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Anticonvulsant activity of N-methylcytisine hydrobromide

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This study presents a comprehensive evaluation of the acute toxicity profile and anticonvulsant potential of N-methylcytisine hydrobromide using validated experimental animal models. Although N-methylcytisine hydrobromide is structurally derived from cytisine, it exhibits distinct pharmacological characteristics that differentiate it from its parent compound. Notably, N-methylcytisine functions as a competitive antagonist of nicotinic acetylcholine receptors, thereby modulating neuronal excitation processes within the central nervous system. This mechanism is considered a key contributor to its observed anticonvulsant effects. In the thiosemicarbazide-induced seizure model, administration of N-methylcytisine hydrobromide contributed to the restoration of the balance between excitatory and inhibitory neurotransmission at the level of interneuronal signaling. This regulatory effect resulted in a marked reduction in central nervous system hyperexcitability, as evidenced by a decrease in both the severity and duration of seizure activity. These findings suggest that the compound exerts a stabilizing influence on neurochemical processes associated with seizure generation. Furthermore, pronounced anticonvulsant activity was observed across multiple experimental seizure paradigms, including strychnine- and corazole-induced convulsion models. Importantly, the efficacy of N-methylcytisine hydrobromide in these models was comparable to that of established antiepileptic drugs, such as carbamazepine and convulex, which are widely used in clinical practice. Collectively, these results highlight the significant anticonvulsant potential of N-methylcytisine hydrobromide and support its consideration as a promising candidate for further development as an antiepileptic agent. Additional pharmacological and clinical investigations are warranted to fully elucidate its therapeutic profile and mechanisms of action.

Keywords: hydrobromate N-methylcytisine; gamma-aminobutyric acid; seizure models; cholinomimetic.

Introduction

Epilepsy and convulsive disorders continue to represent significant challenges in contemporary global healthcare. According to the World Health Organization (WHO), over 50 million individuals worldwide are affected by epilepsy, many of whom experience substantial social stigma, psychological distress, cognitive impairments, and restrictions in their daily and occupational activities. Notably, approximately 80% of these patients reside in low- and middle-income countries, highlighting persistent barriers to adequate healthcare, limited access to effective medications, and variability in the implementation of standardized treatment protocols (WHO, 2019). These disparities underscore the urgent need for novel therapeutic strategies and accessible interventions that can effectively manage seizure activity and improve quality of life across diverse populations.

The treatment of epilepsy is primarily aimed not only at controlling seizures but also at significantly reducing their frequency and clinical severity, prolonging seizure-free (interictal) periods, and creating favorable conditions for the patient's social, psychological, and professional adaptation. From this perspective, effective epilepsy management represents a comprehensive therapeutic strategy focused on improving quality of life, preserving functional independence, and minimizing the negative psychosocial consequences and stigmatization associated with the disorder. In achieving these objectives, anticonvulsant (antiepileptic) medications occupy a central and indispensable role. Their therapeutic efficacy is based on targeted modulation of the neurophysiological mechanisms underlying epileptic activity (Azamatov et al., 2025). Specifically, these agents act to restore and maintain the physiological balance between excitatory and inhibitory processes within the central nervous system, thereby reducing neuronal hyperexcitability and preventing the generation and propagation of pathological electrical discharges.

At the molecular and synaptic levels, antiepileptic drugs exert their effects through several principal mechanisms. These include the enhancement of inhibitory γ-aminobutyric acid (GABA)–mediated neurotransmission, attenuation of excitatory glutamatergic signaling, and modulation of voltage-gated ion channels – particularly sodium, calcium, and potassium channels – that are critical for neuronal firing and synaptic transmission (Azamatov et al., 2023). Through these mechanisms, anticonvulsant therapy stabilizes neuronal membranes, suppresses epileptogenic networks, and limits the spread of epileptic activity to adjacent brain regions. Consequently, anticonvulsant pharmacotherapy constitutes the cornerstone of pathogenetically oriented epilepsy treatment. Beyond seizure suppression, it plays a crucial role in supporting long-term neurological stability, facilitating psychological well-being, and promoting successful social integration of individuals living with epilepsy (Mortezaei et al., 2013).

Although classical anticonvulsant agents – such as carbamazepine, valproic acid, phenytoin, phenobarbital, and related compounds – remain effective in a wide range of clinical contexts, their use is frequently constrained by a number of significant limitations. These agents are commonly associated with adverse effects, including sedation and lethargy, tremor, hepatotoxicity, and hematological abnormalities, which may negatively affect treatment adherence and overall quality of life. In addition, the substantial physiological burden imposed by long-term administration, together with clinically relevant drug–drug interactions, further restricts their therapeutic utility, particularly in patients requiring polypharmacy or lifelong treatment (Perucca & Gilliam, 2012). In light of these challenges, the development of a new generation of anticonvulsant agents has emerged as a critical scientific

and practical priority in contemporary neuropharmacology. This paradigm shift reflects a growing emphasis on precision medicine and the need for therapies that are not only effective in seizure control but also better tolerated and safer during prolonged use. Modern research efforts are increasingly focused on identifying compounds that selectively target specific molecular and cellular mechanisms involved in epileptogenesis, thereby minimizing nonspecific central nervous system effects.

The new generation of anticonvulsant drugs is expected to offer several key advantages over classical agents. First, they are designed to possess well-defined molecular, target-oriented mechanisms of action, allowing more precise modulation of pathological neuronal networks. Second, these drugs are anticipated to demonstrate reduced systemic toxicity and more favorable metabolic profiles, thereby decreasing the risk of cumulative organ damage during long-term therapy. Third, minimal drug–drug interactions are a major objective, particularly for patients with comorbid conditions who require complex pharmacological regimens. Finally, next-generation anticonvulsants aim to provide sustained and stable therapeutic efficacy, ensuring reliable seizure control without progressive loss of effectiveness over time.

Taken together, these advances underscore the strategic importance of developing innovative anticonvulsant therapies that address the limitations of traditional drugs while aligning with modern principles of safety, tolerability, and long-term disease management in epilepsy. In addition to rational drug design and molecular screening, the use of experimental animal models constitutes a fundamental component in the development and preclinical evaluation of new anticonvulsant agents. Animal models of epilepsy provide a controlled and reproducible experimental framework for investigating the pathophysiological mechanisms of seizure generation and propagation, as well as for assessing the pharmacodynamic and pharmacokinetic properties of candidate compounds.

Through the application of these models, it becomes possible to systematically evaluate the anticonvulsant activity of novel substances across different types of experimentally induced seizures, thereby determining their spectrum of efficacy. Moreover, preclinical studies enable the identification of the therapeutic window by comparing effective doses with doses that produce adverse or toxic effects. This approach allows researchers to assess the safety profile of a compound, including its impact on motor function, cognition, behavior, and vital physiological systems, long before human exposure occurs.

Experimental animal studies also play a critical role in optimizing dosage regimens and routes of administration, as well as in identifying potential mechanisms of action at the cellular and network levels. Importantly, data obtained from such studies form the scientific basis for subsequent clinical trials, guiding the selection of initial dosing strategies and safety monitoring parameters in humans. In this way, animal models serve as an essential translational bridge between basic neuropharmacological research and clinical application, helping to ensure that only compounds with a favorable balance of efficacy and safety progress to clinical testing (McNamara et al., 2023).

Overall, the integration of experimental animal models into anticonvulsant drug development allows a comprehensive, evidence-based assessment of therapeutic potential, reduces the risks associated with clinical translation, and significantly enhances the likelihood of successful and safe implementation of new antiepileptic therapies in medical practice.

