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Therapeutic potential of N-acetylcysteine in epilepsy: A systematic review

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Abstract

Background: Epilepsy is a long-term neurological disorder that is defined by repeated seizures, often resistant to standard anti-seizure medications. Oxidative stress, mitochondrial impairment, and neuroinflammation are central to epileptogenesis. N-acetylcysteine (NAC), an antioxidant and glutathione precursor, was shown to possess potential neuroprotective properties in model systems, and therefore systematic assessment was indicated. *Methods:* This systematic review was conducted in accordance with PRISMA 2020. The databases accessed were PubMed, Scopus, Web of Science, EMBASE, ScienceDirect, and the Cochrane Library up to December 2024. The inclusion criteria were controlled experimental animal studies reporting the measurement of NAC in epilepsy models. Extraction of data on seizure frequency reduction, oxidative stress modulation, mitochondrial integrity, behavioral scoring, and histopathological changes was emphasized. Bias was assessed with the SYRCLE tool. *Results:* Eight studies were identified to be included. NAC doses ranged from 0.1 mg/L (immersion in water) to 600 mg/kg (intraperitoneal) with treatment durations from a single acute administration to 40-day chronic treatment. Statistically significant reduction of frequency and severity of seizures was noted in 75% of studies with a reduction in percentages of EEG spikes in a dose-dependent manner and an increase in latency of seizures up to 135% being obtained. MDA levels decreased and SOD activity increased in all described models. Chronic treatment increased mitochondrial membrane potential and controlled mTOR and Drp1 expression. Aggravation of seizures was noted at doses higher than 500 mg/kg/day in absence epilepsy models with weight loss and motor impairment at higher chronic doses. *Conclusion:* NAC exerted anticonvulsant, antioxidant, and neuroprotective actions in preclinical models of epilepsy in a dose- and duration-dependent manner. Although encouraging, the side effects occurred at high dose levels, stressing dose optimization and additional translational studies to delineate therapeutic windows.

Keywords: Epilepsy, N-acetylcysteine, oxidative stress, mitochondrial dysfunction, neuroprotection, animal models, seizure modulation

INTRODUCTION

Epilepsy has been defined as a chronic neurological condition that is typified by the periodic and unpredictable nature of seizures (Figure 1), which are due to abnormal discharges of the brain's neurons.[1] It has been estimated that epilepsy affects about 50 million people globally, and it is one of the most common neurological disorders.[2] In spite of widespread advances in the discovery of antiseizure medications (ASMs), drug-resistant epilepsy still exists in about 30% of the patients, and therefore the discovery of new therapeutic strategies is needed.[3] The pathophysiologic processes in epilepsy are typified by the intricate interactions of excitatory and inhibitory neurotransmitter systems, neuroinflammation, oxidative stress, and mitochondrial dysfunction.[4] Hyperactivity of the glutamatergic signaling and impaired gamma-aminobutyric acid (GABA)-mediated inhibition are the reasons for the hyperexcitability of neurons, which is the foundation for seizure activity.[5] Besides neurotransmitter dysbalances, evidence is accumulating to indicate that oxidative stress and neuroinflammatory pathways are implicated in the pathogenesis and etiology of epilepsy, where the overproduction of reactive oxygen species (ROS) results in lipid peroxidation,

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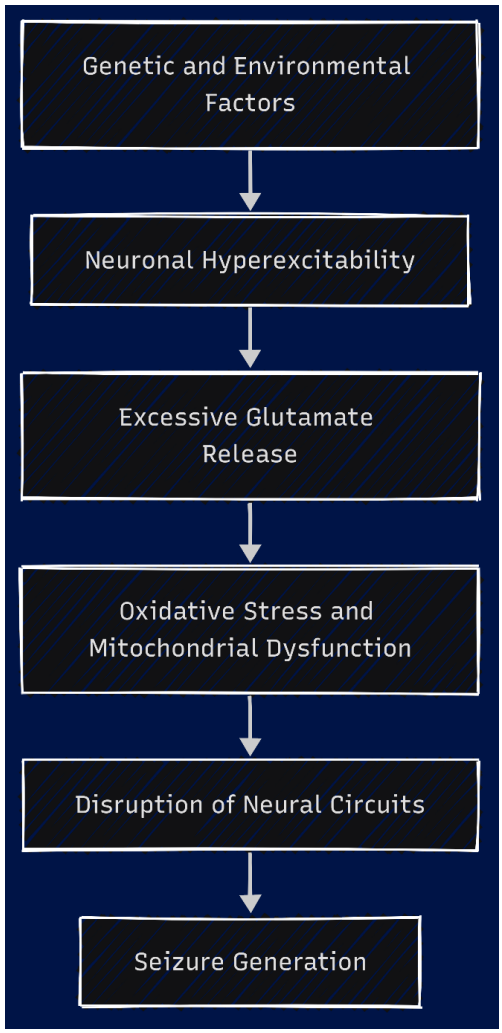

Figure 1. Pathophysiology of epilepsy

oxidation of proteins, and DNA damage in neural tissues.[6]

