SYSTEMATIC REVIEW

Clinical Effectiveness of Muscarinic Receptor-Targeted Interventions in Neuropsychiatric Disorders: A Systematic Review

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Abstract

Background For decades, treatment of mood disorders, psychoses, anxiety and dementia have been confounded by limited efficacy and high rates of treatment resistance. Preclinical and clinical evidence have highlighted disruption of cholinergic signalling in several neuropsychiatric conditions and examined intervention strategies including acetylcholinesterase inhibitors and nicotinic receptor-targeted intervention. However, the effectiveness of these approaches is often curtailed by on-target side effects. Post mortem studies implicate muscarinic receptor dysregulation in neuropsychiatric pathophysiology; therefore, we conducted a systematic review and meta-analysis to investigate the therapeutic efficacy and safety of muscarinic receptor-targeted interventions in adults with neuropsychiatric disorders.

Methods PubMed, EMBASE, PsycINFO, EBSCO and Web of Science were searched using relevant keywords from database inception to 7 August 2022. Randomised, double-blind, placebo-controlled studies were included if they investigated the effect of muscarinic receptor-targeted intervention in adults with a diagnosis of a neuropsychiatric disorder and were published in English. A narrative synthesis approach was adopted to describe the findings. Wherever three or more studies with a similar intervention were available, effect sizes were calculated, and a meta-analysis was performed. Cochrane risk-of-bias-2 tool was utilised to assess the risk of bias, and sensitivity analyses were performed to identify publication bias. Certainty analysis (high, moderate, low and/or very low) was conducted using GRADE criteria.

Results Overall, 33 studies met the inclusion criteria and 5 were included in the meta-analysis. Despite a limited pool with several different interventions, we found therapeutic efficacy of xanomeline (M_1/M_4 agonist) in primary psychotic disorders plus behavioural and psychological symptoms of dementia. Scopolamine showed a significant antidepressant effect in a combined cohort of major depressive and bipolar disorders in the short-term outcome measure, but no effect following cessation of treatment. Results from bias assessments suggest "very low" certainty in the antidepressant effect of scopolamine. Critical limitations of the current literature included low power, high heterogeneity in the patient population and a lack of active comparators.

Conclusion While the results are not definitive, findings on muscarinic receptor-targeted interventions in several mental disorders are promising in terms of efficacy and safety, specifically in treating schizophrenia, mood disorders, and behavioural and psychiatric symptoms of Alzheimer's disease. However, orthosteric muscarinic receptor-targeted interventions are associated with a range of peripheral adverse effects that are thought to be mediated via M_2/M_3 receptors. The orthosteric binding site of muscarinic acetylcholine receptors is remarkably conserved, posing a challenge for subtype-selective interventions; nonetheless allosteric ligands with biased signalling pathways are now in development. We conclude that adequately powered

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prospective studies with subtype-selective interventions are required to determine the clinical effectiveness of muscarinic-receptor targeted interventions for the treatment of neuropsychiatric disorders.

Graphical Abstract
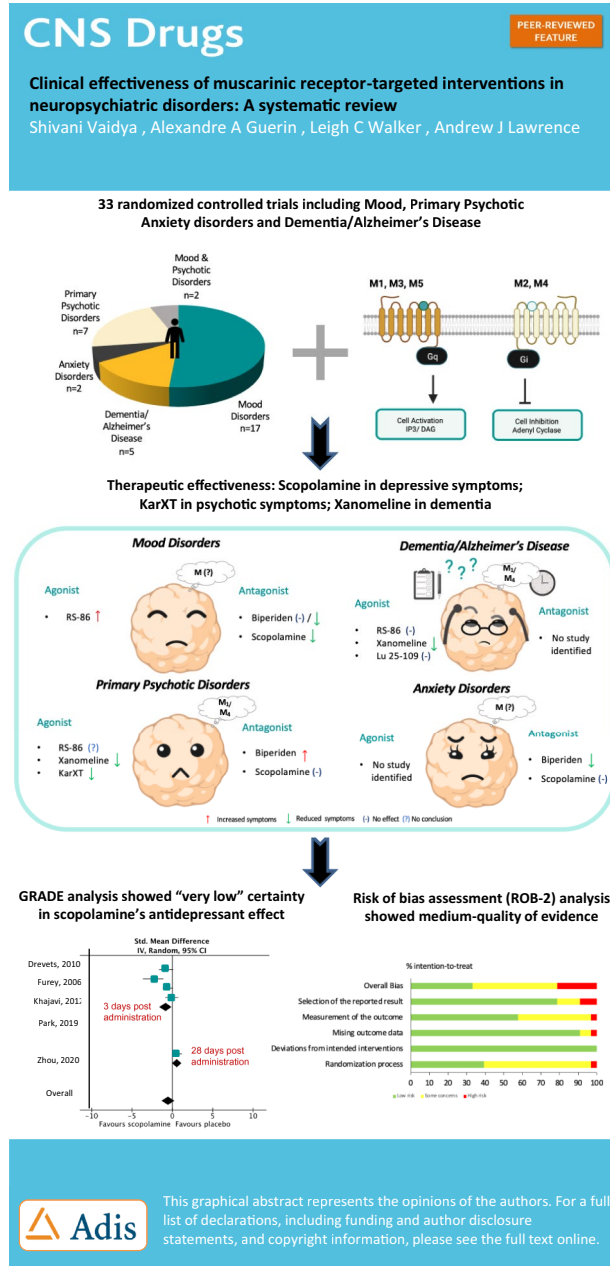
1 Introduction

In the brain, the cholinergic system forms an intricate neural network with three segregated elements based on their localisation and projection pattern [1]; (1) long-range basal forebrain projections, critical for attention, learning, memory, decision-making [2, 3]; (2) projections from the brainstem, which modulate attention, sleep, and motor control [4]; and (3) cholinergic interneurons of the striatum, which play a crucial role in reward and motivation [4, 5]. At the cellular level, acetylcholine signalling is modulated by nicotinic (nAChR; ionotropic receptors) and muscarinic acetylcholine receptors (mAChR; G-protein coupled receptors). To date, there are five cloned mAChR subtypes (M_1, M_2, M_3, M_4, and M_5). M_1, M_3 and M_5 receptors couple primarily to $G_{q/11}$, stimulating phospholipase C and inositol phosphate, mediating an excitatory effect through intracellular calcium influx. In contrast, M_2 and M_4 are coupled with $G_{i/o}$, resulting in an inhibition of the downstream adenyl cyclase activation [6–8].

Due to its widespread innervation and diverse central function, the acetylcholine system has attracted attention as a target for new pharmacotherapies to treat neuropsychiatric disorders with overlapping symptomatology [9, 10]. In 2019, 1 in every 8 or 970 million people in the world suffered from a mental disorder [11]. In the USA, 18.1% (43.6 million) adults experience mental health problems annually [12]. Current clinical strategies to combat dysfunctional cholinergic signalling include acetylcholinesterase inhibitors to avoid the breakdown of Ach- and nAChR-targeted interventions. However, the clinical effectiveness of these compounds is curtailed by on-target adverse effects [13–15]. As detailed below, growing evidence suggests the apparent involvement of muscarinic receptors in certain neuropsychiatric illnesses, including major depressive disorder (MDD), bipolar disorder (BP), schizophrenia, dementia, Alzheimer's disease (AD) and anxiety disorders [16].

1.1 Mood Disorders

The term mood disorder broadly defines all types of depression and BP. In the USA, an estimated 8.4% of adults will have at least one major depressive episode during their lifetime, with the prevalence rate higher in women (10.5%) than men (6.2%) [17]. Major depressive disorder is diagnosed when an individual has persistent feelings of sadness, anhedonia, worthlessness, lack of concentration, suicidal thoughts, appetite and sleep disturbances [18]. On the other hand, people with BP move between two states, from depression to mania. Treatment options mainly include pharmacological interventions such as antidepressants, anticonvulsants, mood stabilisers, and non-pharmacological approaches such as cognitive behavioural therapy, interpersonal psychotherapy, or a combination of these approaches [19]. Despite the accessibility of multiple treatment options, approximately 20–40% of people with MDD do not respond to the primary therapy, and the proportion of non-responders increases further for people with BP [20–22].

The involvement of the acetylcholine system in mood disorders was proposed in 1950, following a study in which psychiatric patients developed depressive-like symptoms after administration of acetylcholine esterase inhibitors [23]. Subsequently, this was assisted by studies showing that depressive symptoms were exaggerated in patients with MDD after administration of physostigmine, an acetylcholinesterase inhibitor [24, 25]. A PET study using [^{18}F]FP-TZTP, a selective M_2 ligand, found reduced receptor availability in the anterior cingulate cortex of patients with BP and MDD [26]. Similarly, haplotype analysis also suggests a possible involvement of M_2 receptors in patients with depression [27]. To date, only one study measured M_3 receptors and found a decrease in density in the frontal but not in the parietal and prefrontal cortex. Collectively, these data provide considerable support for region-specific differences in M_2 or M_3 receptors in patients with mood disorders, but no apparent regulation of M_1 receptor [28, 29].

1.2 Primary Psychotic Disorders

As defined by the International Classification of Diseases 11th Revision (ICD-11), primary psychotic disorders encompass schizophrenia, schizoaffective disorder, and other psychotic disorders. The prevalence of schizophrenia and related psychotic disorders range from 0.25 to 0.64% in the USA, based on a household survey, clinical diagnostic examinations, and health records [30–32]. Schizophrenia is a potentially devastating disorder with the following symptoms: positive (psychotic) symptoms such as disorganised thoughts, hallucination, delusion; negative symptoms

consisting of social withdrawal, affective flattening, alogia, anhedonia; and cognitive impairments in all domains. A vital issue with conventional antipsychotics is limited efficacy for negative symptoms and cognitive impairments, with predominant focus to date on the monoamine hypothesis [33, 34]. Even second-generation neuroleptics, including clozapine and olanzapine, produce only modest improvement in people with chronic and refractory illness [35, 36].

Initial investigation for the function of muscarinic receptors in schizophrenia was conducted using human post mortem brain tissue and radioligand binding assays. These results revealed region-specific alterations in M_1 and M_4 muscarinic receptors in the striatum, prefrontal cortex, hippocampus, anterior cingulate and superior temporal gyrus [37–40]. Studies employing receptor-specific antibodies, in situ hybridisation, and tomographic imaging revealed a region-specific negative correlation between muscarinic receptor availability and symptoms of schizophrenia [40, 41]. Notably, scopolamine (non-selective muscarinic receptor antagonist) induces psychotic-like symptoms, referred to as "anti-muscarinic syndrome" [42, 43]. Based on available evidence from tomographic imaging and post mortem studies, it was proposed that combined agonism of M_1 and M_4 receptors may be more efficacious given that a subgroup of people with schizophrenia also showed M_1 muscarinic receptor deficiency, known as muscarinic receptor deficit schizophrenia (MRDS) [44]. Moreover, intervention with agonist action at M_4 receptors also produced cognitive enhancement and anti-psychotic-like effects in animal models of the schizophrenia [45, 46]. This is a growing area of development with several pharmaceutical companies pursuing active muscarinic programmes (Table 1) for psychiatric disorders, including Karuna therapeutics (KarXT (Xanomeline+Trospium); NCT04659161), Sosei Heptares (HTL0016878; NCT03244228, NCT04849286, NCT04935320) and Cerevel therapeutics (Emraclidine: NCT05227703, NCT05227690).