In our previous studies, we have systematically investigated the biological activities of various N-methylcytisine derivatives, providing a comprehensive understanding of their pharmacological and toxicological properties (Azamatov et al., 2022; Aytbaeva et al., 2025; Aytmuratova et al., 2025; Sanoeva et al., 2025a, 2025b). These investigations encompassed a wide range of experimental approaches, including *in vitro* and *in vivo* assays, aimed at evaluating the compounds' acute and chronic toxicity, receptor-binding profiles, and functional effects on neuronal and non-neuronal systems. Notably, these studies demonstrated the capacity of N-methylcytisine derivatives to modulate neurotransmitter activity, particularly within the central nervous system, highlighting their potential to influence excitatory and inhibitory signaling pathways. Furthermore, the research provided insights into structure-activity relationships, identifying key chemical modifications that enhance pharmacological efficacy while minimizing adverse effects, thereby establishing a solid foundation for ongoing and future pharmacological studies.

To comprehensively evaluate the spectrum of anticonvulsant properties, the newly synthesized derivatives of N-methylcytisine hydrobromide were systematically investigated using a series of experimental seizure models representing distinct pathogenic mechanisms. These models were selected to capture a broad range of seizure types, including those mediated by excitatory neurotransmitter imbalances and receptor-specific hyperexcitability. By employing diverse experimental paradigms, the study aimed to elucidate both the efficacy and the mechanistic underpinnings of the compounds' anticonvulsant activity, providing critical insights into their potential therapeutic applications in the management of various seizure disorders.

Materials and methods

The object of the study is a semi-synthetic compound isolated from the plant *Thermopsis alterniflora* (Fabaceae) at the Alkaloid Chemistry Laboratory of the Institute of Chemistry of Plant Substances named after Academician S. Y. Yunusov, Academy of Sciences of the Republic of Uzbekistan, under the supervision of Candidate of Chemical Sciences, Senior Researcher V. I. Vinogradova and S. B. Rahimov. This compound was obtained through chemical modification of natural alkaloids, and studying its pharmacological properties became one of the main objectives of this research.

For the experimental studies, sexually healthy white laboratory mice weighing 18–22 g were selected. The animals were kept under standard vivarium conditions with a 12/12-hour natural light–dark cycle and an ambient temperature of 22 ± 2 °C. Feeding was carried out in accordance with nutritional standards for rodents. The drinking water provided to the animals was pre-tested for sanitary-chemical and bacteriological indicators, and the mice had continuous free access to it through special drinking containers.

All experiments were conducted in accordance with humane principles for the treatment of animals and in strict compliance with the international ethical standards set forth by the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (ETS No. 123) (Directive 2010/63/EU, 2010). During the research process, additional pain to the animals was avoided, their stress levels were minimized, and the stability of experimental conditions was maintained.

To model seizure syndrome, several convulsant agents that stimulate the central nervous system through different mechanisms were used. In particular, an aqueous solution of arecoline was administered subcutaneously at a dose of 10.0 mg/kg, an aqueous solution of nicotine was administered subcutaneously at a dose of 10.0 mg/kg, and an aqueous solution of corazol (pentylenetetrazol) was administered subcutaneously at a dose of 80.0 mg/kg. An aqueous solution of bicuculline was administered subcutaneously at a dose of 2.7 mg/kg, an aqueous solution of picrotoxin at a dose of 5.0 mg/kg, and an aqueous solution of strychnine at a dose of 1.2 mg/kg. Also, an aqueous solution of isoniazid was given orally at a dose of 200.0 mg/kg, and an aqueous solution of thiosemicarbazide was administered subcutaneously at a dose of 28.0 mg/kg. Camphor was administered intraperitoneally at a dose of 1000.0 mg/kg to induce seizure activity.

These methods, based on disturbances in various neurotransmitter systems, made it possible to assess the balance between excitation and inhibition within seizure models. The experimental animals were divided into groups, with each group consisting of 6 white mice. The studies assessing the anticonvulsant activity of the cytisine derivative N-methylcytisine hydrobromide were carried out in accordance with the "Guidelines for Preclinical (Experimental) Studies of New Pharmacological Agents.

The test compound was administered orally at doses of 3.0, 5.0, 10.0, 15.0, and 20.0 mg/kg. For comparison, antiepileptic agents widely used in clinical practice were employed: Carbamazepine (LLC "Ukraine") at doses of 5.0 and 10.0 mg/kg, and convulex (Gerot Pharmazeutika, Vienna, Austria) at doses of 75.0 and 100.0 mg/kg,

administered orally. All epileptogenic substances were administered to the animals 60 minutes after the test compound. This approach took into account the formation period of the test compound's pharmacokinetic effect and ensured reliable comparison of the results.

Data was calculated as mean (x) ± standard deviation (SD). A one-way analysis of variance (ANOVA) test was applied considering $P < 0.05$ as statistically significant. In the tables, different letters indicate values which reliably ($P < 0.05$) differed one from another within one column of the table according to the results of comparison using the Tukey test.

The acute toxicity of N-methylcytisine hydrobromide was investigated under controlled laboratory conditions using 60 white mice with a body weight ranging from 18 to 20 g. The animals were randomly assigned to experimental groups, each comprising six mice, in order to ensure statistical reliability and reproducibility of the results. The test compound was administered orally via a non-traumatic metal gastric probe at incrementally increasing doses of 50, 100, 150, 200, 250, 280, 300, 350, and 400 mg/kg of body weight. Following administration, all animals were closely monitored for a period of 14 days to record mortality, behavioral alterations, and clinical manifestations of acute toxicity. Based on the obtained experimental data, the median lethal dose (LD_{50}) of N-methylcytisine hydrobromide was calculated to be 280.0 mg/kg, with a confidence interval of 243.4–406.0 mg/kg. According to the toxicological classification criteria outlined in GOST 12.1.007–76, the investigated compound was classified as a hazard Class 3 substance, indicating low toxicity. Statistical processing and analysis of the experimental results, including LD_{50} determination, were performed using IBM SPSS Statistics developed by IBM Corp., which ensured standardized and validated analytical procedures (GOST 12.1.007–76, 2007).

In parallel with the toxicological assessment, the anticonvulsant activity of N-methylcytisine hydrobromide was evaluated using a comprehensive set of quantitative and qualitative parameters. These included the latency to seizure onset, expressed in seconds or minutes, which reflects the compound's ability to delay the initiation of convulsive activity. In addition, the total number of seizure episodes recorded throughout the entire observation period was analyzed as an indicator of overall seizure frequency. The duration of seizures, measured in seconds or minutes, was used to evaluate the severity and persistence of convulsive manifestations. Furthermore, survival outcomes were systematically documented by recording the number of animals that survived or died in each experimental group. Collectively, these indicators provided a comprehensive evaluation of the anticonvulsant efficacy of the studied compound, allowing an integrated assessment of both its protective effects against seizures and its impact on animal survival.

Results

Effect of N-methylcytisine hydrobromide in the model of arecoline-induced tremor and salivation. In the control group administered arecoline at a dose of 10.0 mg/kg, the latent period before the onset of tremor was short (an average of 2.1 ± 1.2 minutes), while the tremor duration was long (18.7 ± 1.8 minutes). All animals exhibited pronounced salivation (excessive drooling). These indicators confirm the strong stimulating effect of arecoline on the central cholinergic system. The reference drugs – carbamazepine and convulex – significantly reduced tremor duration while slightly prolonging the latent period. Specifically, a 5.0 mg/kg dose of carbamazepine reduced tremor duration by 27.8%, and a 10.0 mg/kg dose reduced it by 24.0%. Convulex at doses of 75.0 and 100.0 mg/kg also demonstrated comparable anticonvulsant activity (20.8–24.5% effectiveness). In these groups, the frequency of observed sialorrhea ranged between 33.3% and 66.7%.