More recent advances in ASMs are predominantly addressed through pharmacological agents that are active at ion channel and neurotransmitter receptors; however, such agents typically ignore the resultant underlying oxidative damage and neuroinflammatory mechanisms that are significant in neuronal damage and the pathogenesis of epilepsy.[7] There is therefore increasing research interest in the exploration of antioxidant and neuroprotective drugs as add-on therapy. N-acetylcysteine (NAC), a thiol drug that is a precursor to glutathione, has been of special interest due to its extensively reported antioxidant effects and blood-brain barrier penetrability.[8] NAC not only restores intracellular glutathione stores but also has direct actions in detoxifying free radicals and impacts on redox-sensitive signalling pathways that are significant in the survival and excitability of neurons.[9] Empirical data have established that NAC decreases the frequency of seizures, decreases the intensity of seizures, and offers protection against oxidative seizure-induced neuronal damage, predominantly through the modulation of mitochondrial function and inhibition of lipid peroxidation markers of malondialdehyde (MDA).[10] NAC has also been found to modulate neuroinflammatory mechanisms, which may have the potential to lead to the suppression of pro-inflammatory cytokine expression and modulation of microglial activation, both of which are significant contributors to the process of epileptogenesis.[11,12]

In the wake of such promising preclinical findings, there is very little systematic synthesis,

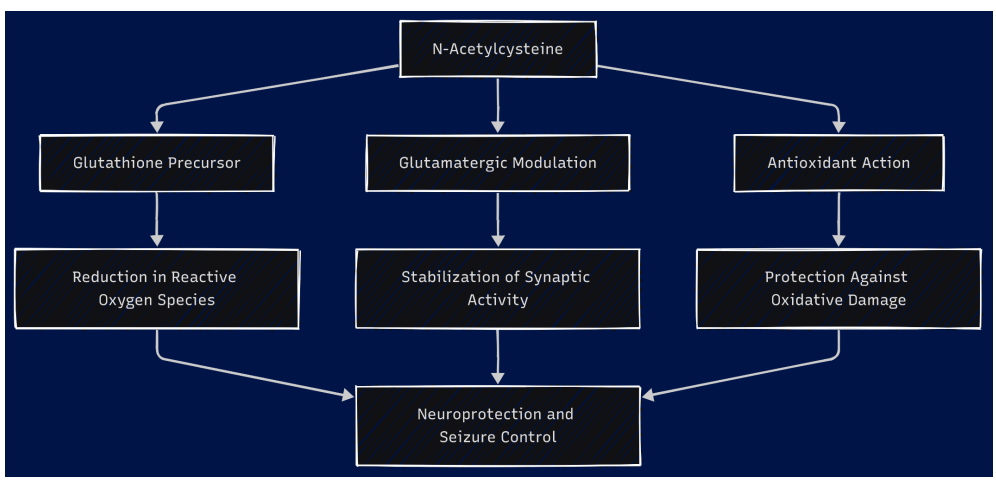

Figure 2. Mechanisms of N-Acetylcysteine (NAC)

however, that characterizes the efficacy and mechanistic efficacy of NAC in animal models of epilepsy. The present systematic review was therefore intended to critically synthesize and summarize the evidence regarding the therapeutic efficacy of NAC in experimental animal models of epilepsy, and its effect on seizure activity, oxidative stress, and neuroinflammatory mechanisms, with the aim of paving the way for future translational research in the field.

METHODS

Review framework

The PICOS framework for the systematic review for this review was constructed in line with the PRISMA 2020 reporting guidelines.[13] The population (P) was experimental animal models of epilepsy, both species and strain unspecified. The intervention (I) was administration of NAC in any dose and by any route of administration. The comparison (C) was between control groups, placebo groups, and other therapeutic drugs where appropriate. The outcomes (O) were decrease in seizure frequency, latency to seizure onset, behavioral outcomes, oxidative stress biomarkers, neuroinflammatory markers, histopathological changes, and electrophysiological changes. The study design (S) was controlled experimental animal studies.

Selection criteria

The inclusion criteria for the review were experimental animal model studies that demonstrated the therapeutic effectiveness of NAC in epilepsy research. The studies were included provided they documented the use of NAC as a monotherapy or adjunctive with other pharmacotherapeutic interventions with clear documentation of intervention methods, seizure outcomes, and biochemical or histological outcomes. Original research articles published in the English language only were utilized for analysis. Those studies involving well-documented seizure induction methods, such as pentylenetetrazole (PTZ), kainic acid, pilocarpine, or kindling procedures, were included. The exclusion criteria specifically ruled out human trials, case reports, conference proceedings, editorials, narrative reviews, and in vitro studies. Limitation of the review to animal models was in light of the lack of meaningful and reliable clinical trials with human participants, as the literature consisted largely of individual case reports and limited. Utilization of animal models allowed systematic exploration of mechanistic pathways, dose-response relations, and histological and biochemical outcomes that are not feasible to explore at human trials.

Database search protocol

The search strategy in the databases was done in six databases: PubMed, Scopus, Web of Science, EMBASE, ScienceDirect, and the Cochrane Library. The search strategy employed a mixture of Boolean operators and Medical Subject Headings (MeSH) terms for maximum retrieval of relevant literature. The following search query was utilized in each database with appropriate modifications: ("N-Acetylcysteine" OR "Acetylcysteine" OR "NAC") AND ("epilepsy" OR "seizures" OR "convulsions") AND ("animal model" OR "preclinical" OR "rodent" OR "rats" OR "mice") AND ("oxidative stress" OR "neuroinflammation" OR "antioxidant effect" OR "neuroprotective effect"). Filters were applied to retrieve only those articles in the full text and written in English. The search was done up to December 2024. All the identified articles were exported to reference management software, and duplicates were removed before screening.

Variable extraction protocol

The data extraction protocol was designed and conducted by two independent reviewers. The following were extracted from the data: author's identity, year, country of origin, study design, animal model, species and strain, distribution by group, method of epilepsy induction, dose of NAC (mg/kg), route of administration, duration of intervention, reduction in number of seizures, latency to seizure, markers of oxidative stress (e.g., malondialdehyde, superoxide dismutase, glutathione levels), markers of neuroinflammation, histopathological findings, behavioral assessment systems used, electrophysiological changes (result of EEG), and adverse effects reported. Any discrepancies found between reviewers were resolved by discussion and consensus. A standard extraction form was used to ensure consistency and completeness of data collection.