1.3 Dementia/Alzheimer's Disease

The clinical syndrome of dementia consists of several subtypes, including AD, vascular, frontotemporal and dementia with Lewy bodies. Alzheimer's Disease contributes to 60–70% of cases [47]. Globally, 55 million individuals have dementia, which is expected to rise to 139 million by 2050 [47]. Along with cognitive impairment, 97% of patients also experience neuropsychiatric symptoms [48], also referred to as 'behavioural and psychological symptoms of dementia (BPSD)', which include lack of motivation, psychotic symptoms, depression, anxiety, agitation, and impulsivity [49, 50]. Current drug therapy for BPSD consists of atypical antipsychotics, antidepressants and anticonvulsants, which may cause severe adverse effects such as excessive sedation, orthostatic hypotension and cognitive slowing [51].

The cholinergic hypothesis of AD suggests that degeneration of cholinergic innervations in the cortex and hippocampus substantially contributes to cognitive dysfunction. In fact, the role of the acetylcholine system in learning and memory was first identified 50 years ago, when the muscarinic receptor antagonist scopolamine produced cognitive impairment in rats [52]. Clinical observation found acetylcholine esterase inhibitors such as tacrine, rivastigmine, donepezil and galantamine improved both cognition and behavioural and psychiatric symptoms. Unfortunately, dose-dependent adverse effects attributed to non-selective activation of acetylcholine receptors in both the central and peripheral nervous system restrict clinical utility [53].

Preclinical and post mortem findings also implicate muscarinic receptors in BPSD [54, 55]. Out of all five subtypes, M_1 receptor is most abundantly expressed in the prefrontal cortex and hippocampus [56]. Recently, M_1 muscarinic receptors have been postulated to be potential therapeutic agents for both cognitive and non-cognitive symptoms of AD [57, 58]. Additionally, the M_4 receptor may also represent a potential target for BPSD because of its widespread expression in the cortex, striatum, thalamus and hippocampus, key brain regulators for emotional processing and motivation [59]. Collectively, these results highlight the potential of muscarinic receptors in the regulation of cognitive and behavioural symptoms observed in AD patients [59].

1.4 Anxiety Disorders

Anxiety disorders are a group of psychiatric disorders comprised of generalised anxiety, panic disorders and various phobias (such as social phobia or claustrophobia). Data from the National Comorbidity Survey Replication suggest that 31.1% of US adults experience an anxiety disorder at least once in their lifetime [60]. A preclinical study revealed pro-depressive and anxiogenic-like effects following infusion of pilocarpine (a cholinergic agonist) in rats. In this study, pilocarpine was infused directly into ventral tegmental area (VTA), as mAChR in the midbrain are critical regulators of anxiety-like behaviour [61]. In contrast, the non-selective mAChR antagonist scopolamine attenuated chronic stress-induced anxiety-like behaviour in mice following intra-hippocampal administration [62]. Additionally, a recent study showed that direct infusion of a selective M_5 negative allosteric modulator into the VTA can modulate both depressive-like and anxiety-related behaviours in rats, implying a potential role for M_5 receptors in the regulation of anxiety-like behaviour [63].

Given this evidence, we therefore aimed to systematically review findings from randomised controlled trials (RCTs) assessing the therapeutic effectiveness of muscarinic-receptor targeted interventions in adults with neuropsychiatric

Table 1 Characteristics of muscarinic-receptor targeted interventions

Name of drug	Muscarinic receptor preference	Pharmacological action	References
RS-86	M_1/M_2	Agonist	[122]
Xanomeline	M_1/M_4	Agonist	[123] [124]
KarXT: A fixed combination of xanomeline and trospium chloride	Xanomeline: M_1/M_4	Agonist	[123] [124]
	Trospium chloride: Non-selective	Peripherally acting antagonist	[125] https://www.medicines.org.uk/emc/medicine/22236 Last accessed: 19th Sept 2022
Biperiden	M_1	Antagonist	[126] [127]
Scopolamine	Non-selective	Antagonist	[126]
Glycopyrrolate	Non-selective	Peripherally acting antagonist	[128]
Lu 25-109	Partial M_1 agonist, M_2 and M_3 antagonist	Agonist	[129]
Drugs in clinical trials			
HTL9936 (NCT02291783)	M_1	Agonist	https://soseiheptares.com/news/70/129/Sosei-subsidiary-Heptares-announces-positive-results-from-Phase-1b-clinical-trial-with-HTL9936-a-first-in-class-selective-muscarinic-M1-receptor-agonist-for-improving-cognition-in-dementia-and-schizophrenia.html Last accessed: 19th Sept 2022
HTL0018318 (NCT03456349)	M_1	Partial agonist	https://soseiheptares.com/news/109/129/Sosei-Provides-Update-on-HTL0018318.html Last accessed: 19th Sept 2022
Talsaclidine (NCT02249403)	$M_1 /M_2/M_3$	Agonist	[130]
HTL0016878 (NCT03244228)	M_4	Agonist	https://soseiheptares.com/news/402/129/First-Subject-Dosed-in-Phase1-Clinical-Study-of-Novel-Selective-Muscarinic-M4-Agonist-in-Development-to-Treat-Major-Symptoms-of-Alzheimers-Disease.html Last accessed: 19th Sept 2022
Emraclidine (CVL-231) (NCT05227703, NCT05443724, NCT05227690)	M_4	Positive allosteric modulator	https://www.cerevel.com/compounds/emraclidine/ Last accessed: 19th Sept 2022

M_1-M_5 muscarinic receptor subtypes 1–5

disorders, including mood disorders, primary psychotic disorders, dementia/AD, and anxiety disorders.

2 Methods

2.1 Protocol and Registration

The study was registered with the International Prospective Register of Systematic Reviews (PROSPERO: CRD42021236260) and followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (PRISMA) statement [64]. To limit the number of studies retained for screening, each step adhered to the PRISMA statement.

2.2 Detailed Search Strategy

Comprehensive systematic computer-based searches were carried out in PubMed, EMBASE, PsycINFO, EBSCO, and Web of Science, from database inception to the 31st of August 2021, with no date or publication type

limitation. Different combinations of keywords along with synonyms and word variants of keywords were used to create a comprehensive search strategy. Relevant search terms included: ("Muscarinic") AND ("Mental Health" OR "Mental disorder" OR "Psychiatric disease" OR "Psychiatric illness" OR "Psychiatric disorder" OR "Psychological disorder" OR "Behaviour disorder" OR "Behavior disorder" OR "Psychosis" OR "Schizophrenics" OR "Schizophrenia" OR "Mental illness" OR "Psychotic disorder" OR "Schizoaffective disorder" OR "Depression" OR "Depressive" OR "Anxiety" OR "Neurotic disorder" OR "Habit" OR "Impulsive disorder" OR "Bipolar disorder" OR "Bipolar" OR "Mood disorder" OR "Learning" OR "Memory" OR "Attention" OR "Neuropsychiatric disorder" OR "Obsessive-compulsive disorder" OR "Personality disorder" OR "Dementia" OR "Alzheimer") AND ("Human" OR "Participants "OR "Subjects" OR "Clinical") in the title, abstract, and keywords. We also manually searched the bibliography of eligible studies and full-text reviews for relevant papers if not incorporated in the primary search strategy. Searches were re-run before submission on 7th August 2022.

2.3 Inclusion Criteria

Articles qualified for inclusion if they: (1) investigated the effect of muscarinic receptor-targeted interventions; (2) included adults with a Diagnostic and Statistical Manual of Mental Disorders (DSM)/ICD diagnosis of neuropsychiatric disorder (schizophrenia, psychotic disorder, schizoaffective disorders, mood (affective) disorder, depression, mania, bipolar disorder, neurotic disorders, anxiety disorder, obsessive-compulsive disorder, habit and impulse behaviour, personality disorder), OR patients with dementia/AD (where the symptomatic measures are both cognitive and non-cognitive function or neuropsychological test); (3) had a comparison group (inactive placebo/active placebo); and (4) were published in English-language in a peer-reviewed journal. There were no restrictions to sample sizes, demographics, trial type (i.e., parallel or crossover) or type of intervention (agonist/antagonist). After initial title and abstract screening, only empirical articles were included in the full-text screening. For further inclusion in the meta-analysis, trials needed to be: (1) randomised controlled trials; (2) have no repeated participants; and (3) measure psychiatric symptoms on a validated disorder-specific psychometric scale.

Full-text articles were excluded if they: (1) were other systematic reviews or meta-analyses, conference proceedings published as abstract only, case reports, book chapters or animal study; and (2) lacked randomisation details (i.e., whether treatment allocation was randomised)

(3) Published in other than English language. We also excluded studies where the population of interest were adolescents and children aged ≤ 17 years or adults with dementia and AD where cognition was the only outcome measure.

2.4 Study Selection

Full-text articles were screened by two independent reviewers (SV and AAG) using the web-based systematic review tool Covidence (Veritas Health Innovation, Melbourne, Australia). Any disagreement between the two reviewers was resolved via discussion to achieve consensus.

2.5 Data Extraction and Outcome Included in the Qualitative Synthesis

A custom datasheet was generated using Microsoft Excel, and all relevant data were extracted by the primary reviewer (SV). All extracted data were verified independently by a second reviewer (AAG). The following study characteristics were extracted: (1) lead author; (2) publication year; (3) country; (4) sample size; (5) sample demographics (age, sex, and ethnicity); (6) study settings; and (7) participant characteristics (any co-morbidity or treatment status). In addition, details of mAChR interventions were extracted, including (1) name of the drug and mechanism of action; (2) dose; (3) route of administration; (4) washout period; (5) frequency; (6) experimental design; (7) comparison group used; (8) adverse effects; (9) retention rates; and (10) main findings of the trial.

The primary outcome measures were the mean and standard deviation of the after-treatment symptomology measurement for active and placebo interventions. We also included studies where rapid eye movement (REM) sleep alteration was a diagnostic measure. It has been hypothesised that REM sleep alterations in depression aid in the progression of depressive symptoms, including cognitive distortions and negative self-esteem [65].

2.6 Quality and Risk of Bias Assessment

The quality of studies included for analysis was assessed using the Cochrane risk of bias assessment tool (ROB-2) for randomised controlled trials [66]. Cochrane ROB-2 assesses five individual domains for bias: (1) randomisation process; (2) deviations from intended interventions; (3) missing outcome data; (4) measurement of the outcome; and (5) selection of the reported result. Each individual category was ranked as low risk, some concern or high risk of bias. Two independent reviewers (SV and AAG) assessed the risk of bias, any disagreements were solved by discussion.

Table 2 Certainty analysis using GRADE

Certainty analysis

Outcome	No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Certainty
Anti-depressant effect	5	Randomised controlled trial	Not serious	Serious[a]	Not serious	Serious[b]	Strongly suspected[c]	⊕⊖⊖⊖ **Very Low**
Anti-anxiety effect	3	Randomised controlled trial	Not serious	Serious[a]	Not serious	Serious[b]	Strongly suspected[c]	⊕⊖⊖⊖ **Very Low**

[a]Down-graded one level for inconsistency: I^2 value represents high heterogeneity (>50%)

[b]Down-graded for imprecision due to lower sample size for standardised mean difference (< 100/group)

[c]Down-graded one level for publication bias: Funnel plot was not created because of low study numbers (<10); however, leave one out sensitivity analysis changes the significance

2.7 Data Synthesis and Analysis

For the systematic review, a narrative synthesis approach was utilised to describe findings from included studies. There are four significant steps included in the Cochrane guidelines [67]. The first step was to develop a hypothesis about the mechanism of intervention. Alteration in acetylcholine release has been widely observed across various neuropsychiatric disorders, and muscarinic receptors modulate the release of acetylcholine both pre- and post-synaptically. Based on these facts, the question was framed. In the next step, we performed a pilot search for relevant studies. In addition to data extraction, discrepancies and factors influencing results were also interrogated to follow step 3. A significant challenge with narrative synthesis is the lack of transparency, which increases the likelihood of bias and reduces the robustness of findings. To prevent that, methods were pre-specified in the PROSPERO protocol and strictly followed.

