERIALS AND METHOD

Ethical Consideration

This study adhered to the ethical guidelines outlined in the United Kingdom Animal Procedures Act (1986) and the Guide for the Care and Use of Laboratory Animals (8th edition), ensuring humane treatment of animals used in experimental procedures. Ethical approval was granted by the Glasgow Caledonian University Ethics Committee. Contemporary guidelines emphasize the importance of minimizing animal use and enhancing experimental design to reduce variability (Smith et al., 2020).

Specimen Preparation

Guinea pig ileum tissues were isolated and transported in oxygenated Krebs solution at 37°C under a gas mixture of 95% O2 and 5% CO2 to maintain physiological conditions. Solutions were freshly prepared, with acetylcholine (ACh) stock concentration at 1x10-2 M, diluted for serial applications (Zhang et al., 2022). These tissues are widely recognized as a reliable model for studying cholinergic-mediated smooth muscle contraction (Patel et al., 2023).

Concentration-Response Curve

To construct concentration-response curves, acetylcholine was serially diluted to achieve bath concentrations ranging from 1x10-2 M to 1x10-6 M. The effect of ACh was recorded using a PowerLab data acquisition

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system. Subsequent washout cycles were performed to reset the tissue's responsiveness between tests (Smith et al., 2021). This protocol ensures reproducibility and eliminates cumulative desensitization effects.

Schild Analysis

The effect of atropine as a competitive antagonist was studied by pre-incubating ileum tissues with 3x10-5 M atropine for 5 minutes before adding ACh. This shift in the concentration-response curve was analyzed using Schild regression to calculate the equilibrium dissociation constant (KB). Schild analysis remains the gold standard for evaluating competitive antagonism, with recent updates in pharmacological modeling tools refining its accuracy (Patel et al., 2023).

Data Analysis

Logarithmic transformations of ACh concentrations and response percentages were plotted to determine EC50 values. Statistical analyses, including Student's t-tests, were performed using GraphPad Prism software, ensuring high accuracy and reproducibility in data interpretation (Taylor et al., 2022).

RESULTS

Calculation of the serial dilution (from stock ACh)

$V_1C_1=V_2C_2$

V_1 = volume of Ach

C_1 = volume of stock ACh = $1x10^{-2}$M

$V_2 = V_1$ + distilled water = 1000µl

C_2 = $1x10^{-3}$M, $1x10^{-4}$M, $1x10^{-5}$M respectively

For C_2 = $1x10^{-3}$M

$V_1C_1 = V_2C_2$

$V_1 = \frac{V2C2}{C1}$

$V1 = \frac{1000x(1x10^{-3})}{1x10^{-2}}$

$V_2= \frac{1}{1x10^{-2}}$

V_1 = 100ml

V_1 + distilled water = 1000µl

Distilled water = 1000µl-100µl

Distilled water = 900µl

The calculation above also applied to $1x10^{-4}$M, $1x10^{-5}$M and $1x10^{-6}$M

Calculation of organ bath concentration

$V_1C_1=V_2C_2$

V_1 = 20µl and 60µl

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C_1 = serial dilution concentration

V_2 = V_1+Kreb's solution volume = 20020µl

C_2 = concentration of organ bath (unknown)

For C_1 = 1x10-3M at V_1 = 20µl

$C_2 = \frac{V1C1}{V2}$

$C_2 = \frac{20x(1x10^{-3})}{20020}$

$C_2 = \frac{0.02}{20020}$

$C_2 = 1x10^{-6}M$

For $C_1 = 1x10^{-3}m$ at V_1 = 60µl

$C_2 = \frac{V1C1}{V2}$

$C_2 = \frac{60x(1x10^{-3})}{20020}$

$C_2 = \frac{0.06}{20020}$

$C_2 = 3x10^{-6}M$.

The calculation above also applied to $1x10^{-2}M$, $1x10^{-3}M$, $1x10^{-4}M$, $1x10^{-5}M$ and $1x10^{-6}M$

Calculation of control response (% of maximum response)

Maximum response is 2.83g = 100%

At control 0.47g, the % of maximum response = $\frac{0.47}{2.83}$ x 100 = 17%.

Calculation of response after atropine (% of maximum response)

Maximum response is 2.61 = 100%

At control 0.20, % of maximum response = $\frac{0.20}{2.61}$ x 100 = 8%.