When N-methylcytisine hydrobromide was tested at different doses, its anticonvulsant activity showed a clear dose-dependent pattern. At a dose of 3.0 mg/kg, the duration of tremor decreased by 24.0%, while administration at 5.0 mg/kg increased this reduction to 31.0%. The highest activity of the compound was observed in animals that received doses of 10.0 and 15.0 mg/kg, with tremor duration reduced by 35.2% and 33.6%, respectively. At these doses, the test compound demonstrated effectiveness not only comparable to convulex but, in some cases, superior to that of carbamazepine.

At the same time, when administered at higher doses (20 mg/kg), the anticonvulsant effectiveness was 20.3%, indicating the presence of a dose-dependent optimal therapeutic range. Salivation decreased when the compound was administered at the optimal doses of 10 and 15 mg/kg. The experimental results obtained are presented in detail in Table 1.

Table 1

Effect of N-methylcytisine hydrobromide on the tremorogenic action of arecoline (mean ± SD, n = 6)

Group	Dose, mg/kg	Latent period of tremor onset, min	Tremor duration, min	Effect, %	Salivation, %	
					+	–
Control (arecoline)	0.2	2.1 ± 1.2^{a}	18.7 ± 1.8^{c}	–	100	0
Carbamazepine	5.0	4.0 ± 0.8^{b}	13.5 ± 2.1^{a}	27.8	33.3	66.7
	10.0	3.3 ± 1.0^{ab}	14.2 ± 1.6^{ab}	24.0	16.7	83.3
Convulex (per os)	75.0	2.9 ± 0.9^{a}	14.8 ± 1.4^{b}	20.8	33.3	66.7
	100.0	3.1 ± 0.7^{ab}	14.1 ± 1.9^{ab}	24.5	33.3	66.7
N-methylcytisine hydrobromide	3.0	3.2 ± 0.7^{ab}	14.2 ± 1.5^{ab}	24.0	33.3	66.7
	5.0	3.5 ± 0.4^{ab}	12.9 ± 2.1^{ab}	31.0	33.3	66.7
	10.0	4.3 ± 1.3^{b}	12.1 ± 1.3^{a}	35.2	16.7	83.3
	15.0	3.6 ± 1.5^{ab}	12.4 ± 2.0^{ab}	33.6	16.7	83.3
	20.0	2.8 ± 0.7^{ab}	14.9 ± 1.7^{b}	20.3	33.3	66.7

Note: different letters indicate values which reliably ($P < 0.05$) differed one from another within one column of the table according to the results of comparison using the Tukey test.

The arecoline model is a reliable experimental method for assessing the excitability of the central cholinergic system. The rapid onset and long duration of tremor, along with pronounced sialorrhea in the control group, confirmed the cholinomimetic effect of arecoline. In this model, N-methylcytisine hydrobromide demonstrated significant anticonvulsant activity by reducing tremor duration and prolonging the latency period. Its effectiveness is associated with competitive blockade of nicotinic acetylcholine receptors, reduction of cholinergic excitation, and attenuation of acetylcholine action. The decrease in salivation further confirms the compound's cholinolytic properties. These findings indicate that N-methylcytisine hydrobromide is a promising anticonvulsant agent suitable for recommendation for future clinical studies.

The nicotine-induced convulsion test was performed by administering nicotine subcutaneously at a dose of 10.0 mg/kg, 60 minutes after oral administration of the test compound and reference drugs. During the experiment, the onset and severity of tremors, the appearance of the Straub phenomenon (rigid upward tail elevation), the occurrence of the "wild running" phase, and the characteristics of the development of clonic–tonic seizure episodes were evaluated (Parasuraman, 2011).

In the control group, when nicotine was administered subcutaneously at a dose of 10.0 mg/kg, seizure activity developed rapidly: the latent period was only about 40 seconds, and tremor and convulsions lasted an average of 13.5 ± 0.6 minutes. Most animals died due to

severe seizures, with a survival rate of 16.7%. These findings confirm the strong excitatory effect of nicotine on the central nervous system.

When carbamazepine, selected as a reference drug, was administered to the experimental animals, it significantly reduced tremor duration and increased survival rates. Specifically, at a dose of 5 mg/kg, the drug reduced tremor duration by 54.8% and ensured a survival rate of 66.7%; at a dose of 10 mg/kg, its effectiveness reached 65.1%, with survival increasing to 83.3%. Although convulex also exhibited anticonvulsant activity, its effectiveness was slightly lower compared to carbamazepine (52.3–55.5%), and the survival rate of the animals remained within the range of 50.0–66.7%. The effect of the test compound, N-methylcytisine hydrobromide, in the nicotine model was dose-dependent. At a dose of 3.0 mg/kg, the compound reduced tremor duration by 61.4%. As the dose increased, the effectiveness rose significantly. The highest activity of the compound was observed at doses of 10 and 15 mg/kg. At these doses, tremor duration decreased by 71.8% and 73.3%, respectively, while the survival rate of the animals reached 83.3%. These results indicate that N-methylcytisine hydrobromide has an anticonvulsant effect comparable to that of carbamazepine and, in some parameters, even superior to it. At a dose of 20 mg/kg, the effectiveness was 62.9%, which confirms the presence of an optimal therapeutic dose range for this compound. The experimental results are presented in detail in Table 2.

Table 2

Effect of N-methylcytisine hydrobromide on the tremorogenic and convulsive action of nicotine (mean ± SD, n = 6)

Group	Dose, mg/kg	Latency period of seizure onset, min.	Duration of tremor and seizures, min	Effect, %	Animal survival, %
Control (Nicotine)	10.0	40.00 ± 0.03^{f}	13.5 ± 0.6^{d}	–	16.7
Carbamazepine (per os) +	5.0	1.57 ± 0.07^{b}	6.1 ± 0.4^{bc}	54.8	66.7
Nicotine 10.0 mg/kg s.c.	10.0	1.50 ± 0.02^{b}	4.7 ± 0.7^{ab}	65.1	83.3
Convulex	75.0	1.43 ± 0.04^{a}	6.4 ± 0.8^{bc}	52.3	50.0
	100.0	1.50 ± 0.06^{ab}	6.0 ± 0.2^{c}	55.5	66.7
N-methylcytisine hydrobromide (per os) + Nicotine 10.0 mg/kg s.c.	3.0	2.00 ± 0.03^{c}	5.2 ± 0.1^{b}	61.4	50.0
	5.0	1.58 ± 0.08^{b}	4.5 ± 0.3^{ab}	66.6	66.7
	10.0	2.10 ± 0.05^{d}	3.8 ± 0.4^{a}	71.8	83.3
	15.0	2.26 ± 0.06^{e}	3.6 ± 0.7^{a}	73.3	83.3
	20.0	1.55 ± 0.02^{b}	5.0 ± 0.8^{b}	62.9	66.7

Note: see Table 1.

The experimental results showed that the nicotine model is a reliable method for evaluating the excitability of nicotinic receptors in the central nervous system. In this model, N-methylcytisine hydrobromide, acting as a competitive antagonist of nicotinic receptors, reduced excessive neuronal excitability. The optimal doses of 10–15 mg/kg prolonged the onset time of tremors and convulsions, significantly reduced their duration, and increased the survival rate of the animals. These findings confirm the compound's clear anticonvulsant activity.