Meta-analyses were performed with the help of Cochrane Review Manager software (Revman Version 5.4, The Cochrane Collaboration 2020). The standardised mean difference (SMD) was used as an outcome measure. The SMD was calculated as the difference in mean outcome between placebo and active intervention, divided by the pooled standard deviation of outcome among participants. In the absence of standard deviation, it was calculated from the number of participants, p-values and standard error. We used pre-crossover data from crossover trials to mimic the parallel trials. By assuming study level variability, we used a random-effects model. A Forest plot was generated to visualise the individual contribution of the study, and I^2 statistics were computed to assess heterogeneity between studies, where < 40% was considered low heterogeneity while > 40% represents moderate to substantial (< 60%) heterogeneity. Leave-one-out sensitivity analyses were performed to estimate influence and bias, whereby SMD values were calculated

by discarding one different observation each time. Meta-regression and funnel plots were considered inappropriate for this study as less than ten studies were included in the quantitative analysis [68]. To assess our confidence in meta-analysis effect estimates, the certainty of the evidence was judged by using the grading of recommendation assessment, development, and evaluation (GRADE) system using the GRADEpro GDT web application (Grade Pro GDT, 2020). The assessed criteria were risk of bias, consistency, directness, precision, and publication bias (Table 2).

3 Results

3.1 Selection of Studies

Initial database searches yielded 4368 potential articles, of which 2525 duplicates were removed (Fig. 1). Following title and abstract screening, full-text for the remaining 287 articles were reviewed for eligibility; of these, 254 were excluded for various reasons (e.g., inappropriate outcome measures, no information about randomisation, conference abstracts and other reviews). In total, 33 studies were included in the systematic review. Five studies were included in the meta-analysis. Table 1 represents the characteristics of each included muscarinic-receptor targeted intervention. Table 3 summarises the study characteristics of the 33 trials included. Studies were divided into groups based on disorder; 17 studies on mood disorders, 7 on primary psychotic disorders, 5 studies on dementia/AD, 2 on anxiety disorders, and the remaining 2 articles included people with both psychotic and mood disorders.

3.2 Quality and Risk of Bias

Table 3 and Fig. 2 show the ROB-2 quality assessment for each study. Seven studies were rated as high risk of bias, 15
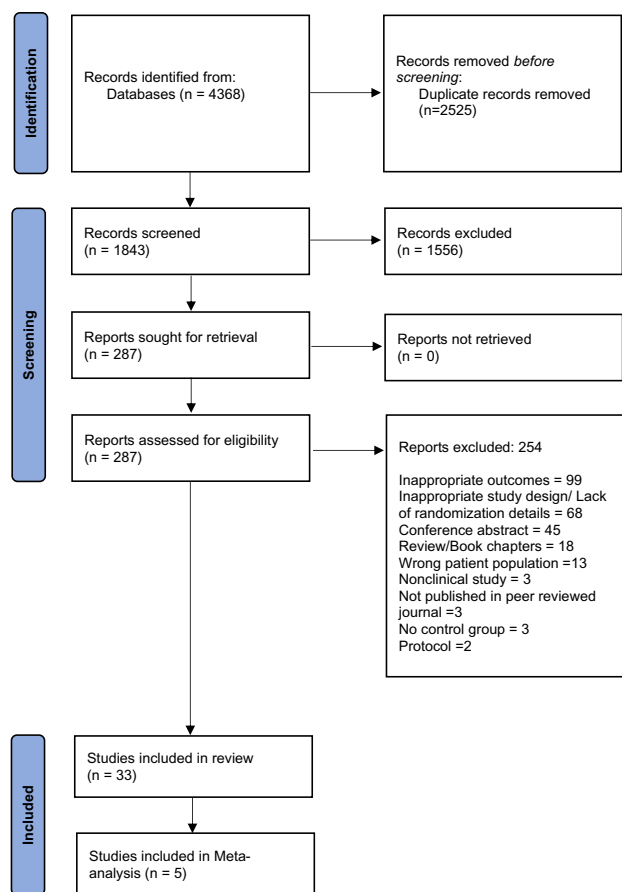
Fig. 1 Preferred Reporting Items for Systematic Reviews and Meta-Analysis guidelines (PRISMA) flow diagram

had some concerns, and 11 were rated as low risk of bias. The percentage similarities between the two reviewers were 82%, and the kappa score was 0.76 indicating substantial agreement. All studies included were randomised, placebo-controlled double-blind trials, except one, where the study was pseudo-randomised [69]. The main limitation across studies was poorly described randomisation details. Only 10 trials explicitly described randomisation methods, and 13 described allocation concealment. The second prime reason for some concern was the lack of information about assessors' blinding, which may lead to bias in study results. In seven studies, the dropout rate exceeded 20% [69–73]. In two studies, the key reason was adverse events, especially in the high-dose group. The reason for dropout was unavailable for two studies. Despite one having a significant effect on the overall visual analogue score (VAS) in people with depression, the reason for dropout was not given [69, 70]. Shekhar and colleagues (2008) reported that participants dropped out in both arms [71]. The KarXT clinical trial mentioned several reasons, including adverse events, consent withdrawal,

withdrawal by an investigator, and forgetting to follow-up [72].

3.3 Mood Disorders

Among the 19 studies in people with MDD or BP (Tables 3 and 4), 11 were conducted in the USA, 6 in Germany, one in Iran and one in China. A total of 538 participants ranging in age from 18 to 72 years, and 66% were female. In all, 12 studies investigated the effect of scopolamine, 5 studies RS-86 and 2 studies investigated the impact of biperiden. Seven scopolamine studies administered the medication intravenously, of which six showed that scopolamine effectively reduced depressive symptoms [74–79]. Further, when administered orally, scopolamine also reduced depressive symptoms in one study [80]. However, a more recent study exhibited no significant difference between scopolamine and placebo treatments post-intravenous administration [81]. In agreement with a more recent study, intramuscular scopolamine injection did not produce a significant difference compared to placebo [82]. However, in all the previous studies, symptom measurement was performed after every three infusions, whereas results were obtained 28 days after scopolamine injections in the intramuscular study. Acute biperiden administration reduced depressive symptoms compared to the placebo group [83]. In contrast, participants who received biperiden for 4 weeks did not significantly differ from placebo [84].

People with MDD frequently display sleep abnormalities, specifically reduced REM latency and heightened REM density [85, 86]. The remaining mood disorder studies focused on muscarinic receptor interventions in the modulation of REM sleep in people with depression. The muscarinic receptor agonist RS-86 reduced REM latency and enhanced REM density in depressed patients compared to placebo [87–89]. Conversely, REM latency was increased in response to the muscarinic receptor antagonist scopolamine compared to placebo [90, 91].

3.4 Primary Psychotic Disorders

Nine studies examined the effect of muscarinic receptor interventions in primary psychotic disorders (Tables 3 and 5). Six studies included people with schizophrenia, two with unspecified psychotic disorders, and one with schizophrenia and schizoaffective disorder. The sample sizes ranged from 11 to 182 participants, for a total of 361 participants (age range: 19–65 years; 31.2% female participants). Biperiden treatment was administered in four studies, and one for each of scopolamine, xanomeline, and KarXT. In the remaining two studies, RS-86 was used to measure disrupted sleep

Table 3 Study characteristics listed alphabetically based on the first author

Lead author and year	Sample size and characteristics		Sex (%)	Study settings and country	Psychiatric diagnosis	Patients characteristics	ROB-2 score
	The total number of patients enrolled	Mean age (SD)					
Battaglia (2001) [70]	N = 12	27.35 (5.1)	M = 50, F = 50	San Raffaelle Hospital, Italy	DSM-III-R and DSM-IV: panic disorder	None of the subjects taking a benzodiazepine, one man qualified for alcohol abuse 4 years before the experiment	High risk
Bakker (2020) [92]	N = 26	27.7 (4.9)	M = 73, F = 27	Netherlands	DSM-IV: Psychotic disorder	Medication-free and were recruited from early detection and intervention programmes	Some concerns
Beckmann (1982) [83]	N = 6	49 (7.8)	F = 100	Germany	ICD-9: Primary affective disorder or MDD	Mainly diagnosed as an affective disorder, consecutively admitted, severely depressed, Duration of illness >15 days. Unipolar n = 4, bipolar n = 1, neurotic depression n = 1 All patients were drug-free at least 7 days before the study	Some concerns
Berger (1989) [104]	N = 16	38 (14)	M = 37.5, F = 62.5	Germany	DSM-III: MDD	Drug-free >1 week Patients are acutely depressed	Some concerns
Bodick (1997) [99]	N = 343	74.9 (6.6)	M = 43, F = 57	USA	NINCDS/ADARA: Mild to moderate AD	Same as Veroff, 1998	Some concern
Brannan (2021) [72]	Total N = 182 Placebo (n = 90) KarXT (n = 92)	Placebo group: 41.6 (10.1) KarXT group: 43.1 (10.1)	Placebo M = 74, F = 26 KarXT M = 80, F = 20	USA	DSM-5: SCZ	Free from anti-psychotic medication 2 weeks before the baseline assessment	Low risk
Bruno (1986) [101]	N = 8	62 (SEM 1.5)	M = 38, F = 62	National Institute of Health, USA	DSM-III: AD with mild to moderately advanced dementia	No centrally active medications received > 2 weeks before study	Some concern

Table 3 (continued)

Lead author and year	Sample size and characteristics			Study settings and country	Psychiatric diagnosis	Patients characteristics	ROB-2 score
	The total number of patients enrolled	Mean age (SD)	Sex (%)				
Craske (2019) [103]	$N = 66$	Placebo group: 23.05 (8.58), 0.5 mg group: 26.53 (10.21), 0.6 mg group: 25.47 (10.56)	M: N = 22, F: N = 35, undeclared $n = 2$, did not answer $n = 1$ (Placebo: M $n = 4$, F $n = 17$, Scopolamine 0.5 mg; M $n = 8$, F $n = 10$, undeclared = 1, Scopolamine 0.6 mg; M $n = 10$, F $n = 8$ transgender $n = 1$, undeclared $n = 1$)	California, USA	DSM-IV/5: Anxiety disorder Social anxiety disorder	Patients have a fear, anxiety disorder and fear of public speaking	Low risk
Drevets (2010) [75]	$N = 22$	P/S group: 30 (7.0), S/P group: 33 (7.1)	M = 45, F = 55	National Institute of Mental Health, USA	DSM-IV: MDD	Chronic illness (>2 years) n = 13, comorbid anxiety n = 8, unresponsive to treatment n = 6	Low risk
Ellis (2014) [78]	$N = 62$	32.4 (9.5)	M = 40, F = 60	National Institute of Mental Health, USA	DSM-IV: BP or MDD	Treatment resistant n=31, treatment-naïve n=31, all the participants were medication-free at least two weeks before the study (MDD n=49, BP-I n=1, BP-II n=12)	Some concerns
Furey (2015) [79]	$N = 16$	32.4 (8.9)	M = 19, F = 81	National Institute of Mental Health, USA	DSM-IV: MDD without psychosis	Unmedicated depressed major depressive disorder patients, 5 patients have a comorbid anxiety disorder	Low risk
Furey (2013) [77]	$N = 15$	32.9 (7.8)	M = 74, F = 26	National Institute of Mental Health, USA	DSM-IV: Recurrent MDD	MDD without psychotic features, 5 MDD patients have comorbid anxiety disorder	Low risk