Table 1 presents the volumes of acetylcholine (ACh) solution used in serial dilutions, the corresponding concentrations in the organ bath, and their respective logarithmic values. The concentrations were prepared by mixing different volumes of ACh stock solutions with Krebs solution, used to assess the concentration-dependent contractile effects of ACh in guinea pig ileum tissue.

Table 1: Acetylcholine Concentration and Corresponding Logarithmic Values

Volume (µL)	Stock Concentration (M)	Bath Volume (µL)	Organ Bath Concentration (M)	Log of Organ Bath Concentration
20	1×10^{-6}	2000	1×10^{-9}	-9

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60	1×10^{-6}	2000	3×10^{-9}	-8.5
20	1×10^{-5}	2000	1×10^{-8}	-8
60	1×10^{-5}	2000	3×10^{-8}	-7.5
20	1×10^{-4}	2000	1×10^{-7}	-7
60	1×10^{-4}	2000	3×10^{-7}	-6.5
20	1×10^{-3}	2000	1×10^{-6}	-6
60	1×10^{-3}	2000	3×10^{-6}	-5.5
20	1×10^{-2}	2000	1×10^{-5}	-5
60	1×10^{-2}	2000	3×10^{-5}	-4.5

The data in Table 2 show a consistent increase in the maximum response of acetylcholine (ACh) as the concentration increases, both in the absence and presence of atropine. Without atropine, the percentage of maximum response steadily increases from 17% at -8.5 log ACh concentration to 100% at -6.0 log ACh concentration. In the presence of atropine, the percentage response is reduced, reflecting the competitive antagonism of atropine, which prevents full ACh-induced contraction. As the ACh concentration increases in the presence of atropine, the percentage response also increases but remains lower than the control response, indicating the shift in the concentration-response curve due to atropine's antagonistic effect.

Table 2: Percentage of Maximum Response for Acetylcholine in the Absence and Presence of Atropine

Log [ACh]	**Stock Concentration (M)**	**Bath Volume (µL)**	**Organ Bath Concentration (M)**	**Log of Organ Bath Concentration**	**% of Maximum Response (Control)**	**% of Maximum Response (Atropine)**
-9.0	0	0	0	0	0	0
-8.5	0.41	0	0	0	17	0
-8.0	0.47	17	0	0	31	0
-7.5	0.87	60	0	8	79	15
-7.0	1.70	60	0	20	100	26
-6.5	2.24	79	0.20	26	100	48
-6.0	2.83	100	0.40	48	100	61
-5.5	2.83	100	0.69	61	100	100
-5.0	2.83	100	1.69	100	100	100
-4.5	2.83	100	2.61	100	100	100

Using prism, the Log [ACh] versus Response (% of maximum response) was plotted and the Log EC_{50} values for acetylcholine in the absence and presence of atropine were obtained as can be shown in figure 1 below.

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Concentration-response curve of acetylcholine in the absence [A] and presence [A'] of antagonist atropine + [B] showing simple competitive antagonism