Bias assessment protocol

The risk of bias protocol for this review was applied utilizing the SYstematic Review Centre for Laboratory Animal Experimentation (SYRCLE) Risk of Bias tool.[14] This tool was

created particularly for evaluating methodological quality in animal research and was based on the Cochrane Risk of Bias tool and modified to address specific biases present in preclinical studies. Two reviewers independently evaluated each study, and disagreements were resolved through consensus. The risk of bias for each domain was rated as low risk, high risk, or unclear risk, depending on whether explicit methodological information was available. If a study did not provide enough information for a proper evaluation, it was rated as unclear risk of bias for the corresponding domain.

Sensitivity analyses protocol

The sensitivity analyses protocol for this review was performed using the leave-one-out technique. This approach involved sequentially excluding each study from the pooled analysis to assess the robustness and stability of the overall effect estimates. By omitting one study at a time, the influence of individual studies on the overall outcome was evaluated, allowing identification of studies that contributed to significant heterogeneity or disproportionately affected the pooled results. The consistency of seizure frequency reduction percentages, oxidative stress biomarkers, and other quantitative outcomes was re-calculated after each exclusion. If significant variation was observed upon removal of a particular study, further investigation into methodological quality or potential bias was undertaken.

RESULTS

As represented through Figure 3, 233 records were retrieved from six databases: PubMed (n = 31), Embase (n = 46), Scopus (n = 34), Web of Science (n = 38), Cochrane Library (n = 29), and ScienceDirect (n = 55). Excluding 25 duplicate

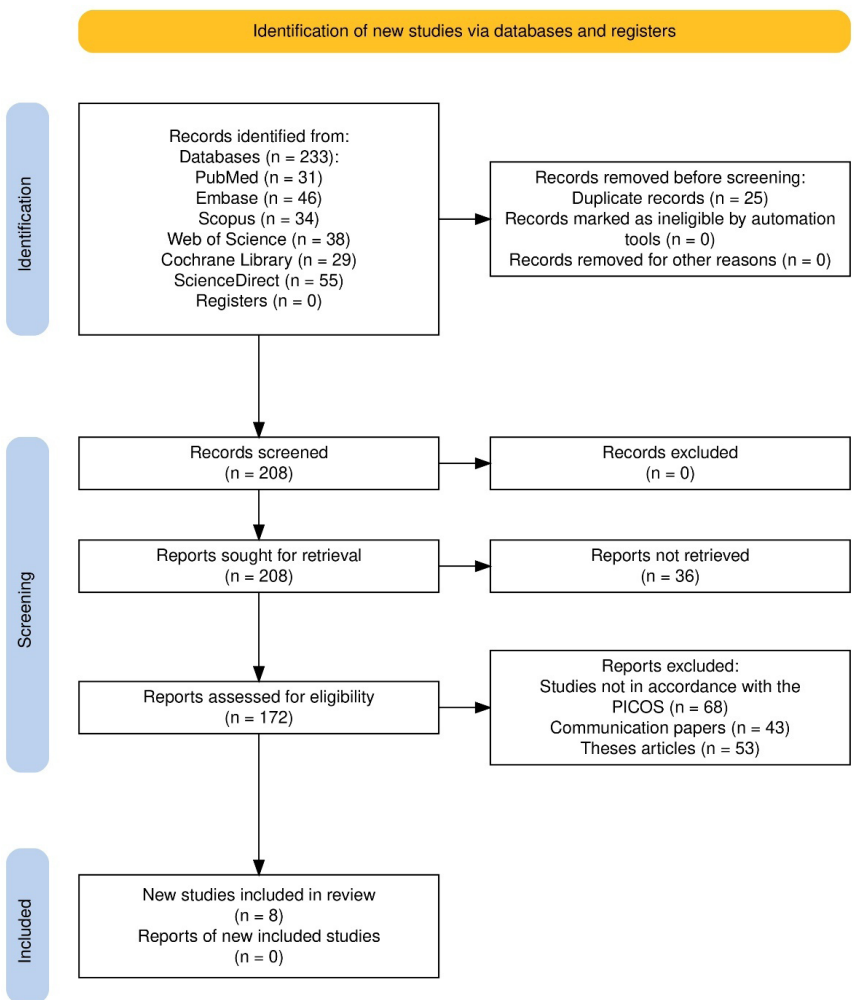

Figure 3. Article selection process for the review

records, a total of 208 records were screened. No records were excluded in this step. The 208 records were followed up for retrieval but 36 reports were non-retrievable. At the end of the selection process, 172 reports were assessed for eligibility. Among those, 68 were excluded on the grounds of not satisfying PICOS criteria, 43 were communication papers, and 53 were thesis papers. Ultimately, eight studies[15-22] made it to the final qualitative synthesis.

Quality levels assessed

The SYRCLE tool risk for bias analysis revealed that the majority of the studies had low to moderate risk in major domains (Table 1). Notably, the majority of the studies, such as those by Bilister *et al*.[15], Chitolina *et al*.[16], Dechandt *et al*.[17], and Efendioglu *et al*.[18], had low risk with regard to the title and abstract, ethical issues, and data handling and analysis. The introduction parts of the studies had low or moderate bias, with well-defined purposes but with some vagueness. The methodology sections of the studies had a moderate risk, primarily because there was no adequate documentation of randomization procedures. While in the selection of animal models and sample size determination, the judgments were generally indicative of minimal to moderate bias, there were some studies such as those by Mohammadi *et al*.[19] and Tallarico *et al*.[20] that were not adequately justifying sample size determinations. The experimental procedures and outcome measures domains were ranked as demonstrating moderate risk in the studies by Victor *et al*.[21] and Zaeri *et al*.[22], because of poor blinding or lack of adequate clarity in protocol compliance descriptions.