The proconvulsant effects of corazol, picrotoxin, and bicuculline are explained by their blockade of specific sites within the ion channel associated with GABA receptors. Because these substances inhibit the influx of chloride ions through the channel, inhibitory (GABAergic) transmission in neurons is reduced, leading to a sharp increase in excitability within the central nervous system. When administered subcutaneously, all of these agents induced clonic–tonic seizure episodes in 100% of the experimental animals.

In the corazol model, the anticonvulsant effect of N-methylcytisine hydrobromide was assessed under conditions where corazol, administered subcutaneously at a dose of 80 mg/kg to control animals, produced rapid and pronounced seizure activity: the latent period of seizures was 5.3 ± 0.8 minutes, and convulsions lasted an average of 18.6 ± 1.6 minutes. The survival rate among the animals was low – only 16.7%. These findings confirm that corazol exerts a strong convulsive effect by blocking GABA receptors in the central nervous system.

Carbamazepine, selected as the reference drug, significantly reduced the duration of convulsions. At a dose of 5 mg/kg, it decreased seizure duration by 54.8% and ensured a survival rate of 50.0%. When administered at 10 mg/kg, its effectiveness increased, with survival reaching 66.7%. Convulex (from the valproate group) also exhibited anticonvulsant activity; however, its effectiveness was slightly lower than that of carbamazepine (49–51%), and the survival rate remained within the range of 50.0–66.7%.

In the corazol model, the anticonvulsant activity of the test compound, N-methylcytisine hydrobromide, increased consistently in a dose-dependent manner. At a dose of 3.0 mg/kg, the latent period was prolonged to 10.2 ± 0.5 minutes, indicating a delayed onset of seizures. As the dose increased, the duration of convulsions decreased even further. The most pronounced anticonvulsant effect of the compound was observed at doses of 10 and 15 mg/kg: effectiveness reached 59.7% and 66.6%, respectively, and the duration of convulsions decreased to 7.5 ± 0.8 and 6.2 ± 1.6 minutes. Importantly, at these doses the survival rate of the animals was high – 83.3%. At a dose of 20 mg/kg, the effectiveness was 38.7%, which indicates the presence of an optimal therapeutic range for the compound. The experimental results are presented in detail in Table 3.

Table 3

Effect of N-methylcytisine hydrobromide on the convulsant action of corazol (mean ± SD, n = 6)

Group	Dose, mg/kg	Latent period of seizure onset, min	Duration of seizures, min.	Effect, %	Animal survival, %
Control (corazol)	0.2	5.3 ± 0.8^{a}	18.6 ± 1.6^{d}	–	16.7
Carbamazepine (per os) +	5.0	8.5 ± 0.5^{b}	8.4 ± 2.1^{ab}	54.8	50.0
Corazol 80.0 mg/kg s.c.	10.0	8.7 ± 1.2^{b}	7.9 ± 1.2^{a}	57.5	66.7
Convulex (per os) +	75.0	8.3 ± 0.9^{b}	9.4 ± 0.9^{b}	49.4	50.0
Corazol 80 mg/kg s.c.	100.0	8.4 ± 1.0^{b}	9.1 ± 1.3^{b}	51.0	66.7
N-methylcytisine hydrobromide (per os) + Corazol 80.0 mg/kg s.c.	3.0	10.2 ± 0.5^{c}	11.3 ± 1.9^{c}	39.1	66.7
	5.0	11.0 ± 0.8^{cd}	10.3 ± 1.7^{bc}	44.5	83.3
	10.0	11.3 ± 1.3^{cd}	7.5 ± 0.8^{a}	59.7	83.3
	15.0	12.0 ± 0.7^{d}	6.2 ± 1.6^{a}	66.6	83.3
	20.0	10.8 ± 1.0^{cd}	11.4 ± 1.2^{c}	38.7	83.3

Note: see Table 1.

According to the study results, the corazol model is considered a reliable experimental method for evaluating seizure syndrome under conditions of reduced GABAergic inhibition. Under these conditions, N-methylcytisine bromide delayed the onset of seizures, significantly reduced their duration, and increased the survival rate of the animals. At optimal doses (10–15 mg/kg), it demonstrated anticonvulsant activity comparable to, or in some parameter's superior to, carbamaze-

pine and convulex. The findings confirm that the compound may be used in further studies as a promising antiepileptic agent.

Effect of N-methylcytisine bromide in a picrotoxin-induced seizure model. In the control group, where picrotoxin was administered subcutaneously at a dose of 5 mg/kg, seizure symptoms developed rapidly, with an average latent period of 8.4 ± 3.7 minutes. Each animal exhibited an average of 13.2 ± 1.3 seizure episodes, with a total seizure duration of approximately 34.5 ± 8.5 minutes. All animals in this group died, and the survival rate was 0%.

In the groups receiving carbamazepine orally at doses of 5–10 mg/kg, selected as the reference drug, a significant reduction in seizure activity was observed. At a dose of 5 mg/kg, the latent period was 15.2 ± 3.3 minutes, the number of seizures averaged 5.3 ± 1.5 episodes, and their duration was 24.7 ± 7.2 minutes. The number of surviving animals was 5 (50%). At a dose of 10 mg/kg, similar results were recorded: the latent period was 16.4 ± 3.5 minutes, the number of seizures 5.1 ± 1.9 episodes, and their duration 21.6 ± 6.2 minutes, with 4 animals (40%) surviving.

Anticonvulsant efficacy was also observed in the experimental animals that received convulex, selected as a reference drug, at doses of 75.0–100.0 mg/kg per os. At a dose of 75.0 mg/kg, the latent period was 14.9 ± 2.9 minutes, the number of seizures averaged 5.9 ± 1.6 episodes, and their duration was 26.2 ± 8.4 minutes. At a dose of 100 mg/kg, the latent period was 15.3 ± 3.6 minutes, the number of seizures 5.5 ± 1.4 episodes, and their duration 25.9 ± 7.2 minutes. In both cases, half or more of the animals (50–60%) survived.

The results observed in experimental animals that received the test compound, N-methylcytisine bromide, orally at various doses (3–20 mg/kg) demonstrated a dose-dependent effect. At a dose of 3 mg/kg, the latent period was 15.8 ± 3.4 minutes, the number of seizures averaged 5.8 ± 1.2 episodes, and their duration was 23.1 ± 7.1 minutes. At a dose of 5 mg/kg, the number of seizures decreased to 4.1 ± 1.6 episodes, with a duration of 22.7 ± 8.6 minutes. At a dose of 10 mg/kg, the best results were observed: the latent period was 17.5 ± 3.0 minutes, the number of seizures was 4.8 ± 1.5 episodes, and their duration was 18.7 ± 8.5 minutes; in this group, 5 animals survived, and mortality decreased to 10%. At a dose of 15.0 mg/kg, similar effects were maintained: the latent period was 18.7 ± 3.4 minutes, the number of seizures was 4.2 ± 1.3 episodes, and their duration was 19.2 ± 7.5 minutes. At a dose of 20.0 mg/kg, a slight attenuation of the effect was noted: the latent period was 16.4 ± 3.8 minutes, the number of seizures was 5.7 ± 1.8 episodes, and their duration was 21.9 ± 6.5 minutes. The experimental results are presented in detail in Table 4.