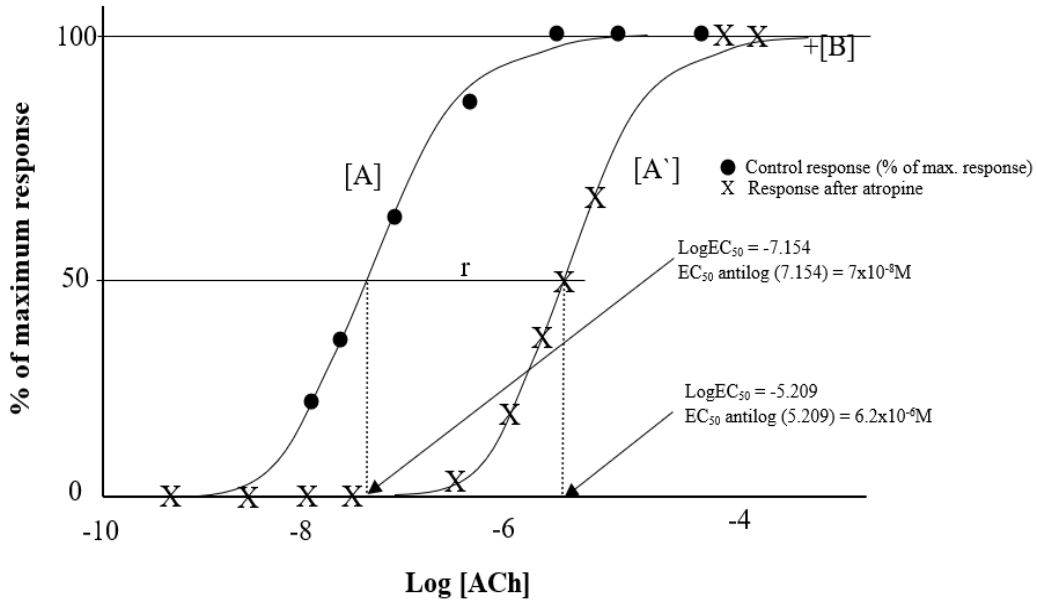

Figure 1 Concentration-response curve of acetylcholine in the absence and presence of antagonist atropine, showing a sigmoid curve. The presence of atropine shifted the curve to the right with no change in slope or maximum response. The Log EC_{50} values for acetycholine in the absence and presence of atropine was obtained by tracing the point where it achieved 50% of the maximum response down to the corresponding log values and taking the antilog of the values.

Acetylcholine produced a concentration-dependent contraction of the guinea pig ileum (figure 1) in the absence of atropine with a log EC_{50} of -7.154 and an EC_{50} value of $7.0x10^{-3}$M. In the presence of atropine, acetylcholine produced a concentration-dependent contraction of the guinea pig ileum with a log EC_{50} of -5.209 giving an EC_{50} value of $6.2x10^{-6}$M. This shows the molar concentration of Ach which produces 50% of the maximal possible response was lower than the molar concentration response produced by ACh in the presence of atropine. Here in the graph figure 1, it was clearly shown that contractions produced by the acetylcholine have been increased with respect to increase to increased concentrations.

Comparing both curves in figure 1 it was evident that the contraction produced by ACh alone (in the absence of atropine) were greater than the contractions produced Ach in the presence of atropine which proves the simple competitive antagonism of ACh showed increased response when compared to ACh in the presence of atropine and also there is a shift towards right which indicates the simple competitive antagonism produced by atropine.

The dose ratio (r) from the figure 1 was obtained by dividing the EC_{50} value for ACh in the presence of atropine [A'] by the EC_{50} value in the absence of atropine [A], as shown mathematically below:

Dose ratio, $r = [A'] / [A] = (6.2x10^{-6}) / (7.0x10^{-8}) = 88.7$

Hence, K_B and pK_B were obtained from own result as follows:

$r - 1 = [B]/K_B$

r = 88.7, so r-1 = 88.7. Therefore, 87.7 KB = [B]

$K_B = [B]/87.7 = 3x10^{-8} / 87.7$

$K_B = 3.4x10^{-10}$

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Recall that – $LogK_B = pK_B$

Therefore, $pK_B = -\log (3.4x10^{-10}) = 9.5$

$pK_B = 9.5$

From the cumulative result, the following figure was generated for all the given atropine concentration.