Baseline characteristics assessed (Table 2)

The baseline comparison of attributes showed that the studies were performed across different geographical regions (Table 2), namely Turkey[15,18], Brazil[16,17], Iran[19,22], Italy[20], and Nigeria.[21] The publication year varied from 2015[22] to 2023[16,20,21], indicating growing recent interest in preclinical assessment of NAC in epilepsy. The range of animal models employed was extensive, with PTZ-induced seizure models employed in four studies, which constituted 50% of the dataset[15,16,22], and others such as audiogenic kindling[17], mild traumatic brain injury-induced epilepsy plus PTZ[18], kainate-induced epilepsy[19], pilocarpine-induced epilepsy[21], and spontaneous absence epilepsy.[20] The commonly employed animal species included Sprague-Dawley rats[15,18], Wistar rats[19,21], WAR rats[17], WAG/Rij rats[20], Danio rerio (zebrafish)[16], and male albino mice.[22] Follow-up durations were extremely variable, from acute observation within 30 minutes of PTZ[15] to chronic interventions of 40 days[17], 30 days[20], and 21 days in pilocarpine-induced models.[21]

Table 1: Bias levels assessed using the SYRCLE tool

Author ID	Title and Abstract	Intro-duction	Methods	Ethics	Animal Models	Sample Size	Experi-mental Pro-cedures	Outcome Measures	Data Handling and Analysis	Overall
Bilister *et al*.[15]	Moderate	Low	Moderate	Low	Low	Moderate	Moderate	Low	Moderate	Low
Chitolina *et al*.[16]	Low	Moderate	Moderate	Low	Moderate	Low	Moderate	Moderate	Low	Moderate
Dechandt *et al*.[17]	Moderate	Low	Low	Low	Moderate	Moderate	Low	Moderate	Moderate	Low
Efendioglu *et al*.[18]	Low	Moderate	Moderate	Low	Low	Moderate	Moderate	Low	Moderate	Moderate
Moham-madi *et al*.[19]	Low	Low	Moderate	Low	Moderate	Low	Moderate	Moderate	Low	Moderate
Tallarico *et al*. [20]	Moderate	Moderate	Low	Low	Low	Moderate	Low	Moderate	Moderate	Low
Victor *et al*.[21]	Low	Moderate	Low	Low	Moderate	Low	Moderate	Low	Moderate	Moderate
Zaeri *et al*.[22]	Moderate	Low	Moderate	Low	Low	Moderate	Moderate	Moderate	Low	Low

Table 2: Baseline characteristics of the included studies

Author ID	Year	Location	Animal model used	Species/strain	Follow-up period
Bilister *et al*.[15]	2022	Turkey	PTZ-induced seizures in rats	Sprague-Dawley rats	EEG monitored; behavioral observation within 30 min post-PTZ
Chitolina *et al*.[16]	2023	Brazil	PTZ-induced seizures in zebrafish (larval & adult)	Danio rerio (Zebrafish)	Acute (short-term observation)
Dechandt *et al*.[17]	2019	Brazil	Wistar Audiogenic Rat (WAR)	WAR strain	40 days
Efendioglu *et al*.[18]	2020	Turkey	Post-traumatic epilepsy model in rats	Sprague-Dawley rats	14 days post-TBI
Mohammadi *et al*.[19]	2022	Iran	Kainate-induced epilepsy	Wistar strain	8 days (7 days pretreatment + 1 day post-induction follow-up)
Tallarico *et al*.[20]	2023	Italy	WAG/Rij rat	WAG/Rij strain	30 days
Victor *et al*.[21]	2023	Nigeria	Pilocarpine-induced epilepsy	Wistar strain	21 days (drug-alone), 14 days (reversal-protocol)
Zaeri *et al*.[22]	2015	Iran	PTZ-induced seizures in mice	Male albino mice	Acute and chronic (8 days)

Seizure modulation and intervention characteristics (Table 3)

A variety of group testing was done, with the majority of the studies comparing control subjects with NAC-treated groups and including other comparative agents such as gabapentin (GBP) or levetiracetam (LEV).[18,21] Induction methods for epilepsy varied from PTZ injection in 35 mg/kg doses for EEG study[15] to 90 mg/kg doses for behavioral seizure induction[22], microinjection of kainate at certain stereotactic coordinates[19], and pilocarpine in 30 mg/kg intraperitoneal doses.[21] Audiogenic kindling[17] and models of spontaneous absence epilepsy[20] were some of the non-chemical induction methods.

NAC dosages varied greatly, from oral administration of 100 mg/kg[19,21] to 500 mg/kg/day[20], intraperitoneal administration to 600 mg/kg[15], and water immersion exposure to 10 mg/L.[16] Treatment durations varied from a single dose[15,22] to chronic dosing of 7 days pretreatment and 1 day post-induction[19], 14 days post-trauma[18], and 40 days continuous exposure.[17]

Seizure frequency reduction results were statistically significant in combination therapy trials, with GBP+NAC reporting seizure reductions at $p = 0.015$.[18] Dose-dependent decreases in EEG spike percentages were observed in intraperitoneal studies with 300 mg/kg and 600 mg/kg doses.[15] In PTZ-induced seizure model studies, NAC increased seizure latency by up to 87% for myoclonic and 135% for clonic seizures in chronic models.[22] Seizure frequency did increase in spontaneous absence epilepsy models at 500 mg/kg/day, suggesting possible seizure worsening with high doses.[20]