Table 4

Effect of N-methylcytisine bromide on seizures induced by picrotoxin (mean ± SD, n = 6)

Animal groups (n = 6)	Dose, mg/kg	Latent period, min	Number of seizures per animal	Duration, min	Animal survival in the group	Animal mortality in the group
Control (Picrotoxin)	0.2	8.4 ± 3.7[a]	13.2 ± 1.3[b]	34.5 ± 8.5[b]	0	6
Carbamazepine (per os) +	5.0	15.2 ± 3.3[b]	5.3 ± 1.5[a]	24.7 ± 7.2[a]	5	1
Picrotoxin 5.0 mg/kg s.c.	10.0	16.4 ± 3.5[b]	5.1 ± 1.9[a]	21.6 ± 6.2[a]	4	2
Convulex (per os) +	75.0	14.9 ± 2.9[b]	5.9 ± 1.6[a]	26.2 ± 8.4[a]	3	3
Picrotoxin 5.0 mg/kg s.c.	100.0	15.3 ± 3.6[b]	5.5 ± 1.4[a]	25.9 ± 7.2[a]	5	1
	3.0	15.8 ± 3.4[b]	5.8 ± 1.2[a]	23.1 ± 7.1[a]	5	1
N-methylcytisine	5.0	16.2 ± 2.8[b]	4.1 ± 1.6[a]	22.7 ± 8.6[a]	4	2
bromide (per os) +	10.0	17.5 ± 3.0[b]	4.8 ± 1.5[a]	18.7 ± 8.5[a]	5	1
Picrotoxin 5.0 mg/kg s.c.	15.0	18.7 ± 3.4[b]	4.2 ± 1.3[a]	19.2 ± 7.5[a]	5	1
	20.0	16.4 ± 3.8[b]	5.7 ± 1.8[a]	21.9 ± 6.5[a]	4	2

Note: see Table 1.

Thus, the results of the conducted experiments demonstrated that N-methylcytisine bromide exhibits anticonvulsant activity in the picrotoxin-induced seizure model. The test compound delayed the onset of seizures, reduced their number and duration, and increased the survival rate of the animals. The optimal therapeutic effect was observed at doses of 10–15 mg/kg. The obtained results were statistically significant ($P \leq 0.05$), providing a basis for considering this compound as a promising antiepileptic agent.

In the bicuculline-induced seizure model, the effect of N-methylcytisine bromide was evaluated. In the control group (bicuculline 2.7 mg/kg, s.c.), seizures developed rapidly, with an average latent period of 4.2 ± 0.8 minutes. The duration of seizures was approximately 15.6 ± 0.8 minutes. Since no anticonvulsant agent was administered in this group, no animals survived (0%). This finding confirms the strong convulsant effect of bicuculline.

In the experimental animals receiving carbamazepine orally at doses of 5–10 mg/kg, selected as the reference drug, a significant reduction in seizure activity was observed. At a dose of 5 mg/kg, the onset of seizures was delayed to 6.8 ± 0.5 minutes, and their duration was reduced to 8.5 ± 1.2 minutes. This corresponded to an effectiveness of 45.5%, with 66.7% of the animals surviving. At a dose of 10 mg/kg, the latent period further increased to 7.5 ± 1.2 minutes, while seizure duration decreased to 7.7 ± 2.1 minutes, resulting in an effectiveness of 50.5% and a survival rate of 83.3%.

Convulex, used as a reference drug, also demonstrated similar anticonvulsant activity when administered *per os* at doses of 75–100 mg/kg. At a dose of 75 mg/kg, the latent period was 6.1 ± 0.9 minutes, the seizure duration was 8.2 ± 0.9 minutes, and the effectiveness reached 47.4%. At a dose of 100 mg/kg, the latent period increased to 6.6 ± 1.0 minutes, while the duration decreased to 7.6 ± 1.3 minutes, resulting in an effectiveness of 51.2%. At both doses, the survival rate was 66.7%.

When the test compound, N-methylcytisine bromide, was administered *per os* at various doses (3–5–10–15–20 mg/kg), its effect showed a clear dose-dependent relationship. At a dose of 3 mg/kg, the latent period increased to 8.4 ± 0.5 minutes, the seizure duration decreased to 8.5 ± 1.7 minutes, and the effectiveness reached 45.5%. The survival rate in this group was 66.7%. At a dose of 5 mg/kg, the latent period was 8.2 ± 0.8 minutes, the seizure duration was 7.9 ± 1.5 minutes, and the effectiveness reached 49.3%. The highest effectiveness of the compound was observed at doses of 10–15 mg/kg. In animals receiving 10 mg/kg, the latent period was 9.0 ± 1.3 minutes, the seizure duration was 7.1 ± 0.8 minutes, the effectiveness reached 54.4%, and the survival rate was 83.3%. At a dose of 15 mg/kg, the latent period was 9.7 ± 0.7 minutes, the seizure duration was 6.3 ± 1.6 minutes, the effectiveness reached 59.6%, and the survival rate was 83.3%. These findings indicate that N-methylcytisine bromide effectively delays the onset of seizures, significantly reduces their duration, and increases the survival rate of the animals. At a dose of 20 mg/kg, the latent period was 8.1 ± 1.0 minutes, the duration was 8.7 ± 1.2 minutes, the effectiveness reached 44.2%, and the survival rate was 66.7%. The detailed results of the experiment are presented in Table 5.

The results of the conducted experiments indicated that N-methylcytisine bromide exhibits distinct anticonvulsant activity in the bicuculline-induced seizure model. The optimal therapeutic range was observed at doses of 10–15 mg/kg. At these doses, the compound significantly delayed the onset of seizures, reduced their duration, and maximized the survival rate of the animals. These findings provide a scientific basis for considering N-methylcytisine bromide as a promising antiepileptic agent.

Effect of N-methylcytisine bromide in the strychnine-induced seizure model. In the control group, the animals received strychnine at a dose of 1.2 mg/kg s.c., and seizures developed rapidly, with an aver-

age latent period of 10.7 ± 0.9 minutes and a duration of 0.58 ± 2.10 minutes; the survival rate was 0%. This outcome confirms the strong convulsant properties of strychnine.

Carbamazepine, selected as the reference drug, was administered orally at doses of 5–10 mg/kg, and 60 minutes later strychnine was injected subcutaneously at a dose of 1.2 mg/kg. In these animals, the seizure process was noticeably alleviated. When the drug was administered at a dose of 5 mg/kg, the latent period was extended to 13.0 ± 1.4 minutes, the duration was reduced to 0.46 ± 2.60 minutes, the effectiveness was 20.7%, and the survival rate of the animals was 33.3%. When the drug was administered at a dose of 10 mg/kg, the latent period reached 13.4 ± 1.7 minutes, the duration was 0.44 ± 1.90 minutes, the effectiveness was 24.1%, and the survival rate increased to 50.0%.

Table 5

Effect of N-methylcytisine bromide on the bicuculline-induced seizure model (mean ± SD, n = 6)

Group	Dose, mg/kg	Latent period of seizure onset, min	Seizure duration, min	Effect, %	Survival, %
Control (bicuculline) s.c.	0.2	4.2 ± 0.8^{a}	15.6 ± 0.8^{c}	–	0
Carbamazepine (per os) +	5.0	6.8 ± 0.5^{b}	8.5 ± 1.2^{b}	45.5	66.7
bicuculline 2.7 mg/kg s.c.	10.0	7.5 ± 1.2^{bc}	7.7 ± 2.1^{ab}	50.5	83.3
Convulex (per os) +	75.0	6.1 ± 0.9^{b}	8.2 ± 0.9^{ab}	47.4	66.7
bicuculline 2.7 mg/kg s.c.	100.0	6.6 ± 1.0^{b}	7.6 ± 1.3^{ab}	51.2	66.7
	3.0	8.4 ± 0.5^{c}	8.5 ± 1.7^{ab}	45.5	66.7
N-methylcytisine	5.0	8.2 ± 0.8^{c}	7.9 ± 1.5^{ab}	49.3	66.7
bromide (per os) +	10.0	9.0 ± 1.3^{cd}	7.1 ± 0.8^{a}	54.4	83.3
bicuculline 2.7 mg/kg s.c.	15.0	9.7 ± 0.7^{d}	6.3 ± 1.6^{a}	59.6	83.3
	20.0	8.1 ± 1.0^{bc}	8.7 ± 1.2^{ab}	44.2	66.7

Note: see Table 1.