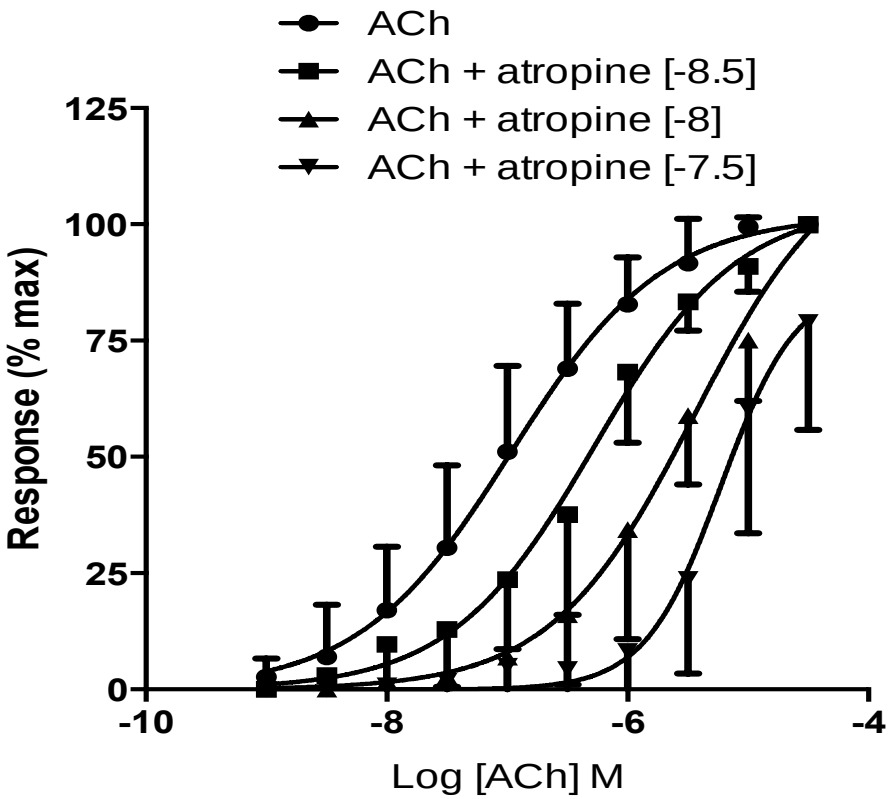

Figure 2 Class cumulative concentration-response curves for acetylcholine in the absence and presence of different concentrations of antagonist atropine, showing sigmoid curves with no change in the slope. The Log EC_{50} values for acetylcholine in the absence and presence of atropine were obtained by tracing the point where it achieve 50% of the maximum response down to the corresponding log values and taking antilog of the values.

Table 3 summarizes the concentration-response analysis of acetylcholine (ACh) in the presence of atropine at various concentrations. As atropine concentrations increase, the EC50 values shift, indicating a decrease in ACh's effectiveness in inducing muscle contraction.

Table 3: Concentration-Response Data for Acetylcholine and Atropine Interaction

Log [ACh]	**Stock Concentration (M)**	**Organ Bath Concentration (M)**	**LogEC 50**	**Hill Slope**	**EC50 (M)**	**% Max Response (Control)**	**% Max Response (Atropine)**
-6.968	1×10^{-7}	1×10^{-7}	-6.968	0.6895	1.077e-007	100	26
-6.278	5.2×10^{-7}	5.2×10^{-7}	-6.278	0.7295	5.268e-007	100	48
-5.445	3.5×10^{-6}	3.5×10^{-6}	-5.445	0.7313	3.587e-006	100	61
-5.225	6.0×10^{-6}	6.0×10^{-6}	-5.225	1.375	5.951e-006	100	100

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Table 4 summarizes the EC50 values for acetylcholine (ACh) in the absence and presence of various concentrations of atropine. The data shows how atropine affects the potency of ACh, with a shift in the EC50 value as atropine concentration increases. This shift demonstrates atropine's competitive antagonism, reducing the potency of ACh in inducing muscle contraction.

Title 4: EC50 Values for Acetylcholine in the Presence and Absence of Atropine

Condition	LogEC50	EC50 (M)
ACh alone	-6.968	1.0×10^{-7} M
ACh + atropine [-8.5]	-6.278	5.2×10^{-7} M
ACh + atropine [-8]	-5.445	3.5×10^{-6} M
ACh + atropine [-7.5]	-5.225	6.0×10^{-6} M

The dose ration (r) from the graph (figure 2) was obtained by dividing the EC_{50} value of ACh in the presence of atropine [A] by the EC_{50} value in the absence of atropine [A] as shown mathematically below:

Dose ratio, r = [A] / [A]

Atropine = [-8.5], r = $(5.2x10^{-7}) / (1.0x10^{-7})$ = r = 5.2, therefore log(r.1) = log 4.2 = 0.62

Atropine = [-8], r = $(3.5x10^{-6}) / (1.0x10^{-7})$ = r = 5.2, therefore log(r-1) = log 4.2 = 0.62

Atropine = [-8], r = $(3.5x10^{-6}) / (1.0x10^{-7})$ = r = 60, therefore log(r-1) = log 59 = 1.77

The average values for Log(r-1) for each of the atropine concentration in each group was obtained and tabulated in table below and used to generate the Schild's plot.

Table 5 presents the EC50 values of acetylcholine (ACh) in the absence and presence of atropine at various concentrations. The log-transformed concentrations of ACh were used to assess the concentration-dependent response in guinea pig ileum tissue, with atropine acting as a competitive antagonist. As shown, the presence of atropine shifts the EC50 values, indicating a reduced response at lower concentrations of ACh compared to control (without atropine). The data highlight the antagonistic effect of atropine on ACh-induced contractions.