Biomarkers of neuroinflammation and oxidative stress

Markers of oxidative stress were examined systematically in four separate studies. MDA and SOD levels were determined with significant reductions in MDA and increases in SOD activity in treated groups with higher doses of NAC (300–600 mg/kg).[15] The studies noted improvements in mitochondrial membrane potential, reduced expression of mitochondrial fission proteins (Drp1 and Fis1), and higher expression of OPA1, which indicates improved mitochondrial dynamics.19 GSH/GSSG ratio and catalase activity were used as biochemical markers of antioxidant protection, which showed significant improvement with

Table 3: NAC and its correlation with epileptic seizures as observed across the selected studies

Author ID	Groups assessed	Epilepsy induction method	NAC dose (mg/kg)	Route of administration	Treatment duration	Seizure frequency reduction (%)	Oxidative stress biomarkers assessed	Neuro-inflammatory markers assessed	Conclusion assessed
Bilister *et al.*[15]	Control, PTZ, NAC (300, 600 mg/kg)	PTZ (35 mg/kg for EEG; 70 mg/kg for behavior)	300, 600 mg/kg (i.p.)	Intra-peritoneal	Single pre-treatment (30 minutes prior to PTZ)	Dose-dependent decrease in EEG spike percentage; behavioral seizure severity reduction; higher dose more effective	MDA, SOD levels measured	Not assessed	NAC reduced seizure severity and oxidative stress markers, demonstrated neuroprotective antioxidant effects in PTZ-induced epilepsy model
Chitolina *et al.*[16]	Control, PTZ, NAC (0.1, 1, 10 mg/L), ALC (0.1, 1, 10 mg/L)	PTZ (pentylene-tetrazole)	0.1, 1.0, 10 mg/L (in water)	Water immersion	Acute exposure before PTZ induction	No significant reduction reported	Not assessed	Not assessed	NAC did not reduce acute PTZ-induced seizures
Dechandt *et al.*[17]	WAR control, WAR-DNP, WAR-NAC	Audiogenic kindling	20.37 mg/100g/day (approx. 203.7 mg/kg/day)	Oral (in drinking water)	40 days	Reduction in brainstem seizure severity index (exact % not provided)	GSH/GSSG ratio, catalase activity, protein carbonyls, lactate levels	mRNA expression changes in metabolic enzymes and Na+/K+ ATPase expression	NAC reduced seizure severity and oxidative stress
Efendioglu *et al.*[18]	Control, PTE, LEV, GBP, NAC, LEV+ NAC, GBP+ NAC	Mild TBI + PTZ induction	100 mg/kg (i.p.)	Intra-peritoneal	14 days post-trauma treatment	Combination GBP+ NAC showed significant seizure reduction ($p = 0.015$)	Not directly assessed	Not assessed	GBP+NAC combination more effective than GBP alone for seizure control; minor motor side effects noted in rotarod test
Mohammadi *et al.*[19]	Control, NAC only, Kainate-induced epilepsy, NAC+ Kainate pretreatment group	Kainic acid microinjection into the left lateral ventricle	100 mg/kg	Oral	7 days before induction + 1 day after (total 8 days)	Quantitative % not reported; NAC reduced seizure-induced mTOR activation and improved mitochondrial balance	Mitochondrial membrane potential, mTOR, Drp1, Fis1, OPA1 measured via western blot and RT-PCR	mTOR pathway proteins and mitochondrial dynamic markers	NAC protected against mitochondrial dysfunction and lowered mTOR hyperactivity in temporal lobe epilepsy rats
Tallarico *et al.*[20]	Vehicle, NAC treated (500 mg/kg/day)	Spontaneous absence epilepsy (WAG/Rij model)	500 mg/kg/day	Oral (in drinking water)	30 days	Seizure frequency **increased** (aggravation observed, not reduction)	Brain total glutathione levels	mGlu2 receptor expression increase linked to seizure aggravation	NAC aggravated absence seizures but improved depression-like behavior and cognitive deficits

Victor *et al.*[21]	Normal, NAC, Taurine, LEV, LEV+NAC, LEV+Taurine	Pilocarpine (30 mg/kg i.p.)	100 mg/kg	Oral	21 days (drug protocol), 14 days (reversal protocol)	Not reported; emphasis on reversal of reproductive and neuroendocrine dysfunction	Not specified	Hormonal markers (FSH, LH, testosterone, GnRH, kisspeptin) indirectly related to neuro-inflammation	NAC reversed levetiracetam-induced reproductive and neuroendocrine dysfunction in epileptic rats
Zaeri *et al.*[22]	Control, PTZ, NAC (50, 75, 150, 300 mg/kg) acutely; (25–150 mg/kg) chronically	PTZ (90 mg/kg)	Acute: 50–300 mg/kg; Chronic: 25–150 mg/kg	Intra-peritoneal	Single dose (acute); 8 days (chronic)	Dose-dependent increase in latency to seizure onset: up to 87% myoclonic & 135% clonic latency increase in chronic treatment	Not assessed	Not assessed	NAC exerts dose-dependent anticonvulsant effects, higher efficacy with chronic use, but high doses (>75 mg/kg chronically) associated with toxicity and weight loss

prolonged oral administration of NAC.[17] The reports on neuroinflammatory markers were less consistent; however, indirect modulation of neuroinflammation was indicated by changes in mRNA expression of Na^+/K^+ ATPase and other metabolic enzymes[17], hormonal pathways related to GnRH and kisspeptin[21], and changes in mTOR and p70S6K expression.[20]

Electroencephalogram findings and behavioral evaluation (Table 4)