Table 3 (continued)

Lead author and year	Sample size and characteristics			Study settings and country	Psychiatric diagnosis	Patients characteristics	ROB-2 score
	The total number of patients enrolled	Mean age (SD)	Sex (%)				
Furey (2010) [76]	N = 52, P/S group (n = 27), S/P group (n = 25)	P/S group: F = 31.2 (7.6), M = 30 (9.1), S/P group: F = 35.7 (7.6), M = 32.8 (10.2)	P/S group M = 44, F = 56, S/P group M = 36, F = 64	National Institute of Mental Health. USA	DSM-IV: BP or MDD	MDD: Male n=17, F n=21; BP: M n=4, F n=10, patients have severe illness >2 years n=31, patients have comorbid anxiety n=20	Low risk
Furey (2006) [74]	N = 19	P/S group: 35.1 (8.5), S/P group: 30.9 (9.2)	P/S F = 70, M = 30, S/P F = 87.5, M = 12.5	National Institute of Mental Health, USA	DSM-IV: BP or MDD	Chronically ill n=12, co-morbid anxiety n=8, unresponsive to previous treatment n=5	Low risk
Gillin (1995) [84]	N = 19	44 (26)	M = 58, F = 42	Mental Health Clinical Research Center in the San Diego VA Medical Center, USA	DSM-III-R: BP or MDD	Drug-free 14 days before the study; Major depression n = 17, bipolar disorder, depressed type n=2 (secondary diagnosis: dysthymia n = 3, alcohol abuse in remission n = 6, substance abuse in remission n = 5, social phobia = 5, panic disorder n = 3, OCD n = 1, PTSD n = 2)	High risk
Hollander (1987) [100]	N = 15	66.1 (54–78)	M = 47, F = 53	Clinical Research Center of the Mount Sinai Hospital; Special Treatment Unit of the Bronx VA Medical Center of the Mount Sinai School of Medicine, USA	Research diagnostic criteria for Alzheimer's disease/NINCDS: Probable AD	All patients were drug free for 2 weeks prior to, and throughout the study	High risk

Table 3 (continued)

Lead author and year	Sample size and characteristics			Study settings and country	Psychiatric diagnosis	Patients characteristics	ROB-2 score
	The total number of patients enrolled	Mean age (SD)	Sex (%)				
Khajavi (2012) [80]	Total: N = 40 Scopolamine n = 20 Placebo n = 20	Scopolamine group: 37.8 (6.7) Placebo group: 36.6 (6.8)	Scopolamine F = 60, M = 40 Placebo F = 65, M = 35	Outpatient clinics of Roozbeh Psychiatric Hospital and National Iranian Oil Company Central Hospital, Iran	DSM-IV-TR: MDD	Citalopram (up to 40 mg) is given in both groups along with scopolamine and placebo during all 6 weeks. As per the author's knowledge, no interaction between escitalopram and scopolamine	Low risk
Liang (2010) [94]	N = 13	Age range: 20–65	M = 42, F = 58	Tri-service general hospital, Taiwan	DSM-IV: SCZ	All the patients are taking routine antipsychotic treatment (includes clozapine) except any other anticholinergic drugs during a 12-week study	Low risk
Newhouse (1988) [69]	N = 9	69.7 (6.1)	M = 67, F = 33	National Institute of Mental Health, USA	DSM-III: Major depression	Elderly patients, drug-free for at least 3 weeks	High risk
Park (2019) [81]	N = 23	36.83 (10.78)	M = 48, F = 52	National Institute of Mental Health USA	DSM-IV: MDD	MDD subjects with more severe and refractory forms of major depressive disorder relative to previous patients, medication-free depressive subjects, comorbid disorders: Anxiety disorder n = 7, OCD n = 3, PTSD n = 3, personal history of alcohol n = 7	Some concerns
Poland (1997) [90]	Depressed N = 14 Remitted/Recovered N = 16	Depressed: 42.6 (3.1) Remitted/Recovered: 39.7 (3.3)	Depressed M = 21, F = 79 Remitted/Recovered M = 31, F = 69	California, USA	DSM-III-R: Major depression	Patients with unipolar major depressive disorder and recovered subjects	Some concerns

Table 3 (continued)

Lead author and year	Sample size and characteristics		Sex (%)	Study settings and country	Psychiatric diagnosis	Patients characteristics	ROB-2 score
	The total number of patients enrolled	Mean age (SD)					
Rao (2004) [91]	N = 10	33.3 (8.8)	F = 100	USA	DSM-IV: MDD	Unipolar major depressive disorder/medication free from last 3 months	High risk
Riemann (1994a) [89]	N = 56	40.6 (13.6)	M = 36, F = 64	Germany	DSM-III: MDD	Major depressive disorder	High risk
Riemann (1994b) [96]	N = 40	Depression: 42.7 (12.1)	M = 35, F = 65	Psychiatric clinic of the University of Freiburg, Germany	DSM-III-R: Unipolar depression, BP I & II	Patients with major depressive disorder; unipolar depression n=34, bipolar depression I or II n = 6, Drug-free 7 days prior to investigation	Some concerns
	N = 43	Schizophrenia: 29.3 (6.7)	M = 70, F = 30	Psychiatric clinic of the University of Freiburg, Germany	DSM-III-R: SCZ	Schizophrenic form subtype n = 5, paranoid schizophrenia n = 10, residual type of disorder n = 21, undifferentiated/unorganized schizophrenia n=7, Patients are drug-free for > 6 days. Patients also have first-degree depressive disorder n = 6; patients have some other psychiatric symptoms (e.g., alcoholism) n = 10	Some concerns
Riemann (1992) [88]	N = 40	40.9 (12.3)	M = 42.5, F = 57.5	Germany	DSM-III: MDD & bipolar disorder "depressed type."	MDD=33, bipolar disorder "depressed type" n = 7	Some concerns

Table 3 (continued)

Lead author and year	Sample size and characteristics			Study settings and country	Psychiatric diagnosis	Patients characteristics	ROB-2 score
	The total number of patients enrolled	Mean age (SD)	Sex (%)				
Riemann (1991) [87]	$N = 11$	33.9 (3.9)	M = 36, F = 64	Germany	DSM-III: MDD	No medication 7 days before treatment	Some concerns
	$N = 11$	33.6 (8.7)	M = 73, F = 27	Germany	DSM-III-R: Residual SCZ	Patients also fulfilled the MDD criteria for a secondary measure n=6, all subjects were free from psychoactive medicine 7 days before	Some concerns
Segev (2019) [73])	$N = 15$	38.7 (12)	M = 71, F = 29	UK	DSM-IV-TR: SCZ or schizoaffective disorder	Receiving clozapine for <2 weeks	Low risk
Shekhar (2008) [71]	$N = 20$	Control: 42.1 (9.2) Xanomeline: 43.4 (9.3)	Control M = 80, F = 20 Xanomeline M = 60, F = 40	Neuroscience Clinical Research Center at Indiana University, USA	DSM-IV: SCZ or schizoaffective disorder	Patients taking atypical antipsychotic $n = 12$, typical antipsychotic $n = 6$, Drug-free from 1 month $n=1$, drug-free from 3 months n=1, medication-free before 3–7 days	Some concerns
Silver (1995a) [93]	$N = 26$	36.7 (8.9)	M = 69, F = 31	Israel	DSM-III-R: Schizophrenia	Chronically medicated schizophrenic patients, all antipsychotic medication continued throughout the study	Some concerns
Silver (1995b) [95]	$N = 26$	36.65 (8.89)	M = 69, F = 31	Israel	DSM-III-R: Schizophrenia	All antipsychotic medication continued throughout the study	Some concerns
Thal (2000) [102]	$N = 496$	75.5 (1)	M = 42, F = 58	29 centres across the USA	National Institute of Neurologic and Communicative Disorders-Alzheimer's Disease and Related Disorders Association criteria: Probable AD	N/A	Low risk

Table 3 (continued)

Lead author and year	Sample size and characteristics		Sex (%)	Psychiatric diagnosis	Study settings and country	Patients characteristics	ROB-2 score
	The total number of patients enrolled	Mean age (SD)					
Veroff (1998) [98]	N = 343	74.9 (6.6)	M = 43, F = 57	NINCDS/ADRDA: Mild to moderate AD	Lily research laboratories, California, USA	Use of central nervous system drugs were prohibited 1 month before study	Some concern
Zhou (2020) [82]	N = 66	Saline: 27.1 (7.0), Low dose: 25.7 (5.3), High dose: 26.5 (5.6)	M = 35, F = 65	DSM-IV: MDD	Beijing Anding Hospital, Capital Medical University, China	Permitted non-benzodiazepines for severe insomnia and benzodiazepines for significant anxiety but not 8 h before assessment	High risk

AD Alzheimer's disease, *ADRDA* AD and associated disorders association, *BP* bipolar disorder, *BP-I* bipolar disorder type-I, *BP-II* bipolar disorder type-II, *DSM* Diagnostic and Statistical Manual of Mental Disorders, *F* female, *ICD* International Classification of Diseases, *M* male, *MDD* major depressive disorder, *N* total numbers of participants, *n* participants in subgroup, *N/A* not available, *NINCDS* National Institute of Neurological and Communicative Disorders and Stroke, *OCD* obsessive-compulsive disorder, *PTSD* posttraumatic stress disorder, *P/S* placebo/scopolamine group, *RDS* research diagnostic criteria, *SCZ* schizophrenia, *SD* standard deviation, *SEM* standard error of the mean, *S/P* scopolamine/placebo group
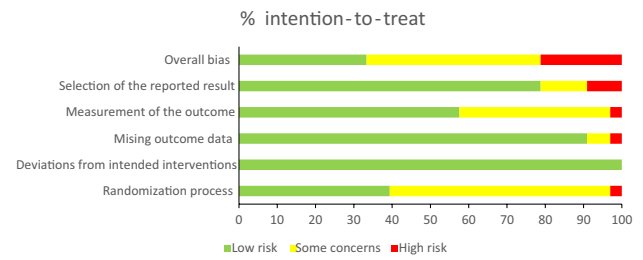

Fig. 2 Risk of bias assessment using ROB-2 tool. The weighted summary plot suggests that most studies fall under the category of "some concern" due to insufficient details about the randomisation process

parameters in psychiatric patients but not for primary psychiatric symptom measurements.

Administration of biperiden (4 mg, orally) produced significant impairment in Weschler memory scale, Benton visual retention test and paired-associate learning on Cambridge Neuropsychological Test Automated Battery in people with a primary psychotic disorder compared with controls [92, 93]. In contrast, one of the four studies showed no changes in Mini-Mental State Examination [93, 94]. Additionally, no significant difference was found in positive and negative symptoms [95]. Like biperiden, scopolamine had no significant impact on anxiety and depressive symptoms in subjects with a psychotic disorder [73], suggesting the potential efficacy of scopolamine is limited to patients with mood disorders.

Shekhar et al. 2008, studied the efficaciousness of xanomeline in subjects with schizophrenia, finding a beneficial effect of xanomeline in both positive and negative symptoms compared with placebo [71]. In the cognitive domain, improvements were most robustly observed in verbal and short-term memory function. In line with xanomeline, the response to KarXT treatment in schizophrenia demonstrated a lower score on the positive and negative syndrome scale, clinical global impressions scale-improvement, and Marder negative symptoms scale [72].