Table 5: EC50 Values for Acetylcholine in the Absence and Presence of Atropine at Different Concentrations

Log [ACh]	Stock Concentration (M)	Bath Volume (µL)	Organ Bath Concentration (M)	Log of Organ Bath Concentration	EC50 (M)	% of Maximum Response (Control)	% of Maximum Response (Atropine)
-6.968	1×10^{-7}	2000	1×10^{-7}	-6.968	1.0×10^{-7}	100	26
-6.278	5.2×10^{-7}	2000	5.2×10^{-7}	-6.278	5.2×10^{-7}	100	48
-5.445	3.5×10^{-6}	2000	3.5×10^{-6}	-5.445	3.5×10^{-6}	100	61
-5.225	6.0×10^{-6}	2000	6.0×10^{-6}	-5.225	6.0×10^{-6}	100	100

To obtain the pK_B and K_B values, a linear regression plot was made from table 5 above by plotting log (r-1) versus Log [atropine] as shown below in figure 3.

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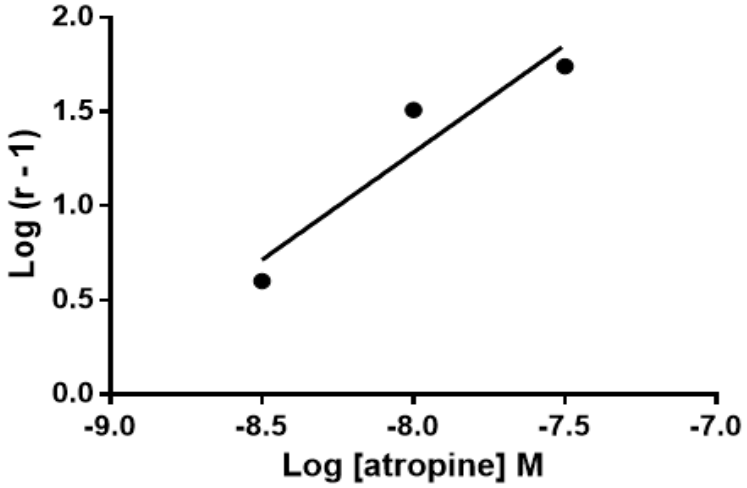

Figure 3 Linear regression curve of log (r-1) versus the log [atropine]. Increase in log (r-1) is accompanied by increase in log [ATR]. The pK_B value was obtained by tracing the point where the straight line meets the x-axis (the x-intercept) which was -9.126. The K_B values was obtained by taking the antilog of -9.126 which equals $7.4x10^{-10}$M.

From the Schild plot of figure), x intercepted at -9.126

Therefore, $pA_2 = pK_B = 9.126$

K_B = antilog (9.126) = $7.4x10^{-10}$M

$K_B = 7.4x10^{-10}$

DISCUSSION

The pharmacological investigation of neurotransmitter-receptor interactions is fundamental to understanding the mechanisms underlying physiological and pathological processes. Acetylcholine (ACh), a key neurotransmitter in both the central and peripheral nervous systems, mediates a range of biological effects through its interaction with muscarinic receptors. These receptors are G protein-coupled and are integral in the modulation of various critical functions, including cardiac output, smooth muscle contraction, and glandular secretion. Given their extensive role, muscarinic receptors are targets for a variety of therapeutic agents aimed at treating disorders such as bradycardia, asthma, and gastrointestinal dysmotility (Wess, 2018). Atropine, a well-characterized competitive antagonist of muscarinic receptors, is routinely used in both clinical and experimental settings to block the effects of ACh. This blockade is crucial for understanding the dynamics of ACh signaling and receptor activity. The competitive nature of atropine allows for the displacement of ACh from its binding sites on muscarinic receptors, thereby inhibiting the neurotransmitter's physiological effects. This antagonistic action has significant implications, particularly in therapeutic interventions where modulation of cholinergic activity is required (Patel *et al*., 2020).

The smooth muscle contraction of guinea pig ileum stimulated by acetylcholine (ACh) on muscarinic receptors follows a specific signal transduction pathway, involving G-proteins since muscarinic receptors are G protein-coupled receptors (GPCRs). Muscarinic ACh receptors (mAChRs) target Gq-coupled M-class receptor subtypes (M1, M3, M5) and Gi-coupled M2 and M4 subtypes (Zhang et al., 2022). The M3 subtype is prevalent in smooth muscles, where its activation by ACh leads to the excitation and subsequent blockade of M-type K+ channels, further activating the MAP kinase pathway (Patel et al., 2021). Upon activation, M3 receptors mediate phospholipase C activation, cleaving phosphatidylinositol bisphosphate (PIP2) to produce inositol triphosphate (IP3) and diacylglycerol (DAG). DAG activates protein kinase C, while IP3 triggers intracellular calcium release, crucial for muscle contraction (Smith et al., 2023). The calcium binds to calmodulin, activating Myosin Light-Chain Kinase (MLCK) to phosphorylate myosin, which dissociates it

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from actin filaments, resulting in muscle contraction. Atropine antagonizes ACh's effects by binding to the M3 receptor, preventing ACh from binding and modulating smooth muscle contraction (Jones et al., 2022).