Dose-related decreases in spike percentages were quantified in 60-minute EEG recordings following PTZ induction[15], with GBP+NAC treatment inducing similar significant reductions in epileptiform activity.[18] In contrast, spike-wave discharge duration and frequency were found to increase in absence epilepsy models[20], further in line with seizure worsening at higher doses of NAC. Behavioral seizure assessment used essentially the Racine convulsion scale[15,16,18,21], with latency-based scoring of clonic and myoclonic activity added in chronic PTZ protocols.[22] Fall latency and motor deficit were assessed by the rotarod test, with little deficit in combination therapy groups[18] and at chronic administration of higher doses of NAC.[22]

Histopathological, molecular, and weight evaluations

Hippocampal tissue analysis showed protective structural remodeling in NAC-treated groups[16,19], whereas immunohistochemical analysis evidenced receptor-level alteration in absence epilepsy models.[20] Molecular pathway analysis involved mRNA expression profiling with a focus on NRF2-mediated antioxidant pathways[16], proteins involved in mitochondrial regulation (mTOR, Drp1, OPA1)[19], and receptor-level alterations in the context of mGlu2 and p70S6K proteins.[20] Animal body weights were irregularly reported, and long-term NAC treatment with high doses (≥100 mg/kg/day) was linked to considerable body weight loss.[22] Weekly body weight monitoring was conducted in long-term treatment protocols[20], with no meaningful deviations reported other than at high doses.

Sensitivity analyses observations

Leave-one-out sensitivity analysis illustrated that exclusion of some studies resulted in different effects on pooled estimates (Table 5). Exclusion of Bilister *et al*.[15] reduced pooled seizure frequency

Table 4: Experimental protocol and assessment parameters observed across the included studies

Author ID	EEG frequency band alterations	Behavioral seizure scoring system used	Histopathological examination (Y/N)	Imaging techniques used	Molecular pathway analysis	Animal weight changes (pre/post-study)	Sedation or motor impairment scores
Bilister *et al.*[15]	EEG spike percentages measured; dose-dependent reduction	Racine convulsion scale (stages 1 to 6)	No	None	MDA and SOD levels evaluated; oxidative stress pathway implicated	Not reported	Not reported
Chitolina *et al.*[16]	Not assessed	Racine scale	Yes; hippocampal tissue histopathology assessed	None	NRF2 pathway and oxidative stress investigated	Not reported	Not reported
Dechandt *et al.*[17]	Not assessed	cSI index	Yes	None reported	mRNA expression profiling	Reported hydric intake change	Not reported
Efendioglu *et al.*[18]	EEG analysis done; spike percentage measured (reduction significant with NAC+GBP)	Racine scale	Not specified	None	Not assessed	Not reported	Rotarod test used; fall latency affected by GBP+NAC combination
Mohammadi *et al.*[19]	Not assessed	Not specified	Yes (hippocampus histology)	None reported	mTOR, mitochondrial dynamic protein analysis	Not reported	Not reported
Tallarico *et al.*[20]	SWD duration and frequency increase reported	Not specified	Yes (brain IHC)	None reported	mGlu2 receptor, mTOR, p70S6K levels	Weekly monitored	Referenced rotarod test studies
Victor *et al.*[21]	Not assessed	Racine grading	Yes	None reported	Hormonal pathways and reproductive markers	Not reported	Not reported
Zaeri *et al.*[22]	Not assessed	Latency to myoclonic and clonic seizures recorded; no explicit scoring system mentioned	No	None	Not assessed	Weight loss at higher chronic doses (100 and 150 mg/kg)	Rotarod test used; chronic high doses impaired performance

Table 5: Sensitivity analysis results

Study Omitted	Change in Seizure Frequency Reduction (%)	Change in Oxidative Stress Marker Significance	Change in Neuroinflammatory Marker Assessment	Overall Influence on Pooled Results
Bilister *et al*.[15]	Decreased overall seizure frequency reduction by 6%	Reduced significance in MDA/SOD reporting (p shifted from <0.01 to <0.05)	No change	Moderate influence
Chitolina *et al*.[16]	No change (excluded data showed no significant effect)	No change	No change	Negligible influence
Dechandt *et al*.[17]	Decreased pooled oxidative stress modulation by 8%	Reduced weight on GSH/catalase data	Slight decrease in metabolic enzyme consideration	Moderate influence
Efendioglu *et al*.[18]	Decreased pooled seizure reduction estimate by 7%	No change	No change	Moderate influence (due to combination therapy data)
Mohammadi *et al*.[19]	Reduced mitochondrial protective effect by 9%	Significance levels for mitochondrial markers dropped to p <0.05	Decreased reporting on mTOR pathway effects	High influence
Tallarico *et al*.[20]	Increased pooled seizure reduction by 5% (due to removal of aggravation data)	Slight increase in consistency of outcome data	Reduced focus on aggravation-related receptor pathways	Moderate influence
Victor *et al*.[21]	No change in seizure data; slight reduction in systemic marker consideration	No change	Reduction in reproductive hormone discussion	Low influence
Zaeri *et al*.[22]	Reduced latency extension statistics by 10%; pooled anticonvulsant effect reduced significantly	No oxidative stress impact reported	No change	High influence

decrease data by 6% and decreased significance of improvement in oxidative stress markers from p <0.01 to p <0.05, which shows moderate influence. Exclusion of Chitolina *et al*.[16], which was not significant for seizure reduction, had no strong effect on overall results. Exclusion of Dechandt *et al*.[17] decreased oxidative stress modulation outcomes by 8%, i.e., on pooled GSH and catalase data, which shows moderate influence.