Two studies also examined the effect of RS-86 on sleep parameters in schizophrenia patients compared to placebo [87, 96]. Similar to observations in individuals with depressive disorders, RS-86 significantly reduced REM latency in this population compared with placebo [87, 96].

3.5 Dementia/Alzheimer's Disease

In total, five studies were included, investigating muscarinic-receptor-targeted interventions in cognitive and psychiatric symptoms of dementia. Among them, four studies included patients with mild to moderate AD, and one study included probable AD participants. Probable AD was diagnosed when a patient had cognitive gradual decline without a history of illness that could cause mental impairment without AD

Table 4 Outcomes of mood disorders (mixed cohort of MDD and BP)

Lead author, year	Study design	Interventions and comparator (Dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Beckmann (1982) [83]	Acute, double-blind, randomised, placebo-controlled	Intervention: biperiden 10 mg IV 4 days; Control: placebo; Washout: 24 h	No psychiatric or somatic side effects reported	HAM-D MADRS EWL-K	Biperiden significantly ($p < 0.01$) reduced MADRS and EWL-K score compared to placebo. Overall, HAM-D score did not significantly differ between two groups; only retardation, agitation factors, depressed mood, and work and interests improved ($p = 0.01$, $p = 0.05$, $p = 0.01$, $p = 0.05$, respectively).	100
Berger (1989) [104]	Randomised, double-blind, cross-over	Intervention: RS-86 1.5 mg oral Once; Control: placebo; Washout: 24 h	Increased salivation post RS-86 administration	REM sleep (latency, duration and density) Both depressive and remissive	RS-86 significantly reduced REM latency ($p < 0.001$) compared to placebo. In contrast, first REM period density ($p < 0.0001$), total REM density ($p < 0.012$) and first REM period ($p < 0.26$) were increased post RS-86 compared to placebo in depressives. No significant effect in remissive on the parameters mentioned above post-RS-86 compared to placebo	100

Table 4 (continued)

Lead author, year	Study design	Interventions and comparator (Dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Drevets (2010) [75]	Double-blind, randomised, placebo-controlled, cross-over	Intervention: scopolamine 0.004 mg/kg IV 3 sessions Control: placebo Washout: 3–5 days	Drowsiness, dry mouth, blurred vision, light headedness, dizziness	MADRS HAM-A, CGI-I VAS YMRS POMS	Scopolamine significantly reduced MADRS score change from baseline (p = 0.011) and CGI-I score ($p < 0.001$) compared to placebo in block 1 However, no difference was found between two groups after cross-over Due to baseline variation HAM-A score, differences in each group (scopolamine and placebo) were measured separately. A significant reduction was observed in HAM-A in the 2nd block in the P/S group (p = 0.001) and 1st block the in S/P group ($p < 0.023$) compared to the baseline No significant difference in VAS and POMS score relative to placebo	91.30
Ellis (2014) [78]	Double-blind, randomised, placebo-controlled, cross-over	Intervention: scopolamine 0.004 mg/kg IV 3 sessions Control: placebo Washout: 3–5 days	N/A	MADRS	Scopolamine significantly the reduced MADRS score ($p < 0.001$) compared to placebo This reduction was higher in treatment-naïve patients compared to treatment-resistant patients (p = 0.04)	93.54

Table 4 (continued)

Lead author, year	Study design	Interventions and comparator (Dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Furey (2015) [79]	Double-blind, randomised, placebo-controlled, crossover	Intervention: scopolamine 0.004 mg/kg IV 3 sessions Control: placebo Washout: 3–5 days	N/A	MADRS Selective attention task	Significant reduction ($p < 0.001$) in MADRS score compared to baseline at the study end. No data are given For selective attention task, scopolamine did not produce a significant effect on implicit emotional processing compared to baseline	93.75
Furey (2013) [77]	Double-blind, randomised, placebo-controlled, crossover	Intervention: scopolamine 0.004 mg/kg IV 3 sessions Control: placebo Washout: 3–5 days	N/A	MADRS Working memory tasks	Scopolamine significantly reduced MADRS score ($p < 0.001$) compared to baseline In working memory tasks, scopolamine decreased reaction time in the emotional task and increased in identity tasks ($p = 0.05$) compared to placebo	93.33
Furey (2010) [76]	Double-blind, randomised, placebo-controlled, crossover	Intervention: Scopolamine 0.004 mg/kg IV 3 sessions Control: placebo Washout: 3–5 days	Side effects after scopolamine: Dizziness, lightheaded, blurred-vision, drowsy, dry mouth, fatigue, feel drugged	MADRS HAM-A/HARS CGI-I	Scopolamine significantly reduced MADRS, HAM-A and CGI-I scores ($p < 0.001$) in females compared to placebo Whereas less significant reduction was observed in MADRS ($p = 0.043$) and CGI-I ($p = 0.046$) in males and no change in HAM-A score	Completed treatment 100 Completed follow up: 84.61

Table 4 (continued)

Lead author, year	Study design	Interventions and comparator (Dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Furey (2006) [74]	Randomised, double-blind, cross-over	Intervention: scopolamine 0.004 mg/kg IV 3 sessions Control: placebo Washout: 3–5 days	Drowsiness, dry mouth, blurred vision, light headedness, dizziness, hypotension Drowsiness and dry mouth also reported after placebo	MADRS HARS/HAM-A CGI-I VAS YMRS POMS Selective attention task	Scopolamine significantly reduced MADRS ($p <$ 0.001) and CGI-I ($p =$ 0.002) scores compared to placebo in block 1 Due to baseline variation HAM-A score, Difference in each group was measured separately. Significant reduction (p < 0.001) was observed in HAM-A score in 2nd block in P/S group and in 1st block in S/P group compared to baseline No difference was found between two groups in block 2 In selective attention task, performance accuracy was significantly reduced ($p = 0.02$) No significant effect of scopolamine was observed in VAS, YMRS and POMS scale	94.73
Gillin (1995) [84]	Randomised, double-blind, parallel	Intervention: biperiden 4–12 mg/day oral For 4 consecutive weeks Control: glycopyrrolate (active placebo)	Mild dry mouth and mild constipation (both groups)	HDRS-17 POMS REM sleep [latency, density, total (%)]	There was no significant difference in HDRS, POMS and REM latency between biperiden and glycopyrrolate at the end of 4th week	94.73
Khajavi (2012) [80]	Two-centre, randomised, placebo-controlled, double-blind, parallel	Intervention: scopolamine 0.5 mg BID Oral 6 weeks Control: placebo	Dry mouth, blurred vision, dizziness was more common in the scopolamine group compared to the placebo group	HDRS	Scopolamine significantly reduced HDRS score ($p = 0.021$) compared to placebo after 6 weeks	100

Table 4 (continued)

Lead author, year	Study design	Interventions and comparator (Dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Newhouse (1988) [69]	Double-blind, pseudorandom, placebo-controlled	Intervention: scopolamine 0.1 mg, 0.25 mg, 0.5 mg IV once Control: Placebo Washout: 48 h	Pulse rate shows a decreasing trend with dose, Increase in pupillary size, dry skin and dry mucus membrane Attentional impairment, drowsiness and mild confusion, Restlessness at higher dose	VAS BPRS Vigilance Selective-reminding words Continuous performance task POMS BPI NSRS Pfeiffer Short Portable Mental Status Questionnaire, Cognitive battery	Scopolamine significantly increased VAS score ($p < 0.01$), BPRS score ($p < 0.01$), NSRS anxiety sub-score ($p = 0.02$) at 0.5 mg relative to placebo No significant changes in BPI and POMS All the cognitive parameters were impaired at 0.5 mg ($p < 0.05$) compared to placebo	100 (9/9) For all other tests 66.66 (6/9) For VAS
Park (2019) [81]	Randomised, placebo-controlled, cross-over	Intervention: scopolamine 0.004 mg/kg IV 3 sessions Control: placebo Washout: 3–5 days	Dry mouth, constipation, blurred vision, drowsiness, and nervousness	MADRS HAM-A	There was no significant difference in antidepressant and antianxiety effects between scopolamine and placebo	82.60
Poland (1997) [90]	Double-blind, randomised, placebo-controlled	Intervention: scopolamine 0.0045 mg/kg IM Twice Control: placebo Washout: 24 h	N/A	REM sleep (latency, density, duration)	Scopolamine significantly ($p < 0.05$) increased 1st REM period latency and decreased overall REM duration relative to placebo	100
Rao (2004) [91]	Randomised double-blind, cross-over	Intervention: scopolamine, 0.0015 mg/kg, IM, twice Control: placebo Washout: 1 week	None noted	REM sleep (latency, density, total duration)	Scopolamine significantly ($p < 0.001$) increased 1st REM period latency and decreased 1st REM period density ($p < 0.005$) and overall, REM duration ($p < 0.001$) relative to placebo	100

Table 4 (continued)

Lead author, year	Study design	Interventions and comparator (Dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Riemann (1994a) [89]	Randomised, double-blind, cross-over	Intervention: RS-86 1.5 mg Oral, once Control: placebo Washout: 24 h	N/A	REM latency	RS-86 significantly reduced REM sleep latency (p = 0.0001) compared to placebo	100
Riemann (1994b) [96]	Randomised, double-blind cross over	Intervention: RS-86 1.5 mg Oral, once Control: placebo Washout: 24 h	N/A	REM sleep (latency, duration and density)	RS-86 significantly reduced REM sleep latency (p = 0.0001) compared to placebo	100
Riemann (1992) [88]	Randomised, double-blind, cross-over	Intervention: RS-86 1.5 mg Oral, once Control: placebo Washout: 24 h	N/A	REM latency	RS-86 significantly (p < 0.001) reduced REM latency compared to placebo	100
Riemann (1991) [87]	Randomised, double-blind-cross over	Intervention: RS-86 1.5 mg Oral, once Control: placebo Washout: 24 h	N/A	REM latency	RS-86 significantly reduced REM latency (p = 0.0072) compared to placebo	100
Zhou (2020) [82]	Double blind, randomised, three-arm, 4 week follow-up, parallel design	Intervention: Scopolamine: 0.3 mg (OD) 0.3 mg (BID) IM 3 days Control: placebo 10 mg/day of escitalopram was administered for first 3 days as add-on and all also for remaining 25 days until the last follow-up	Blurred vision, dizziness, somnolence, nausea, fatigue, drowsiness, dry mouth, anxiety, insomnia, and tachycardia	HDRS-17 MADRS YMRS CGI-I QIDS-SR-16 GAD-7	There was no significant effect of scopolamine on any of the symptoms	83.3

BID twice daily, *BPI* Beck Depression Inventory, *BPRS* Brief Psychiatric Rating Scale, *CGI-I* Clinical Global Impression – Improvement scale, *EWL-K* The Janke and Debus self-rating questionnaire, *GAD-7* Generalised Anxiety disorder scale, *IM* intramuscular, *IV* intravenous, *HAM-A/HARS* Hamilton Anxiety Rating Scale, *HAM-D* Modified Hamilton Rating scale, *HDRS* The Hamilton Rating Scale for Depression, *MADRS* Montgomery and Åsberg Depression Rating Scale, *N/A* not available, *NSRS* National Institute of Mental Health Self-Rating Scale, *OD* once daily, *POMS* Profile of Mood States, *QIDS-SR-16* Quick Inventory of Depressive symptomatology, *REM* rapid eye movement, *VAS* visual analogue scale, *YMRS* Young Mania Rating Scale