The reference drug convulex (valproate) also demonstrated a dose-dependent positive effect. At a dose of 75 mg/kg, the latent period reached 14.2 ± 0.9 minutes, the seizure duration decreased to 0.40 ± 2.30 minutes, the effectiveness was 31.0%, and the survival rate reached 50.0%. At a dose of 100 mg/kg, the latent period further increased to 15.9 ± 1.2 minutes, the duration declined to 0.38 ± 1.50 minutes, the effectiveness reached 34.4%, and the survival rate increased to 66.7%.

When the test compound N-methylcytisine bromide (*per os*) was evaluated across the 3–5–10–15–20 mg/kg range, a stable anticonvulsant effect was observed. At a dose of 3 mg/kg, the latent period reached 16.2 ± 1.5 minutes, the seizure duration decreased to 0.43 ± 2.10 minutes, the effectiveness was 25.8%, and the survival rate was 50.0%. At a dose of 5 mg/kg, the latent period was 16.0 ± 0.8 minutes, the seizure duration was 0.40 ± 2.00 minutes, the effectiveness was 31.0%, and the survival rate reached 66.7%.At a dose of 10 mg/kg, the latent period was 16.9 ± 1.6 minutes, the duration was 0.35 ± 1.60 minutes, the effectiveness was 39.6%, and the survival rate was 66.7%. At a dose of 15 mg/kg, the parameters were close to maximal: the latent period was 16.5 ± 1.1 minutes, the duration was 0.37 ± 2.30 minutes, the effectiveness was 36.2%, and the survival rate was 66.7%. At a dose of 20 mg/kg, the latent period was 15.8 ± 1.4 minutes, the duration was 0.42 ± 1.90 minutes, the effectiveness was 27.6%, and the survival rate was 50.0%. The differences compared with the control group were considered statistically significant at $P \leq 0.05$. The experimental results are presented in detail in Table 6.

Table 6

Effect of N-methylcytisine bromide on strychnine-induced seizures (mean ± SD, n = 6)

Group	Dose, mg/kg	Latent period of seizure onset, min	Seizure duration, min	Effect, %	Survival, %
Control (strychnine)	0.2	10.7 ± 0.9^{a}	0.58 ± 2.1^{a}	–	0
Carbamazepine (per os) +	5.0	13.0 ± 1.4^{b}	0.46 ± 2.6^{a}	20.7	33.3
strychnine 1.2 mg/kg s.c.	10.0	13.4 ± 1.7^{b}	0.44 ± 1.9^{a}	24.1	50.0
Convulex (per os) +	75.0	14.2 ± 0.9^{b}	0.40 ± 2.3^{a}	31.0	50.0
strychnine 1.2 mg/kg s.c.	100.0	15.9 ± 1.2^{c}	0.38 ± 1.5^{a}	34.4	66.7
	3.0	16.2 ± 1.5^{c}	0.43 ± 2.1^{a}	25.8	50.0
N-methylcytisine	5.0	16.0 ± 0.8^{c}	0.40 ± 2.0^{a}	31.0	66.7
bromide (per os) +	10.0	16.9 ± 1.6^{c}	0.35 ± 1.6^{a}	39.6	66.7
strychnine 1.2 mg/kg s.c.	15.0	16.5 ± 1.1^{c}	0.37 ± 2.3^{a}	36.2	66.7
	20.0	15.8 ± 1.4^{bc}	0.42 ± 1.9^{a}	27.6	50.0

Note: see Table 1.

The experiments demonstrated that N-methylcytisine bromide exhibits pronounced anticonvulsant activity in the strychnine-induced seizure model. The compound significantly prolongs the onset of seizures, shortens their duration, and increases the survival rate of the animals. The highest therapeutic efficacy is observed within the 10–15 mg/kg dose range, while a slight decrease in activity is noted at 20 mg/kg. These findings provide a solid scientific basis for considering N-methylcytisine bromide as a promising antiepileptic agent.

When administered at high doses, isoniazid suppresses GABAergic inhibitory processes in the central nervous system, inducing a rapidly developing seizure syndrome in animals (Treiman, 2020). In the control group, the latent period of seizure onset after isoniazid administration averaged 24.5 ± 2.1 minutes, the seizure duration was 0.30 ± 0.80 seconds, and the survival time until death was only 5.9 minutes. These findings confirm the strong proconvulsant effect of isoniazid.

For comparison, the standard anticonvulsant drugs carbamazepine and convulex (valproate) prolonged the time to seizure onset and increased the survival duration of the animals. In the carbamazepine-treated groups, the latent period extended to an average of 34.0–35.4 minutes, while the survival time until death increased to 16.2–20.1 minutes. Convulex demonstrated a similar level of protective efficacy, with survival times ranging from 15.6 to 17.8 minutes.

The test compound N-methylcytisine bromide demonstrated a dose-dependent anticonvulsant activity in the isoniazid-induced seizure model. The compound was evaluated at doses of 3–5–10–20 mg/kg. At doses of 3 and 5 mg/kg, the onset of seizures was prolonged to 38.0 and 37.2 minutes, respectively, which corresponds to a protective effect comparable to that of carbamazepine and convulex. At doses of 10–15 mg/kg, the compound produced the highest effect, prolonging the latent period of seizure onset to 41.4 minutes and increasing the survival time of the animals to 25.0–26.6 minutes. These parameters indicate an efficacy comparable to, or even exceeding, that of the reference antiepileptic drugs. At a dose of 20 mg/kg, the effect slightly decreased (latent period 35.7 minutes, survival time 22.7 minutes), suggesting that the optimal therapeutic range for the test compound lies between 10 and 15 mg/kg. The experimental findings are presented in detail in Table 7.

The experimental results showed that in the isoniazid-induced seizure model, seizures developed rapidly in the control group and the survival time of the animals was short, confirming that this model re-

liably reproduces epileptic syndrome. The standard anticonvulsant agents used for comparison (carbamazepine and convulex) prolonged the onset of seizures and improved survival outcomes. N-methylcytisine bromide demonstrated an even stronger protective effect, particularly within the 10–15 mg/kg range, significantly extending the latent period and increasing the survival time until death. These findings provide a scientific basis for considering N-methylcytisine bromide as a promising antiepileptic agent.

Table 7

Effect of N-methylcytisine bromide on isoniazid-induced seizures (mean ± SD, n = 6)

Group	Dose, mg/kg	Latent period of seizure onset, min	Seizure duration, s	Animal survival time until death, min
Control (isoniazid)	0.2	24.5 ± 2.1^{a} (6/0)	0.30 ± 0.8^{a}	5.9
Carbamazepine (per os) +	5.0	34.0 ± 1.9^{b} (6/0)	0.18 ± 0.6^{a}	16.2
isoniazid 200.0 mg/kg i.p.	10.0	35.4 ± 2.0^{b} (6/0)	0.15 ± 0.9^{a}	20.1
Convulex (per os) +	75.0	32.6 ± 1.8^{b} (6/0)	0.16 ± 1.0^{a}	15.6
isoniazid 200.0 mg/kg i.p.	100.0	33.2 ± 2.2^{b} (6/0)	0.14 ± 0.7^{a}	17.8
	3.0	38.0 ± 1.9^{c} (6/0)	0.15 ± 0.9^{a}	18.6
N-methylcytisine	5.0	37.2 ± 1.9^{bc} (6/0)	0.14 ± 0.5^{a}	23.5
bromide (per os) +	10.0	41.4 ± 2.1^{d} (6/0)	0.11 ± 0.7^{a}	26.6
isoniazid 200.0 mg/kg i.p.	15.0	40.0 ± 1.7^{cd} (6/0)	0.12 ± 0.4^{a}	25.0
	20.0	35.7 ± 1.8^{bc} (6/0)	0.15 ± 0.8^{a}	22.7

Note: see Table 1.

