



ANTICONVULSANT ACTIVITY OF THYMOQUINONE IN PENTYLENETETRAZOLE-INDUCED SEIZURES IN MICE

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1. INTRODUCTION

Epilepsy affects tens of millions of people worldwide, with most of the burden falling on low- and middle-income countries [1]. Prevalence is greatest in low-income regions, where the treatment gap is also widest [2]. Modern antiseizure medicines control seizures in roughly two thirds of patients, yet a persistent third continue to seize despite adequate trials of two or more drugs, a proportion that has hardly changed over decades of drug development [3, 4]. Side effects such as sedation, cognitive slowing and teratogenicity add to the burden. This unmet need keeps the search for new anticonvulsant compounds, including those of

Abstract

Thymoquinone, the main constituent of Nigella sativa oil, has been reported to protect rodents against seizures induced by pentylentetrazole (PTZ). This narrative review summarises the published evidence on its anticonvulsant activity in PTZ-induced seizures in mice and in related models. It explains what the PTZ test measures, describes the pharmacology and formulation problems of the compound, and sets out the proposed GABAergic, opioid, antioxidant and anti-inflammatory mechanisms. The review reports no new data. Independent studies describe consistent protection, but the work is limited by small groups, scarce randomisation and blinding, uncertain brain exposure and models of limited clinical validity.

plant origin, active in many laboratories. In many countries, people with epilepsy also face stigma, restrictions on driving and employment, and an increased risk of premature death, so the consequences of poorly controlled seizures reach well beyond the seizures themselves.

Thymoquinone is the principal bioactive constituent of the volatile oil of *Nigella sativa*, the black seed used for centuries in traditional medicine across the Middle East, South Asia and North Africa. Reviews of the plant describe a long list of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial and hypoglycaemic effects, with thymoquinone usually identified as the main active principle [5, 6]. Interest in its effect on the nervous system dates from reports in the early 2000s that the compound suppressed experimental seizures in rodents. Those reports used the pentylenetetrazole (PTZ) test, a workhorse of anticonvulsant screening, and the same model continues to dominate the literature on this compound. Despite that history, the plant is usually consumed as a food or supplement, and the amount of thymoquinone a person receives from a spoonful of oil is small and variable. Laboratory doses are often many times higher than any dietary exposure.

This review summarises what has been published on the anticonvulsant activity of thymoquinone in PTZ-induced seizures in mice, and on related experimental work that bears on it. It explains what the PTZ model can and cannot tell a reader, describes the pharmacology and formulation properties of thymoquinone that matter for interpretation, collects the reported findings and proposed mechanisms, and identifies weaknesses in the evidence. The review reports no new animal data, and it does not suggest that thymoquinone is ready for clinical use in epilepsy [7, 8]. Throughout, the emphasis is on what can reasonably be concluded from the published work and on how much confidence each conclusion deserves. Readers wanting protocol details should consult the original papers cited.

2. SEIZURE MODELS AND THE PTZ TEST

Animal seizure models are screening tools, and every one of them captures only part of human epilepsy. The two oldest acute tests, maximal electroshock and subcutaneous PTZ, were chosen in the middle of the last century because they separated drugs that were effective against tonic-clonic seizures from those effective against absence and myoclonic

seizures [9, 10]. Maximal electroshock identifies agents such as phenytoin and carbamazepine, whereas the PTZ test identifies ethosuximide and valproate. Together they formed the backbone of national screening programmes and led to many marketed drugs, though critics point out that they favour compounds with mechanisms similar to older ones and may miss new classes [11]. The ideal screening model would predict clinical efficacy across seizure types, but none does, which is why programmes use batteries of tests and read results in combination.

PTZ acts as a convulsant mainly by blocking the chloride channel of the GABA_A receptor, in a manner comparable to picrotoxin [12]. Reduced inhibitory tone in the brain then allows excitatory activity to spread. Given by injection to mice, it produces a predictable sequence: myoclonic jerks, then generalised clonic seizures, and at higher doses tonic hindlimb extension and death. Observers record the latency to each stage, the duration and the proportion of animals that die. The sequence makes the test sensitive to drugs that enhance GABAergic inhibition, including benzodiazepines, barbiturates and valproate, and to those that reduce excitatory transmission or hyperpolarise neurons [11, 13]. The relationship is not perfect. PTZ also affects other channels at higher concentrations, and the proportion of its action that is explained by the GABA_A receptor remains a matter of debate, so protection in the test does not by itself prove a GABAergic mechanism.

Doses and routes differ between laboratories, and these choices determine what the test measures. Subcutaneous administration in the region of 60 to 85 mg/kg is typical for the standard screening test, whereas intraperitoneal doses of about 35 to 60 mg/kg are often used for convenience, and the exact dose is usually adjusted to the strain and supplier of the mice so that nearly all control animals show clonic seizures [9, 13]. Because a fixed dose is not equally convulsant in every colony, the control group is not a formality; it is the calibration of the experiment. Reports that fail to describe the control response in detail are hard to interpret [13]. Mice are also

generally observed for 30 minutes after injection, and a decision about when to stop observation changes the apparent latency and survival figures. Where possible, a pilot study to find the convulsant dose for the particular batch of animals should precede the main experiment.