Table 5 Outcomes of primary psychotic disorders

Lead author, year	Study design	Interventions and comparisons (dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Bakker (2020) [92]	Counter-balanced, randomised-controlled trial	Intervention: biperiden 4 mg Oral, once Control: placebo	N/A	PAL of CANTAB	Biperiden significantly impaired all cognitive loads of learning ($p < 0.001$ for 4 and 6 pairs, $p = 0.002$ for 8 pairs) and memory ($p < 0.004$) phase except 6 and 8 pair association in the memory phase	100
Brannan (2021) [72]	Phase 2, randomised, double-blind, placebo-controlled	Intervention: KarXT 50–125 mg xanomeline + 20–30 mg trospium, with the option to reduce to 100 mg + 20 mg Oral, BID 5 weeks Control: placebo	Constipation, nausea, dry mouth, dyspepsia, vomiting	CGI-I PANSS Marder negative symptom sub-score	KarXT significantly reduced ($p < 0.001$) PANSS total score, PANSS positive and negative symptom sub-scores and Marder negative symptom sub-score There was no significant difference in CGI-I score between the groups	79.67
Liang (2010) [94]	Randomised, double-blind, cross-over, fixed dose	Intervention: biperiden 2 mg BID Oral, 4 weeks Control: glycopyrrolate Washout: 4 weeks	Constipation, inner unrest	MMSE DRS	No significant difference was found in DRS score across two groups Biperiden significantly reduced MMSE score at week 4 compared to baseline ($p = 0.008$)	92.30
Riemann (1994b) [96]	Randomised, double-blind cross over	Intervention: RS-86 1.5 mg Oral, once Control: placebo Washout, 24 h	N/A	REM sleep (density, latency, duration)	RS-86 significantly reduced REM sleep latency ($p = 0.0001$) compared to placebo	100
Riemann (1991) [87]	Randomised, double-blind cross over	Intervention: RS-86 1.5 mg Oral, once Control: placebo Washout: 24 h	N/A	REM Latency	RS-86 significantly reduced REM sleep latency ($p = 0.0072$) compared to placebo	100

Table 5 (continued)

Lead author, year	Study design	Interventions and comparisons (dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Segev (2019) [73]	Randomised, double-blind, cross-over, placebo-controlled	Intervention: scopolamine 0.3 mg/day oral 28 days Control: placebo Cross-over: 7 days	GIT-1, neurological-2, psychological-2, Musculo-skeletal-2	HADS anxiety score HADS depression score Toronto nocturnal hypersalivation scale EQ5D-quality of life analogue scale	Scopolamine significantly ($p < 0.001$) improved hypersalivation compared to placebo While no significant difference was found in the HADS anxiety scale, HADS depression scale and EQ5D scale across two groups	73.33
Shekhar (2008) [71]	Randomised, double-blind, placebo-controlled	Intervention: xanomeline up to 75 mg TID Oral, 28 days Control: placebo	Salivation, diarrhoea, nausea, vomiting, GI distress, dizziness, increase in liver function, sweating, flatulence	CGI-I BPRS PANSS Cognitive measure (processing speed, vigilance, working memory, verbal learning, visual learning) Simpson-Angus rating scale Abnormal involuntary movement scale Barnes rating scale for drug-induced akathisia	Xanomeline significantly reduced total BPRS scores ($p < 0.05$) and total PANSS scores ($p = 0.039$) compared to placebo In the cognitive test battery, subjects in the xanomeline group showed improvements most robustly ($p < 0.05$) in measures of verbal learning and short-term memory function compared to placebo No significant difference was observed across groups in any other scales	75
Silver (1995a) [93]	Double-blind, cross-over, randomised	Intervention: biperiden 4 mg/day (2 mg BID) Oral, 2 weeks Control: amantadine Washout, 1 week	N/A	MMSE BVRT WMS	Biperiden significantly reduced BVRT ($p < 0.003$) and visual subscale of WMS ($p \leq 0.02$) score, with a trend to poorer scores on WMS total ($p = 0.086$) and the digit span ($p = 0.065$) and logical memory ($p = 0.06$) subscales. No significant difference in MMSE score between the two groups	100

Table 5 (continued)

Lead author, year	Study design	Interventions and comparisons (dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Silver (1995b) [95]	Double-blind, cross-over, randomised	Intervention: biperiden 4 mg/day (2 mg BID) Oral, 2 weeks Control: amantadine Washout, 1 week	N/A	SANS BPRS SAPS SAS	No significant difference was found between biperiden and amantadine in any of the scales	100

BID twice daily, *BPRS* Brief Psychiatric Rating Scale, *BVRT* Benton Visual Retention test, *CANTAB* Cambridge Neuropsychological Test Automated Battery, *CGI-I* Clinical Global Impression – Improvement scale, *DRS* Drooling Rating Scale, *EQ-5D* EuroQol five-dimension scale, *GIT* Gastrointestinal tract, *HADS* Hospital Anxiety and Depression Scale, *HAM-A* Hamilton Anxiety Rating Scale, *MMSE* Mini-Mental State Examination, *N/A* not available, *PAL* paired associate learning, *PANSS* The Positive and Negative Syndrome Scale, *REM* rapid eye movement, *SANS* Scale for the Assessment of Negative Symptoms, *SAPS* Scale for the Assessment of Positive Symptoms, *SAS* Simpson-Angus scale, *WMS* Wechsler Memory Scale Mental Health Self-Rating Scale, *POMS* Profile of Mood States, *QIDS-SR-16* Quick Inventory of Depressive symptomatology, *REM* rapid eye movement, *TID* three times daily, *VAS* visual analogue scale, *YMRS* Young Mania Rating Scale

[97]. All studies were performed in the USA. The number of patients varied from 8 to 66, while two studies had 343 participants (age range 60–82 years, 57.5% females) [98, 99]. Trial durations ranged from 8 days to 24 weeks, and interventions included RS-86, Lu 25-109, and xanomeline tartrate. Data for outcome measures were analysed using intention-to-treat to prevent attrition bias. Participant characteristics and outcome interventions information are summarised in Tables 3 and 6.

Two studies with RS-86, one assessing cognitive and psychiatric symptoms on the Alzheimer's Disease Assessment Scale (ADAS) [100] and another one with neuropsychiatric tests including verbal memory, a visuospatial form of memory and attention [101] did not find a significant difference in any of the scales compared to placebo. One study investigated the efficacy of Lu25-109 using the activities of the daily living scale, behaviour symptom scale and cognition subscale of ADAS, and found no significant change [102]. Two large studies of medium quality assessed xanomeline tartrate. One study found a substantial difference in the neuropsychological test battery but not in the cognitive subscale of AD [98]. In another study, psychiatric symptoms were also assessed along with cognition, and the intervention group with the highest dose significantly improved BPSD compared to placebo and lower dose groups. At the same time, ADAS-cognition score was unaffected [99].

3.6 Anxiety Disorders

To date, only two clinical trials have examined the effect of muscarinic receptor-targeted interventions in anxiety disorders – one in people with panic disorder and one in people with social anxiety disorder [70, 103] (Tables 3 and 7). The sample size in the two studies was 12 and 66 participants, respectively (age range 18–55 years, 54% female). The first study found that biperiden was effective in reducing anxiety symptom severity in people with panic disorder against placebo [70]. In contrast, the second study showed that scopolamine had no effect on self-reported social anxiety disorder symptoms. However, scopolamine caused a significant reduction in skin conductance responses, which are a less subjective measure of anxiety levels. In addition, this study examined a combination of scopolamine and exposure therapy, where scopolamine augmented extinction in severely anxious participants, highlighting the importance of muscarinic receptors in anxiety-related behaviour [103]. The same study also showed that scopolamine increased the error rate in the mnemonic similarity task at the dose of 0.5 mg, suggesting impaired cognition.

Table 6 Outcomes of dementia/Alzheimer's disease

Lead author, year	Study design	Interventions and comparator (dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Bodick (1997) [99]	6-month, randomised, double-blind, placebo-controlled, parallel-group trial	Intervention: Xanomeline Tartrate 25 mg (TID) 50 mg (TID) 75 mg (TID) Oral, 24 weeks Control: placebo	Syncope, nausea, vomiting, increased salivation, increased diaphoresis, mild to moderate increase in hepatic and biliary transaminase enzyme	ADAS-Cog CIBIC+, ADSS, NOSGER	Xanomeline at highest dose of 75 mg significantly improved CIBIC+ (p = 0.01), NOSGER score (p = 0.03) In ADSS score, higher improvement (p < 0.002) was evident for vocal outbursts, suspiciousness, delusions, agitations and hallucinations No significant improvement was observed in ADAS-cog scale compared to placebo	<30
Bruno (1986) [101]	Double blind, placebo controlled, randomised	Intervention: RS-86 Started at 0.5 mg/day Titrated up to 5 mg/day Maximum mean dose/day=4.6 mg (4–5 mg) 4 times a day 8 days oral Control: placebo Washout: 5 days	Diaphoresis, hypersalivation	Neuropsychological tests Story recall for immediate memory Word learning Dichotic listening Visual form of discrimination test Pictorial memory Recurring figures Reaction time	No significant difference between RS-86 and placebo in any of the tasks	100
Hollander (1987) [100]	Double blind, cross-over, randomised, placebo-controlled	Intervention: RS-86 Dose varies between patients till Maximum tolerated Dose range = 0.75–1.5 mg Oral, 2 weeks Control: placebo	Chills, excessive sweating, flushing, depressed mood, prolongation of P-R interval on EKG, tremor, hypersalivation, and confusion	Cognitive and noncognitive symptoms using ADAS	No statistical significance difference was observed in any of the ADAS symptomatic subscales	80 3 patients dropped out due to severe adverse events (syncope, abdominal distress, seizure)

Table 6 (continued)

Lead author, year	Study design	Interventions and comparator (dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Thal (2000) [102]	Multisite 6-month, randomised, double-blind, placebo-controlled, parallel group trial	Intervention: Lu 25-109 25 mg (TID) 50 mg (TID) 100 mg (TID) oral 24 weeks Control: placebo	Dizziness, nausea, diarrhoea, fatigue, sweating, anorexia, increased salivation, vomiting, loss of weight, and asthenia	ADAS-Cog ADCS-ADL BEHAVE-AD	No significant differences in any of the outcome measures compared with placebo	61 Higher discontinuation rate in high dose group due to adverse effects
Veroff (1998) [98]	Multisite 6-month, randomized, double-blind, placebo-controlled, parallel-group trial	Intervention: xanomeline tartrate 25 mg (TID) 50 mg (TID) 75 mg (TID) Oral, 24 weeks Control: placebo	Not reported	CNTB ADAS	Xanomeline significantly improved CNTB summary score ($p < 0.05$), but not ADAS	61

AD Alzheimer's Disease, *ADAS-Cog* The Alzheimer's Disease Assessment Scale-Cognitive subscale, *ADCS-ADL* AD in basic and instrumental activities of daily living, *ADSS* AD symptomatology scale, *BEHAVE-AD* Behavioural Pathology in AD Rating Scale, *CIBIC+* AD Cooperative Study-Clinical Global Impression of Change, *CNTB* Computerised Neuropsychological Test Battery, *NOSGER* Nurses' Observation Scale for Geriatric Patients