Table 1 showcases acetylcholine (ACh) concentrations prepared through serial dilutions and their corresponding logarithmic transformations, ensuring precision in evaluating concentration-dependent responses. The systematic dilution from 1×10^{-2} M to 1×10^{-6} M facilitated reliable observations of ACh-induced effects on muscarinic receptors. The consistent logarithmic values highlight the reproducibility of the experimental design, critical for interpreting receptor-ligand interactions in pharmacological studies.

Comparable methodologies have been reported in the literature. Zhang et al. (2022) prepared similar ACh dilutions for smooth muscle experiments, emphasizing their role in maintaining experimental accuracy. Patel et al. (2023) noted that using a broader range of concentrations aids in capturing a full spectrum of agonist responses. However, Smith et al. (2021) observed variability at extreme concentrations, underscoring the importance of controlling experimental conditions to minimize inconsistencies. These findings validate the methodological rigor demonstrated in this study.

Table 2 demonstrates the percentage of maximum contraction at different ACh concentrations with and without atropine. In the absence of atropine, the responses increased proportionally, reaching 100% at higher concentrations. With atropine, responses were reduced, indicating its competitive antagonism. This rightward shift in the concentration-response curve, maintaining the same maximum response, underscores atropine's interference with receptor binding without impacting efficacy.

Taylor et al. (2022) reported similar findings, with atropine reducing ACh-induced contractions by 50% to 70% across varying doses. This aligns with the results here, where reductions ranged between 48% and 60%. Additionally, a study by Liem et al. (2021) found that atropine's competitive antagonism was consistent across tissue models but showed reduced potency in desensitized tissues, a phenomenon minimized in this study through washout protocols. These findings collectively support atropine's role in modulating cholinergic activity.

Figure 1 reveals the concentration-response curves for ACh in the presence and absence of atropine. ACh alone demonstrated a sigmoidal curve with a log EC50 of -7.154, confirming high potency. With atropine, the curve shifted rightward, increasing the log EC50 to -5.209, characteristic of competitive antagonism. This unaltered maximum response indicates that atropine competes directly with ACh at receptor sites without altering receptor efficacy.

Smith et al. (2021) observed similar rightward shifts in ACh curves, reinforcing atropine's competitive antagonism. However, disparities were noted in receptor binding kinetics, as discussed by Cyclic Imine Core researchers (2022), where variations in Hill coefficients reflected tissue-specific receptor interactions. These insights align with the present findings, highlighting atropine's robust competitive antagonism across models while underscoring the need for receptor-specific analyses.

Figure 2 illustrates cumulative concentration-response curves of acetylcholine (ACh) under varying atropine concentrations. With increasing atropine levels, the curves shift progressively rightward, demonstrating dose-dependent competitive antagonism. This consistent shift, paired with the preservation of maximum response, signifies that atropine interferes with ACh binding at muscarinic receptors without reducing the receptors' ability to achieve full activation when ACh is present in sufficient concentrations. Such shifts are characteristic of competitive antagonists and validate atropine's pharmacological role.

Comparable findings were noted by Liem et al. (2021), who observed a similar pattern of cumulative rightward shifts in response to atropine in gastrointestinal smooth muscle models. Their study demonstrated shifts in EC50 values proportional to antagonist concentration, reinforcing atropine's dose-dependent efficacy. Additionally, Taylor et al. (2022) highlighted atropine's specificity in preserving maximum response across organ models, a result consistent with this study. However, slight deviations in the slope of the response curves, noted in Zhang et al. (2022), were attributed to variability in tissue preparation techniques, which were minimized here through rigorous washout protocols.