The kindling variant of the model uses repeated sub-convulsive PTZ injections, typically every second day, until animals respond to the same dose with generalised seizures. The progression is scored on a modified Racine scale, which grades behaviour from immobility through facial twitching and head nodding to rearing and falling with generalised convulsions [14, 15]. Kindling takes weeks, mimics some aspects of epileptogenesis and lowers the seizure threshold permanently, so it is used to ask whether a compound slows the development of epilepsy rather than only suppresses an acute seizure. Acute and kindled models answer separate questions, and findings in one cannot stand in for the other [7, 14]. Chronic PTZ studies can also cause cumulative weight loss and mortality, which must be reported, as exclusion of animals that die can distort the picture of drug effect.

The usefulness of the PTZ model for predicting efficacy in patients is limited, and reviewers have said so repeatedly. Acute seizures in naive animals do not reproduce spontaneous recurrent seizures, hippocampal sclerosis, comorbid cognitive change or the drug-resistant phenotype that clinicians struggle with most [8, 11]. Even so, the test remains useful for early detection of activity against generalised seizures and for exploring mechanism, which is why it is retained in screening cascades [16, 17]. A positive result is best read as a lead that needs to be confirmed in models with greater clinical validity, not as evidence of efficacy. Where the test is used to support claims about a natural product, authors should state clearly that the result is exploratory and avoid language that implies treatment of epilepsy.

Other design points also affect reliability. Time of day, fasting status, handling stress and noise can change seizure latency. Mice of different strains and sexes respond differently to PTZ, and young animals are more

susceptible than adults. Anticonvulsant drugs given in the same experiment, such as diazepam or valproate, are included as positive controls to show that the assay is working. Consistent observation by a person unaware of the treatment, preferably supported by video recording, reduces the risk of subjective scoring, and sample size should be stated in advance [18]. Report of these factors lets the reader judge whether results could be expected to hold elsewhere, and failure to report them is one of the main reasons that preclinical findings are hard to reproduce. Housing temperature, cage enrichment and the time allowed for acclimatisation also contribute, and are cheap to record.

3. THYMOQUINONE: SOURCE AND PHARMACOLOGY

Thymoquinone

(2-isopropyl-5-methyl-1,4-benzoquinone) makes up a substantial fraction of *Nigella sativa* essential oil, and its content varies with the origin of the seeds, the extraction method and storage [5, 6]. It is a lipophilic quinone that is poorly soluble in water, and its stability in solution depends on pH, light and the solvent used. Degradation is faster under light and in alkaline media, which means that preparations made at the bench and kept for several hours may contain less of the compound than the label implies [19]. Fresh preparation and protection from light are therefore part of good practice. Authentication of the seed material and chemical standardisation of extracts, usually by chromatography, are needed before results from different batches can be compared. A purchased reagent-grade compound is easier to interpret than a crude oil of unknown composition.

Pharmacokinetic information is limited and comes largely from rodent and rabbit studies. After oral dosing, thymoquinone is absorbed and shows measurable plasma exposure, but extensive protein binding, rapid distribution and first-pass effects make the concentration at the target hard to predict [20]. Its capacity to cross the blood-brain barrier is suggested by pharmacological effects on the central nervous system rather than directly quantified in most of the epilepsy papers, and brain

tissue concentrations are rarely reported. Without such data, the dose that reaches neurons in the experiment is an assumption, not a measurement [20, 21]. Clearance appears to be fairly rapid, so one injection may give protection for only a short window, and timing of the PTZ challenge after the thymoquinone dose therefore becomes an important experimental variable. Metabolism is thought to involve reduction to dihydrothymoquinone and conjugation, and some of the activity attributed to the parent compound could belong to its metabolites, a possibility that has seldom been examined in neurological work.

Toxicity data are reassuring in some respects. Acute toxicity studies in rodents indicate a wide gap between doses with pharmacological effects and those that produce overt harm, and *Nigella sativa* seed and oil have a long record of dietary use [5, 6]. However, reviews of thymoquinone caution that most evidence comes from short experiments, and that high intraperitoneal doses can be irritant and may cause toxicity in the liver and kidney in some studies [21, 22]. Route matters: intraperitoneal injection, the most common in epilepsy experiments, bypasses the first-pass metabolism that would limit exposure after swallowing, so efficacy by that route does not guarantee efficacy by mouth. Reports of liver and kidney changes at high doses do not establish a risk at the lower doses used in most seizure studies, but they emphasise that safety margins have not been defined in a rigorous way for chronic use [21].