Table 7 Outcomes of anxiety disorders

Lead author and year	Study design	Interventions and comparisons (dose/route of administration)	Adverse effects	Main outcome measures	Key findings	Retention rate (%)
Battaglia (2001) [70]	Randomised, double-blind, cross-over	Intervention: biperiden hydrochloride 4 mg Oral, once Control: placebo Washout: 48 h	Not available	VASA Panic symptoms list-III (PSL-III-R)	Biperiden significantly reduced CO_2 exaggerated %VASA ($p \leq 0.003$) and %PSL-III-R ($p \leq 0.005$) compared to placebo	75
Craske (2019) [103]	Double-blind, randomised, placebo-controlled	Intervention: scopolamine 0.5 mg, 0.6 mg Intranasal Once Control: placebo	Drowsiness 3 patients	Paired associate learning (cue-context learning) MST SUDS	Not significant The difference was observed in SUDS during extinction and paired-associate learning task across groups, while errors were significantly increased in MST at 0.5 mg scopolamine ($p < 0.05$)	90.90

MST mnemonic similarity task, *SUDS* subjective units of distress scale, VASA visual analogue scale of anxiety
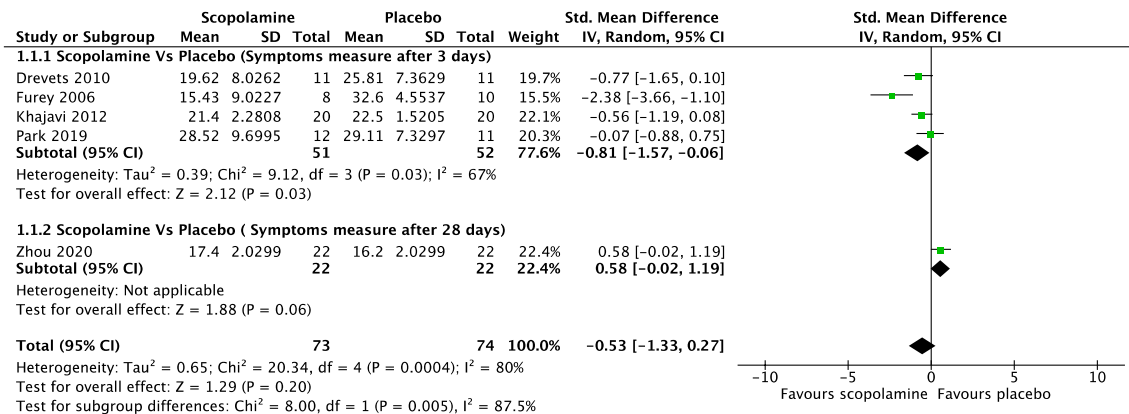

Fig. 3 Forest plot of the scopolamine's antidepressant effect in patients with mood disorders. There was no significant overall antidepressant effect ($p = 0.20$). *CI* confidence interval, *IV* inverse variance, *SD* standard deviation

3.7 Meta-Analysis

Five studies were incorporated in the meta-analysis of the antidepressant effect of scopolamine in a combined cohort of MDD and BP patients (Fig. 3). Three of the studies were crossover [74, 75, 81], and two were parallel design [80, 82]. Due to the possible carry-over effects of scopolamine, we used the last observation of pre-crossover data (following the 3rd scopolamine administration). In the parallel study, we used the 4th observation to equalise the observation parameters with crossover trials. In all four studies mentioned above, outcome measures were available after three infusion days. The results available for the fifth study were at

a different time point (after 28 days), so we performed a sub-group analysis. Overall, a non-significant anti-depressant effect was observed for studies of scopolamine compared with placebo (5 studies, $N = 147$) (SMD = -0.53; 95% CI = [$-1.33, 0.27$]; $z = 1.29$, $p = 0.20$) with substantial heterogeneity ($I^2 = 80\%$). However, when sub-group analysis was performed, all studies with a short duration of 3 days showed a significant anti-depressant effect (4 studies, $N = 103$) (SMD = -0.81; 95% CI = [$-1.57, -0.06$]; $z = 2.12$, $p = 0.03$) with substantially higher heterogeneity ($I^2 = 67\%$). This became non-significant when including the study with 28 days of observation (1 study, $N = 44$) (SMD = 0.58; 95% CI = [$-0.02, 1.19$]; z = 1.88, $p = 0.06$). Within
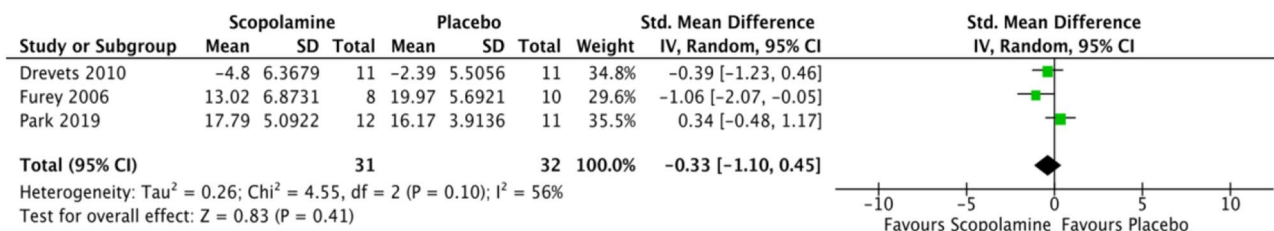
| | Scopolamine | | | Placebo | | | | Std. Mean Difference |
Study or Subgroup	Mean	SD	Total	Mean	SD	Total	Weight	IV, Random, 95% CI
Drevets 2010	−4.8	6.3679	11	−2.39	5.5056	11	34.8%	−0.39 [−1.23, 0.46]
Furey 2006	13.02	6.8731	8	19.97	5.6921	10	29.6%	−1.06 [−2.07, −0.05]
Park 2019	17.79	5.0922	12	16.17	3.9136	11	35.5%	0.34 [−0.48, 1.17]
Total (95% CI)			31			32	100.0%	−0.33 [−1.10, 0.45]

Heterogeneity: $Tau^2 = 0.26$; $Chi^2 = 4.55$, df = 2 (P = 0.10); $I^2 = 56\%$
Test for overall effect: Z = 0.83 (P = 0.41)

Fig. 4 Forest plot of the scopolamine's antianxiety effect in patients with mood disorders. There was no significant difference was found between the two treatment arms ($p = 0.41$) for the anxiolytic effect of scopolamine. *CI* confidence interval, *IV* inverse variance, *SD* standard deviation

the short-term duration studies (symptoms measured after 3 days), leave-one-out sensitivity analyses indicated significant changes in inferences leaving out study 1 [75] and study 3 [80], whereby removal of these studies increased the *p*-value ($p = 0.11$).

In contrast, scopolamine had no overall effect on anxiety in individuals with mood disorders (3 studies, SMD = − 0.33; 95% CI = [− 1.10, 0.45]; $z = 0.83$, $p = 0.41$), and moderate heterogeneity ($I^2 = 56\%$) (Fig. 4). Leave-one-out sensitivity analyses showed that omitting study 3 [81] decreased the *p*-value significantly (from 0.41 to 0.04). Using GRADE analysis, we rated the certainty of scopolamine's antidepressant and anxiolytic effect as "very low" in people with mood disorders.

3.8 Reported Adverse Events

Adverse events were most commonly reported with scopolamine treatment, even at a low dose (0.004 mg/kg). Common adverse events were dry mouth, dry skin, dry mucus membranes, constipation, drowsiness, blurred vision, light-headedness, dizziness, hypotension, fatigue, feeling drugged [74–76, 80, 81, 103]. At higher doses (0.5 mg/kg), scopolamine also showed a reduction in pulse and increased psychiatric symptoms such as visual hallucinations, disorganised speech, and cognitive impairments at the acute drug response phase [69]. Only two studies reported adverse events during biperiden treatment, including mild dry mouth (also reported in the placebo group) and constipation [84, 94]. In studies of primary psychotic disorders, xanomeline increased salivation, diarrhoea, nausea, vomiting, gastrointestinal distress, dizziness, liver function, sweating, and flatulence, likely through the activation of peripheral muscarinic acetylcholine receptors [71]. However, a few patients from the placebo group also showed some gastric disturbances. When xanomeline was combined with trospium (KarXT), side effects were minimal but included nausea, constipation, dry mouth, vomiting and dyspepsia [72]. No severe adverse incidents were stated in any of the studies. Increased salivation was the only adverse effect reported with oral RS-86 administration in MDD patients [104]. In

contrast, side effects were higher in AD patients, including, diaphoresis, hypersalivation, depressed mood, confusion, prolonged P-R interval and tremors [100].

4 Discussion

This systematic review and meta-analysis examined the therapeutic efficacy of muscarinic-targeted interventions for neuropsychiatric disorders. Here, we reviewed 33 clinical trials to examine their effectiveness in treating mood, primary psychotic, dementia, or anxiety disorders. The most common medications tested for mood disorders were the nonselective muscarinic antagonist scopolamine, and biperiden, an M_1 mAChR preferring antagonist. Meta-analysis revealed that scopolamine did not show significant improvement in depressive symptoms compared to placebo. For schizophrenia, most studies used biperiden, followed by the M_1/M_4 mAChR preferring agonist xanomeline and the antagonist scopolamine. Xanomeline significantly reduced positive and negative symptoms; however, safety was improved when combined with a peripheral mAChR antagonist trospium (KarXT). Neither scopolamine nor repeated-dose biperiden improved symptoms in people with schizophrenia. In patients with AD, treatments included RS-86, xanomeline tartrate and Lu 25-109; only xanomeline significantly improved psychiatric and behavioural symptoms of AD. No conclusive findings can be drawn for anxiety disorders as only two clinical trials were available, and neither examined the same compound.

4.1 Mood Disorders

The most substantial evidence of efficacy for muscarinic-targeted interventions was observed in mood disorders. Eight RCTs reported scopolamine's efficacy relative to placebo in patients with mood disorders regardless of diagnosis. The effect was rapid as symptom alleviation was seen 3–5 days following the first scopolamine administration [74, 105]. Compared to typical antidepressants, which can take several weeks to become effective, this time span is beneficial

to individuals with depression [74]. The rapid antidepressant effect of scopolamine was observed in both sexes; however, one study showed the magnitude was higher in women than men, which may be driven by renal elimination rate. Although there are no studies examining pharmacokinetic differences of scopolamine in men versus women, results from drugs with weak anticholinergic activity such as amantadine and digoxin suggest slower clearance in females compared to males, due to sex differences in renal tubule secretion by organic cation transporters [106]. A second possibility is p-glycoprotein transporter polymorphisms may lead to variability in efficacy [76, 107]. This potential difference may also be explained by sex differences observed in the A/T 1890 SNP in *CHRM2* [108].

One study documented substantial symptom improvement when scopolamine was combined with the selective serotonin reuptake inhibitor (SSRI) citalopram, rather than citalopram alone, despite no evidence of interactions between SSRI and anticholinergic agents [80]. On the other hand, scopolamine failed to alleviate depressive symptoms in treatment-resistant patients with higher severity Montgomery-Åsberg Depression Rating Scale scores [81]. Three reasons have been suggested to explain treatment-resistance [109]. First, pathophysiological adaptations are more serious, so standard doses are ineffective. Second, progressive neurobiological changes alter the diagnosis. For example, a patient initially presented with MDD, and subsequently BP or worsening symptoms emerged. A drug targeting the initial diagnosis may not generate an appropriate therapeutic response in such cases. Third, different underlying neurobiological mechanisms may exist between treatment-responsive and treatment-resistant patients. All the studies from Furey and colleagues displayed scopolamine's antidepressant effect at a low dose (0.004 mg) [74–79]. In contrast, Newhouse et al. (1988) showed no significant difference in Beck depression inventory score at a higher dose range (0.1–0.5 mg) [69]. One plausible reason for this difference is pseudo-resistance, where some treatments display a bell-shaped dose-response curve. Pseudo-resistance can be defined as a patient being non-responsive to the treatment, but the criteria for treatment resistance are not fulfilled [109].