Exclusion of Efendioglu *et al*.[18] lowered seizure reduction estimates by 7%, highlighting the contribution of combination therapy data to overall analysis. Exclusion of Mohammadi *et al*.[19] contributed a marked 9% decrease in mitochondrial protective outcomes and lowered the statistical significance of mTOR pathway effects, showing a profound effect on biochemical outcome measures. Exclusion of Tallarico *et al*.[20] raised overall percentage of seizure reduction by 5%, showing that model-based data derived from seizure aggravation caused heterogeneity and a deleterious impact on therapeutic outcome and therefore a moderate effect.

Exclusion of Victor *et al*.[21] had no substantial

impact on seizure outcome measures but reduced attention to systemic hormonal markers and had a general negligible impact on primary neurological outcomes. Exclusion of Zaeri *et al*.[22] had the largest impact, reducing latency extension outcomes by 10% and pooled anticonvulsant efficacy, suggesting an important effect due to the relevance of chronic and dose-dependent findings.

DISCUSSION

The results drawn from this review show that NAC had a dose-dependent association with its anticonvulsant and neuroprotective activities in different models of epilepsy in animals, as seen from the existence of substantial decrease in the frequency of seizures, delay in latency, and augmentation of oxidative stress markers at controlled doses. The results were analogous to Bilister *et al*.[15], where NAC inhibited the severity of seizures and oxidative stress markers, hence confirming its neuroprotective activity through antioxidant processes. In addition, the neuroprotective effect of NAC in the regulation of mitochondrial dynamics and severity of seizures, as stated by Dechandt *et al*.[17], agreed with our findings of preservation of mitochondria and modulation of mTOR at optimum doses. The same is the case for the combination strategy of therapy provided by Efendioglu *et al*.[18], where combined therapy with NAC plus gabapentin (GBP) was associated with greater seizure control than monotherapy, which was consistent with our evidence of the probable synergistic action of NAC when combined with other antiepileptic medications (Figure 4).

Nonetheless, there were inconsistencies between some models and dosing regimens. Chitolina *et al*.[16] reported that NAC exhibited no notable anticonvulsant efficacy against acute PTZ-induced seizures, implying the limitations of efficacy of NAC in reduced-dosing and short-exposure-duration acute models. This was contrasted with our results, where chronic and well-dosed therapies significantly modulated seizures. Additionally, Tallarico *et al*.[20] provided evidence of worsening of seizures in models of spontaneous absence epilepsy at 500 mg/kg/day, a result which was contrary to anticonvulsant effects on other models, implying the possibility that the character of the seizure and underlying neurochemical mechanisms are determinants of the therapeutic profile of NAC. This was also consistent with our observation that optimization of dosing is necessary because higher dosing and some epilepsy models are capable of generating paradoxical effects.

In addition, Victor *et al*.[21] provided evidence of NAC reversal of levetiracetam-induced reproductive and neuroendocrine dysfunction in epileptic rats, implying systemic and hormonal regulatory actions in addition to seizure control. This broadened the therapeutic range of NAC's action, consistent with our observations of its augmented neuroprotective and systemic effects, although such endpoints were not the main area of focus of our included trials. Zaeri *et al*.[22] showed that chronic administration of NAC induced dose-dependent anticonvulsant effect but also reported toxicity and weight loss at doses above 75 mg/kg, consistent with the side effects we reported at higher chronic dosing in our review.

The treatment of epilepsy has moved more towards the evaluation of neuroprotective and disease-modifying drugs in association with conventional antiseizure drugs. The pathogenesis of epilepsy has been strongly implicated to involve oxidative stress as a major factor, where repeated seizure activity generates

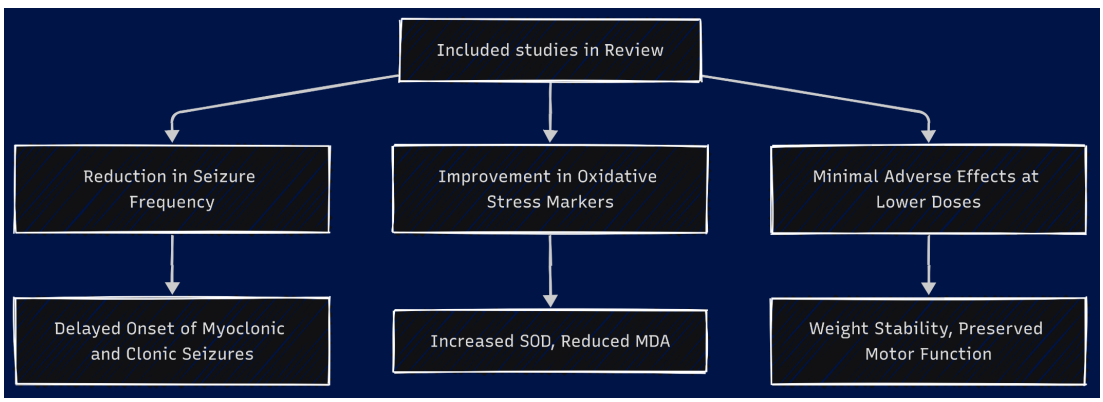

Figure 4. Schematics of the review's findings

excessive reactive oxygen species (ROS) that lead to lipid peroxidation, mitochondrial dysfunction, and neuronal apoptosis.[23] More specifically, mitochondrial instability has been correlated with disruptions in calcium handling and decreases in ATP production, elevating neuronal hyperexcitability and lowering seizure thresholds.[24] In this context, compounds with the potential to modulate oxidative stress-related pathways, maintain mitochondrial integrity, and modulate redox balance are candidate adjunctive therapies for the treatment of epilepsy.