Seizures induced by thiosemicarbazide are characterized by a reduction in γ-aminobutyric acid (GABA) synthesis in the central nervous system and excessive activation of the glutamatergic system. The suppression of GABAergic inhibition and increased influx of calcium ions through NMDA/AMPA receptors lead to prolonged neuronal depolarization, excitotoxicity, and intensification of seizure activity.

N-methylcytisine bromide likely modulates neurotransmitter systems through cholinergic (nicotinic) receptors, supporting the activity of GABAergic interneurons and helping to restore the excitation–inhibition balance. As a result, excessive glutamate release is reduced, NMDA-mediated Ca^{2+} influx is limited, ion channel activity becomes stabilized, and oxidative stress is attenuated. Together, these combined effects contribute to the anticonvulsant action observed in the thiosemicarbazide model.

When administered at high doses, thiosemicarbazide reduces GABA synthesis in the body, leading to a weakening of inhibitory processes in the central nervous system. As a result, animals develop a rapidly emerging and long-lasting convulsive syndrome. In the control group, the latent period of seizure onset averaged 31.0 ± 2.1 minutes, and the seizure duration reached 19.5 ± 0.8 minutes, confirming the severe course of the pathological process. The standard anticonvulsants selected for comparison (carbamazepine and convulex) markedly prolonged the onset time of seizures and reduced their duration. When administered at doses of 5–10 mg/kg, carbamazepine extended the latent period to 52.5–55.7 minutes and reduced the seizure duration by half (to 10.7–10.9 minutes). Accordingly, the overall anticonvulsant effectiveness of carbamazepine was 43.9–45.0%. Convulex, administered at doses of 75–100 mg/kg, demonstrated protective effects comparable to those of carbamazepine (effectiveness 42.0–43.5%).

The test compound N-methylcytisine bromide demonstrated a dose-dependent anticonvulsant activity in the thiosemicarbazide-induced seizure model. At doses of 3 and 5 mg/kg, the onset of seizures was prolonged to 53.0 and 55.5 minutes, respectively, while the seizure duration was markedly reduced (11.5–10.8 minutes). These parameters corresponded to an effectiveness comparable to that of carbamazepine and convulex (42.5–44.3%). At doses of 10–15 mg/kg, the compound produced the highest anticonvulsant effect: the latent period of seizure onset increased to 59.7–58.0 minutes, and the seizure duration decreased to 9.2–9.4 minutes. The overall protective efficacy reached 52.8–51.7%. At a dose of 20 mg/kg, the effectiveness slightly declined (45.2%), indicating that the optimal therapeutic range lies between 10 and 15 mg/kg. The experimental data are presented in Table 8.

Table 8

Effect of N-methylcytisine bromide on thiosemicarbazide-induced seizures (mean ± SD, n = 6)

Group	Dose, mg/kg	Latent period of seizure onset, min	Seizure duration, min	Effect, %
Control (thiosemicarbazide)	0.2	31.0 ± 2.1^{a}	19.5 ± 0.8^{c}	–
Carbamazepine (per os) +	5.0	52.5 ± 1.8^{c}	10.9 ± 0.5^{b}	43.9
thiosemicarbazide 28.0 mg/kg s.c.	10.0	55.7 ± 1.5^{d}	10.7 ± 0.9^{ab}	45.0
Convulex (per os) +	75.0	47.2 ± 2.3^{b}	11.3 ± 1.2^{ab}	42.0
thiosemicarbazide 28.0 mg/kg s.c.	100.0	50.7 ± 2.1^{bc}	11.0 ± 0.1^{b}	43.5
	3.0	53.0 ± 1.9^{c}	11.5 ± 0.5^{b}	42.5
N-methylcytisine	5.0	55.5 ± 1.7^{cd}	10.8 ± 1.0^{ab}	44.3
bromide (per os) +	10.0	59.7 ± 2.0^{e}	9.2 ± 1.0^{a}	52.8
thiosemicarbazide 28.0 mg/kg s.c.	15.0	58.0 ± 1.6^{de}	9.4 ± 1.0^{ab}	51.7
	20.0	55.4 ± 2.5^{cd}	10.6 ± 0.3^{b}	45.2

Note: see Table 1.

The thiosemicarbazide-induced seizure model clearly demonstrates increased neuronal excitability, with prolonged seizure episodes observed in the control group. The standard anticonvulsant agents (carbamazepine and convulex) exerted a protective effect by delaying seizure onset and reducing seizure duration. N-methylcytisine bromide, particularly within the 10–15 mg/kg dose range, exhibited even stronger anticonvulsant activity, indicating therapeutic efficacy comparable to or exceeding that of the standard drugs. These findings support the potential use of this compound as a promising pharmacological agent in epilepsy therapy.

Based on the obtained results, N-methylcytisine bromide may be recommended as a new anticonvulsant agent with GABA-modulating, Na^{+}-channel-blocking, and neuroprotective properties. Camphor-induced seizures are primarily characterized by increased neuronal excitability resulting from reduced GABA-A-receptor –mediated inhibition and enhanced depolarization via voltage-gated Na^{+} and Ca^{2+} channels. Therefore, achieving an anticonvulsant effect requires, on the one hand, maintaining and enhancing GABAergic inhibition (supporting GABA-A-receptor activity), and on the other hand, stabilizing the activity of ion channels (Na^{+}/Ca^{2+}) and limiting excessive Ca^{2+} influx to reduce membrane excitability (Colmers et al., 2024).

Camphor belongs to the group of agents that induce rapidly developing convulsive syndromes. In the control (pathology) group, after camphor administration, the latent period of seizure onset was 5.8 ± 0.9 minutes, the animals exhibited an average of 9.2 ± 1.3 clonic–tonic seizures, and the total duration of convulsions reached 53.5 ± 4.6 minutes. All animals in this group died, corresponding to 100% lethality.

A pronounced protective effect was observed with the reference anticonvulsants carbamazepine and convulex. At doses of 5–10 mg/kg, carbamazepine prolonged the latent period to 11.3–13.5 minutes, reduced both the number and duration of seizures, and decreased lethality to 83.3–66.7%. Convulex demonstrated a similar level of effectiveness (lethality 83.3–66.7%).

The test compound N-methylcytisine bromide demonstrated a clear dose-dependent anticonvulsant effect in the camphor-induced seizure model. At doses of 3–5 mg/kg, the latent period increased to 12.6–14.3 minutes, the number of seizures showed a slight reduction, and lethality remained at 83.3%. At doses of 10–15 mg/kg, the highest protective efficacy was observed: the latent period was prolonged to 16.6–17.2 minutes, the number of seizures decreased to 5.2–5.7 episodes, the total duration of convulsions was significantly reduced (35.2–31.8 minutes), and lethality declined to 66.7% and 50.0%, respectively. At a dose of 20 mg/kg, a slight attenuation of the effect was noted, indicating that the optimal therapeutic range is between 10 and 15 mg/kg. The experimental data are presented in Table 9.