Lastly, the antidepressant effect of scopolamine was non-significant in the meta-analysis, and this result contradicts the findings from another review. From the sub-group analysis, it was also evident that significant alleviation of depressive symptoms is observed after 3 days; however, after 28 days it is no longer significant [110]. Various reasons suggest extreme caution should be taken before scopolamine is recommended as a therapeutic agent. The latter review included different papers which had the same datasets, and they also mentioned this as the biggest limitation. We excluded those studies because the number of repeated participants is much higher than the newly enrolled participants. For example,

in the study with the highest sample size ($N = 62$) [78], data from 52 participants were previously reported [74–76]. Second, the significant antidepressant effect from short-duration studies of scopolamine did not survive our sensitivity analyses, which were not performed by McCaffery and colleagues. Third, GRADE analysis suggested a "very low" certainty of the evidence. Fourth, most scopolamine trials were conducted by the same group of authors. Lastly, the majority of studies were either acute or short-term problems (< 6 weeks); it should be noted that chronic use of scopolamine shows abuse liability [111] and can have significant impact on memory [112]. Therefore, while these data suggest scopolamine may help reduce depressive symptoms in individuals with mood disorders, the dose and route of administration need to be optimised to obtain a clinical response that can produce beneficial results without limiting on-target side effects. More independent replication with a larger sample size would be required to confirm the findings. In the future, novel allosteric modulators, which selectively target central M_2 or M_3 mAChR with negligible peripheral side effects might show clinical effectiveness in patients with mood disorders [113].

In addition, two RCTs examined the effectiveness of biperiden. Acute biperiden administration showed a significant antidepressant effect. However, a 6-week trial did not demonstrate the substantial antidepressant effect of biperiden compared with placebo [84]. From a clinical perspective, however, long-term M_1 receptor antagonism is likely to be problematic, as detailed below. Results from RS-86 studies showed reduction in REM-latency and increase in REM-density, suggesting a possible role of muscarinic receptors in sleep disturbances accompanied by MDD.

4.2 Primary Psychotic Disorders

Muscarinic targeted inventions showed promising results in schizophrenia and psychotic disorders, particularly alleviating positive and negative symptoms. However, there were not enough RCTs to perform a meta-analysis, highlighting the need for additional trials to ascertain the efficacy of such interventions fully. Preliminary data from studies using biperiden suggest that antagonism of the M_1 receptor produces mild to moderate impairment in learning and memory processes in subjects with primary psychotic disorders [92, 93]. As mentioned, biperiden is an M_1 mAChR preferring antagonist, and cognitive impairment results suggest that down-regulation of the M_1 receptor could be a significant contributor to cognitive symptoms of schizophrenia. Although scopolamine has been used to generate "cognitive dysfunction", it has been observed that, along with cognitive dysfunction, scopolamine also produces impairment in attention and motivation [114]. This effect is due to the non-selective nature of scopolamine. Based on these

findings, it was proposed that scopolamine could be used to model cognition dysfunction in AD. Notably, biperiden could be a "cleaner" way to develop a "cholinergic deficit model of cognitive dysfunction" [114]. Previous literature indicates that cognitive impairment may be a vulnerability marker for psychotic disorders as it is also observed in healthy people with high genetic risk [115]. On the contrary to biperiden, xanomeline, an M_1/M_4 receptor orthosteric agonist, showed significant improvement in positive and negative symptoms of schizophrenia [71]. However, the therapeutic use of xanomeline is limited due to severe gastric side effects, driven by its potential to bind to peripheral muscarinic receptors. The combination of xanomeline with trospium (KarXT) shows a similar therapeutic potential of xanomeline in treating psychotic disorders, with minor side effects compared to xanomeline alone, due to the ability of trospium to block peripheral muscarinic receptors without crossing the blood-brain barrier and thereby alleviate gastrointestinal side effects [72]. Indeed, KarXT has recently shown positive results in the Phase 3 EMERGENT-2 Trial (see https://investors.karunatx.com/news-releases/news-release-details/karuna-therapeutics-announces-positive-results-phase-3-emergent). Based on the KarXT results, M_1/M_4 receptor-targeted treatments appear to be a novel potential therapeutic approach in primary psychotic disorder patients. In addition to KarXT, a Phase 1b clinical trial is ongoing for the positive allosteric modulator of M_4 receptor, emraclidine in patients with schizophrenia and is expected to finish by 2024 (NCT05227703, NCT05227690). Notably, when targeting the orthosteric site, it is essential to consider that drug response to the orthosteric agonist of the M_1 receptor was altered in the subgroup of patients with MRDS phenotype. Still, no difference was observed in response to an allosteric agonists, suggesting that positive allosteric modulators with bias signalling may provide a better approach for all types of schizophrenic patients [116]. In this regard, there are several preclinical studies that support positive allosteric modulators of M_4 receptors as novel anti-psychotics [117, 118]. For RS-86 in schizophrenia, a significant decline in REM latency was observed, which is similar to the depression [87, 96], while the results for REM density are the opposite. The authors speculated that this REM latency reduction is due to subpopulations of people with schizophrenia also having secondary depression. In a subsequent study, no significant difference emerged when samples were split with and without secondary depression [87, 96], which suggests that REM latency measurement alone is not a sufficient marker to conclude any specific neuropsychiatric disorder.

4.3 Dementia/Alzheimer's Disease

RS-86 was ineffective for cognition and psychiatric symptoms in patients with mild to moderate AD. Even the highest

tolerable dose of 10 mg showed no significant improvement [119]. Similar to RS-86, Lu 25-109 failed to produce a significant difference in cognitive or behavioural symptoms of AD; rather, symptoms worsened at the highest dose of 300 mg, relative to placebo. A potential reason could be that Lu 25-109 also acts as an M_2/M_3 receptor antagonist, leading to a higher discontinuation rate [102]. There is evidence that xanomeline tartrate (75 mg, TID) is effective for psychiatric symptoms [99]. The results were equivocal for cognitive symptoms as improvement was observed in the neuropsychological test battery but not from the ADAS-cognition subscale [98]. Also, there was a difference in MMSE scores across treatment groups during baseline measurement, which can be a confounding factor for measuring actual treatment effect. The primary concern across most of the dementia studies is the lack of assessor's blinding information.

In summary, except for xanomeline, no other muscarinic receptor agonist with a higher selectivity for the M_1 receptor produced significant improvement in behavioural or psychiatric symptoms of dementia. These results are in line with other previous trials with oxotremorine, pilocarpine and RS-86 [102]. Favourable results from xanomeline suggest a potential role of the M_4 receptor in behavioural and psychiatric disturbances associated with dementia. The hypothesis behind antipsychotic efficacy is that activating the M_4 receptor reduces hyperdopaminergic activity, an effect confirmed in patients with schizophrenia [59]. Xanomeline may also attenuate the beta-amyloid plaque burden by increasing the secretion of non-amyloidogenic form of precursor protein and may thereby slow down the disease progression [99].

4.4 Anxiety Disorders

Results concerning anxiety disorders are equivocal. Scopolamine treatment did not significantly differ in self-reported distress in public speaking compared to placebo [103]. In contrast, scopolamine administration reduced anxiety rating scale scores in people with mood disorders relative to placebo [74]. While these preliminary studies show some promising results, no conclusions can be drawn, and additional studies are needed to determine the role of muscarinic receptors in anxiety disorders. Preclinical studies point out the possible involvement of the M_5 receptor in anxiety [63].

4.5 Strengths and Limitations

In this systematic review, all included studies were randomised (except one pseudo-randomised study), placebo-controlled and double-blind studies. These are considered to produce highest quality of evidence for medical research. A recent review showed a role for cholinergic therapeutics in depressive/manic symptoms [110]. However, they included interventions that can also target nicotinic receptors. So,

to our knowledge, this is the first systematic review with a registered protocol assessing the exclusive role of muscarinic receptor-targeted interventions in neuropsychiatric disorders. For risk of bias assessment, we used Cochrane ROB-2 software. Cochrane ROB-2 is a structured tool that helps assess bias in each domain of the study, such as trial design, conduct and reporting rather than overall RCT. To identify publication bias, we undertook sensitivity analyses. We also performed GRADE analysis for meta-analysis findings, a critical approach in assessing the certainty of evidence. A shortcoming in the available data is that only five studies were included for meta-analysis, so we cannot formally evaluate bias due to missing results (such as funnel plot generation) and meta-regression. However, sensitivity analyses allowed us to assess the robustness of the findings.

There are critical limitations in the literature reviewed in the present study. First, sample sizes were small ($N < 30$) in most included articles; caution is required as underpowered studies may increase error and reduce reliability. Second, numerous studies used different doses and routes of administration. While many studies focused on repeated dosing, some tested acute administration. Acute treatment with biperiden showed improvement in depressive symptoms, and opposite to that, studies lasting 6 weeks failed to see this same efficacy, highlighting the need for optimal treatment protocols. Third, most studies did not report the ethnicity of participants, which can be a limiting factor for the generalisability of findings [120]. Lastly, active comparators/controls are lacking in a number of studies. For instance, in dementia studies, higher rates of adverse events in the treatment but not the placebo condition may have unblinded both participants and assessors; thus, having no active placebo can compromise the integrity of the trial [98, 99].

5 Conclusion and Future Directions

While the results are not yet definitive, findings on muscarinic receptor-targeted interventions in several mental disorders are promising in efficacy and safety, specifically in treating schizophrenia, mood disorders such as MDD and BP and behavioural and psychiatric symptoms of AD. However, several methodological discrepancies question the ability to generalise findings. The most favourable evidence is the combination of M_1/M_4 agonist xanomeline and trospium (although inconclusive for meta-analysis) in schizophrenia because of a higher safety profile. Weak evidence also tentatively suggests a role for scopolamine in mood disorders, more specifically major depressive and bipolar disorders. For BPSD only xanomeline tartare showed significant improvement, but more evidence is required to validate the results.

In conclusion, the major challenge with orthosteric muscarinic receptor-targeted interventions is a wide range of peripheral adverse effects thought to be mediated via binding to M_2/M_3 receptors. The orthosteric binding site of mAChR is remarkably conserved, making it challenging to develop subtype-selective interventions. However, many allosteric ligands with biased signalling pathways are in development [16]. Positive allosteric modulators are compounds that increase the co-bound orthosteric pharmacological effect of the ligand. Allosteric modulators with biased signalling pathways can achieve subtype selectivity and be devoid of side effects (off-target or on-target), a key attribute for targeting mAChR in psychiatric disorders [121]. Comprehensive knowledge of pathways and their engagement in the disease could change the therapeutic use of muscarinic targeted interventions. Despite representing a critical target for neuropsychiatric disorders, most of the included studies in this review were less contemporary and receptor non-selective, which makes it difficult to predict clinical effectiveness. In future studies, more subtype-selective interventions are needed to establish clinical effectiveness with limited side effects.

Declarations

Conflict of interest None.

Ethics approval Not Applicable.

Consent to participate Not Applicable.

Consent for publication Not Applicable.

Availability of data and material Not Applicable.

Code availability Not Applicable.

Author's contributions AJL and LCW conceived the study; SV and AAG performed the analysis; all authors wrote the manuscript. We confirm that all authors have read and approved the final submitted manuscript and agree to be accountable for the work.

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