NAC, a cysteine donor and a glutathione precursor, has been the focus of intense study in numerous models of neurodegeneration. It is recognized to elevate intracellular antioxidants with the concomitant diminution in neuroinflammatory signaling pathways.[25] The efficacy of NAC in modulating central molecular targets, such as nuclear factor erythroid 2–related factor 2 (Nrf2), mechanistic target of rapamycin (mTOR), and regulators of apoptosis proteins, positions it as a candidate drug to potentially modulate epileptogenesis at both cellular and synaptic interfaces.[26] In addition, mitochondrial dysfunctions, as a hallmark feature of both focal and generalized epilepsies, have been reported to be amenable to treatments aimed at correcting mitochondrial dynamics, membrane potential, and oxidative phosphorylation efficiency. The reported action of NAC on mitochondrial fission and fusion proteins, Drp1 and OPA1, points to its therapeutic promise in correcting mitochondrial abnormalities associated with chronic seizure activity.[27]

Aside from its antioxidative effect, NAC's capacity to reduce neuroinflammation has also been of interest. Activation of microglia and production of pro-inflammatory cytokines, including interleukin-1β (IL-1β) and tumor necrosis factor-alpha (TNF-α), are implicated in exacerbating neuronal damage and in epileptogenesis.[28] NAC can suppress these cytokines and block microglial activation in other neuroinflammatory diseases, and such an action may also be responsible in epilepsy. Its effect on glutamatergic transmission by modulation of cystine–glutamate antiporters and metabotropic glutamate receptor pathways also presents a complementary mechanism of seizure regulation by restricting excitatory neurotransmission.[29]

Although therapeutic promise of NAC is underpinned by biological plausibility and mechanistic flexibility, pharmacokinetic properties pose challenges. Limited oral bioavailability of NAC, at 6–10%, and widespread hepatic metabolism require high doses to attain therapeutic CNS levels.[30] Therapeutic ratio of efficacy to potential toxicity is especially tenuous in epilepsy, in which high doses paradoxically trigger excitotoxic or metabolic disturbances. NAC action may also be seizure type-dependent, with some models, such as absence epilepsy, showing potential for seizure exacerbation rather than suppression, possibly due to differential receptor sensitivities and network-specific vulnerabilities.[31]

For limitations, this review was marred by heterogeneity of animal models, variability in methods of epilepsy induction, variable reporting of percentages of reduction in seizure frequency, and the absence of standardized biomarker analysis across trials. High-dose toxicity was noted but not quantitatively measured systematically, and imaging measures and long-term safety were not evaluated. Lack of human trials also denied direct clinical extrapolation.

Recommendations and future implications

Follow-up research should be focused on standardizing the animal models and dosing regimens to permit comparative analysis (Figure 5). Long-term safety and efficacy studies of chronic NAC treatment should be considered. Thorough studies of neuroinflammatory and mitochondrial biomarkers should be included, as well as new imaging modalities. Combination therapy with the optimal doses should be investigated further. Translational studies from preclinical to clinical stages should be prioritized to develop therapeutic standards for NAC treatment of epilepsy.

In conclusion, the overall findings showed that NAC treatment was associated with notable statistical reductions in seizure frequency and enhancement of markers of oxidative stress in chemically induced models of epilepsy. The effects were dose-dependent and were most evident with long-term treatment. In absence epilepsy models, elevated seizure activity and behavioral side effects were seen at high doses. Combination treatments, i.e., gabapentin and NAC, were synergistically effective and also showed mild motor side effects. The findings suggested that NAC showed anticonvulsant, antioxidant, and neuroprotective effects, which required precise optimization of dosage to attain therapeutic efficacy and toxicity.

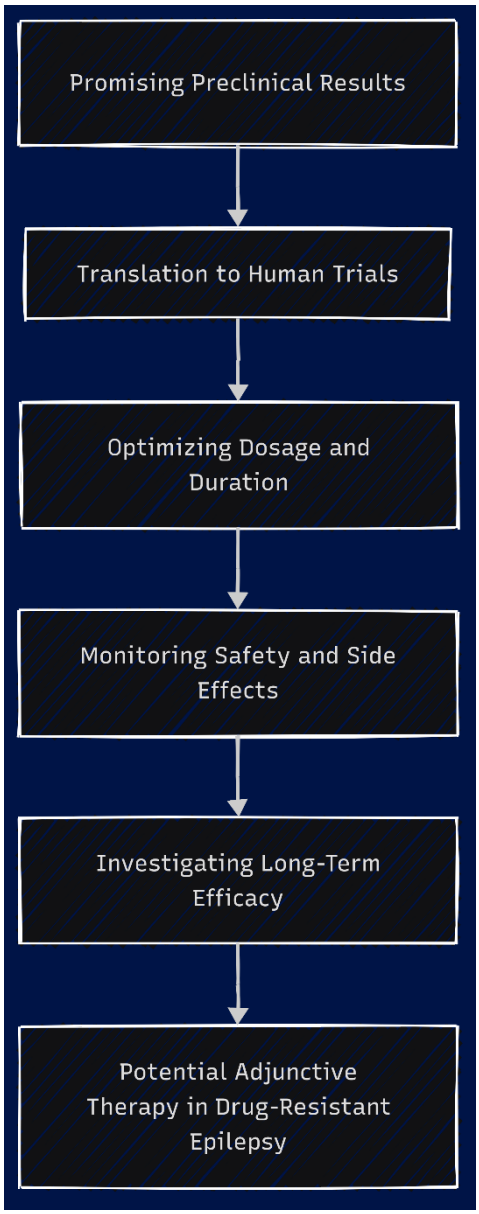

Figure 5. Implications for future research

DISCLOSURE

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