Table 9

Effect of the test compound N-methylcytisine bromide on camphor-induced seizure syndrome in mice (mean ± SD, n = 6)

Animal groups	Dose, mg/kg	Latent period, min	Number of clonic and tonic seizures per animal	Duration, min	Animal mortality in the group, %
Camphor (control pathology)	0.2	5.8 ± 0.9^{a}	9.2 ± 1.3^{b}	53.5 ± 4.6^{b}	100.0
Carbamazepine (per os) +	5.0	11.3 ± 1.1^{b}	6.9 ± 1.8^{a}	38.6 ± 7.2^{a}	83.3
camphor 1.0 g/kg s.c.	10.0	13.5 ± 1.3^{c}	6.1 ± 2.3^{a}	37.2 ± 8.5^{a}	66.7
Convulex (per os) +	75.0	10.6 ± 0.6^{b}	7.2 ± 1.9^{a}	39.5 ± 7.6^{a}	83.3
camphor 1.0 g/kg s.c.	100.0	11.5 ± 0.8^{b}	7.0 ± 2.1^{a}	38.1 ± 8.2^{a}	66.7
	3.0	12.6 ± 1.1^{bc}	6.4 ± 1.5^{a}	37.5 ± 7.8^{a}	83.3
N-methylcytisine	5.0	14.3 ± 0.9^{c}	6.5 ± 1.6^{a}	37.1 ± 6.1^{a}	83.3
bromide (per os) +	10.0	16.6 ± 0.7^{d}	5.7 ± 1.9^{a}	35.2 ± 5.8^{a}	66.7
camphor 1.0 g/kg s.c.	15.0	17.2 ± 1.0^{d}	5.2 ± 2.1^{a}	31.8 ± 6.6^{a}	50.0
	20.0	15.0 ± 0.6^{cd}	5.9 ± 1.7^{a}	36.4 ± 7.3^{a}	66.7

Note: see Table 1.

The obtained experimental results showed that the camphor-induced seizure model reliably reproduces a severe convulsive syndrome. Although standard anticonvulsants (carbamazepine and convulex) slightly reduced the severity of seizures, the mortality rate remained high. In contrast, N-methylcytisine bromide, particularly at doses of 10–15 mg/kg, delayed the onset of seizures, reduced their intensity, and increased the survival probability of the animals. These findings indicate that this compound may serve as a promising antiepileptic agent for the treatment of epilepsy and convulsive states.

Discussion

The present study provides robust experimental evidence that N-methylcytisine bromide possesses stable and reproducible anticonvulsant activity across a wide range of seizure models representing distinct but interconnected pathogenetic mechanisms of epilepsy. By employing cholinergic, GABAergic, glutamatergic, and ion channel-dependent paradigms, the study was able to delineate the compound's pharmacodynamic profile in a mechanistically meaningful manner.

In arecoline- and nicotine-induced seizure models, which primarily reflect excessive activation of central cholinergic pathways, N-methylcytisine bromide significantly prolonged seizure latency and reduced tremor duration. These findings strongly support the hypothesis that the compound acts as a competitive antagonist at nicotinic acetylcholine receptors, thereby limiting excessive cholinergic excitation. Given that hyperactivation of nicotinic receptors promotes neuronal synchronization and lowers seizure threshold, attenuation of this pathway represents a relevant antiepileptic mechanism, particularly in seizure types associated with enhanced cholinergic tone.

In models characterized by impaired GABAergic inhibition, including those induced by pentylenetetrazol, picrotoxin, and bicuculline, N-methylcytisine bromide consistently reduced seizure severity and improved survival. Since these convulsants interfere with GABA_A receptor-mediated chloride conductance, the observed protective effects indicate that the compound indirectly supports inhibitory neurotransmission. This action may involve stabilization of neuronal membrane potential, modulation of voltage-gated Na^{+} and Ca^{2+} channels, or functional facilitation of GABAergic interneuron activity, thereby restoring the excitation–inhibition balance within neuronal networks.

Pronounced anticonvulsant efficacy was also demonstrated in strychnine-, isoniazid-, and thiosemicarbazide-induced seizure models, which are associated with suppression of inhibitory tone and excessive glutamatergic excitotoxicity. In these paradigms, N-methylcytisine bromide markedly delayed seizure onset and reduced mortality, suggesting an ability to limit pathological Ca^{2+} influx and downstream excitotoxic cascades. Such effects are indicative of a neuroprotective component that extends beyond symptomatic seizure suppression and may contribute to long-term stabilization of neuronal function.

Across all experimental models, the highest anticonvulsant efficacy was consistently observed within the 10–15 mg/kg dose range. The partial attenuation of activity at 20 mg/kg suggests the presence of an optimal therapeutic window and may reflect receptor-level desensitization, pharmacodynamic saturation, or compensatory adaptive mechanisms within the central nervous system. Identification of this dose range is critical for rational dose selection in subsequent preclinical and clinical studies.

Comparative evaluation with established antiepileptic drugs demonstrated that the efficacy of N-methylcytisine bromide was comparable to, and in some parameters exceeded, that of carbamazepine and valproate. Importantly, while classical antiepileptic agents predominantly act through a single dominant mechanism, N-methylcytisine bromide appears to exert a broader, multimodal pharmacological action. This profile suggests potential advantages in the management of epilepsy forms characterized by complex or mixed pathophysiology, including pharmacoresistant and multifactorial seizure disorders.

Toxicological assessment revealed that N-methylcytisine bromide belongs to a low-toxicity class, indicating a favorable balance between efficacy and safety. Taken together, the results of this study indicate that N-methylcytisine bromide restores neuronal excitation–inhibition homeostasis through combined cholinergic modulation, indirect enhancement of inhibitory neurotransmission, and stabilization of ion channel function. These properties support its consideration as a promising candidate for further translational development as a novel antiepileptic agent.

Conclusion

The results of this study demonstrated that N-methylcytisine bromide exhibits stable anticonvulsant activity across experimental seizure models with different underlying pathogenic mechanisms. The compound reliably reduced neuronal excitability under conditions of impaired GABAergic inhibition, enhanced cholinergic excitation, destabilized ion channel function, or increased glutamatergic excitotoxicity. Its therapeutic effect was dose-dependent, with the highest efficacy observed in the 10–15 mg/kg range: seizure onset was significantly delayed, the duration and intensity of seizures were reduced, and the survival rates of the animals improved.

Compared with the reference groups, the anticonvulsant effect of N-methylcytisine bromide was comparable to, and in some parameters exceeded, that of clinically used carbamazepine and valproate

(convulex). According to toxicological assessment, the compound belongs to a low-toxicity class, indicating a favorable safety profile for potential long-term use.

The observed pharmacodynamic outcomes suggest the following possible mechanisms of action of the compound: modulation of nicotinic acetylcholine receptors, support or restoration of GABAergic inhibition, limitation of Ca^{2+} influx, and reduction of oxidative stress. Together, these factors contribute to restoring the excitation–inhibition balance within neuronal networks.

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Institutional Review Board Statement. The animal study was conducted in accordance with the International Convention for the Protection of Vertebrate Animals used for Experimental and Scientific Purposes (Strasbourg, 1986), and the Institutional Scientific Board of the Institute of the Chemistry of Plant Substances, Academy of Sciences of Uzbekistan, approved the protocol based on the annual working plan of the Department of Pharmacology and Toxicology (Protocol No. 1 from 17 January 2025).

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