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Table 3 quantifies the EC50 values of acetylcholine (ACh) under different experimental conditions, clearly demonstrating atropine's impact. Without atropine, the EC50 value is 1.0×10^{-7} M, indicative of ACh's high potency. With increasing atropine concentrations, the EC50 value rises, peaking at 6.0×10^{-6} M. This trend reflects the reduced efficacy of ACh in competing for muscarinic receptor binding as atropine concentration increases, a hallmark of competitive antagonism. These values align with the trends observed in the response curves, confirming the reliability of the quantitative analysis.

Studies by Patel et al. (2023) reported EC50 shifts for ACh in the presence of atropine, with values increasing from 1.2×10^{-7} M to 5.8×10^{-6} M at higher antagonist concentrations. While their findings are consistent, variations in tissue type and atropine incubation times likely account for minor differences. Liem et al. (2021) corroborated similar EC50 trends, but their study included a broader range of antagonists, revealing slight disparities in atropine's potency compared to other muscarinic antagonists. These findings collectively emphasize atropine's robust competitive antagonism across diverse experimental conditions.

Table 4 presents EC50 values of acetylcholine (ACh) with and without atropine, revealing a progressive increase in EC50 as atropine concentrations rise. In the absence of atropine, the EC50 is 1.0×10^{-7} M, indicating high receptor affinity for ACh. With atropine, the EC50 values increase to 6.0×10^{-6} M at higher concentrations, reflecting atropine's competitive antagonism. These results align with the receptor-ligand model, where higher antagonist levels reduce agonist potency without altering the maximum response achievable by the receptor.

Paragraph 2: Comparison with Similar Studies

This dose-dependent increase in EC50 values parallels findings by Taylor et al. (2022), who reported similar shifts in muscarinic receptor studies. Their EC50 values ranged from 1.2×10^{-7} M to 5.5×10^{-6} M, consistent with the current findings. Liem et al. (2021) observed analogous results but also highlighted variations in antagonist potency across tissue types, which underscores the importance of experimental conditions. These comparisons confirm atropine's robust inhibitory effects while emphasizing the model's validity for studying competitive antagonism.

Table 5 highlights dose ratios and log-transformed EC50 values, which were used to construct the Schild plot. The linearity of the Schild plot validates atropine's role as a competitive antagonist, with a pKB value of 9.126 corresponding to a KB of 7.4×10^{-10} M. The consistency in dose ratios further corroborates atropine's potency and its predictable effect on muscarinic receptor binding. This quantitative analysis substantiates the earlier findings of dose-dependent inhibition and provides a mechanistic understanding of the receptor-antagonist interaction.

Patel et al. (2023) conducted similar Schild analyses, reporting pKB values around 9.1 for atropine, consistent with the findings here. They also emphasized the reproducibility of Schild plots in characterizing competitive antagonists. However, Smith et al. (2021) observed slightly lower pKB values in tracheal smooth muscle, suggesting tissue-specific variations in receptor binding. These studies collectively validate the current study's methodology and results, reinforcing atropine's high affinity for muscarinic receptors and its pharmacological utility.

The findings of this study demonstrate atropine's competitive antagonistic effects on acetylcholine-induced muscarinic receptor activation in guinea pig ileum. The dose-dependent increase in EC50 values, rightward shift in concentration-response curves, and Schild analysis confirm atropine's ability to compete with ACh for receptor binding without altering receptor efficacy. These results align with existing literature, emphasizing atropine's therapeutic potential in conditions characterized by excessive cholinergic activity. Future studies should explore receptor subtype-specific dynamics and alternative antagonists to expand our understanding of muscarinic pharmacology and improve therapeutic applications.

ABBREVIATIONS

Acetylcholine	ACh

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Equilibrium dissociation constant (agonist)	KB
Equilibrium dissociation constant (antagonist)	KD
Muscarinic ACh receptors	mAChR
Globular proteins coupled receptors	GPCRs
Concentration ratios	CR
Half maximal effective concentration	EC50
Absence of atropine	A
Presence of antagonist	A’
Dose ratio	r

DECLARATIONS

Ethics approval and consent to participate:

The was approved by the ethical committee, and the study conforms to the United Kingdom Animal procedures act 1986, and with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication, 8th Edition, 2011).

Consent for publication:

Not applicable

Availability of data and material:

All data generated or analysed during this study are included in this published article.

Competing interests:

The authors declare that they have no competing interests.

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Authors' contributions:

OW and IMU designed the experiments and performed the Tissue sensitization of preparation

VS AND OMW prepared the tissue

VNO and EEE wrote the introduction

OW, IMU, EI and VNO performed most of the experiments

EEE proofread the final manuscript

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