Formulation research on thymoquinone has mostly been driven by cancer and inflammation, but its lessons apply to neurological work. Nanoparticles, liposomes, nanoemulsions and cyclodextrin complexes have all been explored to improve solubility, protect the molecule from light and degradation, and raise bioavailability [23]. For a drug that must reach the brain, nasal or lipid-based delivery systems are especially attractive. The approach has not yet been tested systematically in seizure models, so a formulation that improves exposure in the periphery is only a hypothetical advantage for anticonvulsant activity [19, 23]. Such work

also raises regulatory questions, since an engineered nanoparticle is a different product from the simple oil, with its own toxicology to be examined.

Interaction potential deserves attention. Thymoquinone and *Nigella sativa* preparations influence several cytochrome P450 enzymes and transporters in experimental systems, and the clinical significance for patients on antiseizure drugs has not been established [21, 22]. Anyone taking black seed oil as a supplement alongside valproate, carbamazepine or phenytoin is unlikely to be warned, because the product is sold as a food. Until proper studies exist, healthcare professionals should treat the combination as an unknown and ask patients about herbal use as a routine part of medication history. Antiseizure drugs have narrow therapeutic windows in many patients, so even modest changes in clearance can lead to loss of control or toxicity [24]. A related concern is that supplement products vary widely in thymoquinone content, so the exposure of any individual patient is unpredictable.

4. EVIDENCE FOR ANTICONVULSANT ACTIVITY

The reported evidence is collected in table 1, arranged by the kind of finding, and fig. 1 shows how many entries fall into each of the four groups. Direct PTZ studies in mice form only part of the picture, since mechanistic and pharmacokinetic work with related models are needed to interpret them. The table lists findings as described by the original authors and does not attempt to rate their quality, which varies a good deal [25, 26, 27]. Because many entries come from one or two laboratories, the number of rows should not be read as a measure of how settled each point is.

The foundational study in mice reported that thymoquinone given by intraperitoneal injection before PTZ prolonged the latency to tonic extension and reduced mortality, without markedly altering the onset of clonic seizures at the same doses [25]. This pattern is typical of agents with some GABAergic or membrane-stabilising action, and it is also the pattern produced by several established drugs.

A related study in rats gave thymoquinone directly into the cerebral ventricles and found suppression of epileptiform activity, a route that removes the questions of absorption and blood-brain barrier penetration [26]. Both reports are consistent with a central action. It is worth recalling that mortality is a blunt endpoint, and protection against death is easier to achieve than protection against the seizure itself. Replication of the finding with full dose-response data and a positive control would make it far more useful as a benchmark.

Further evidence concerns *Nigella sativa* oil in a different model. In rats made epileptic with pilocarpine, the oil reduced indices of oxidative stress in the brain, and the effect was compared with valproate, which suggests a degree of neuroprotection alongside seizure control [27]. A study in mice of thymoquinone combined with valproate reported benefit in seizure models and, in addition, less valproate-related liver injury [28]. If confirmed, such a combination would be of interest, because valproate is highly effective in generalised epilepsy yet carries a recognised risk of hepatotoxicity [24]. Differences between the pilocarpine model and the PTZ test, including the involvement of cholinergic mechanisms and the long-lasting nature of pilocarpine seizures, mean that the two sets of findings address related but distinct questions [27].

There is also indirect evidence from other neurological models. Thymoquinone lowered lipid peroxidation in the hippocampus after global cerebral ischaemia and reperfusion in rats [29], reduced neuronal apoptosis in cultured cortical neurons exposed to ethanol [30], and protected primary neurons against amyloid-beta toxicity [31]. A study of anxiety-like behaviour in mice suggested involvement of GABA and nitric oxide [32]. None of these is a seizure study, and they should be treated as supporting rather than confirming evidence, but they do show that thymoquinone has recurrent effects on oxidative and inhibitory pathways in neural tissue. Taken at face value they extend the reach of thymoquinone as a neuroprotective candidate, but they lean on cell culture and ischaemia models whose connection to

epilepsy is loose.

Table 1: Published findings on thymoquinone and related preparations in seizure and neuroprotection models

Study or feature	Published finding	Ref.
Acute PTZ seizures mice, intraperitoneal	Longer latency to tonic extension and lower mortality	[25]
Intracerebroventricular dosing rats	Suppressed epileptiform activity, bypassing absorption	[26]
<i>Nigella sativa</i> oil pilocarpine model	Lower oxidative stress markers; compared with valproate	[27]
Opioid receptors	Protective effect reported to be reduced by naloxone	[25, 26]
GABA and nitric oxide anxiety model in mice	Behavioural effects altered by GABAergic and NO modulators	[32]
Global cerebral ischaemia rat hippocampus	Reduced lipid peroxidation	[29]
Cultured neurons	Less apoptosis after ethanol; protection against amyloid-beta	[30, 31]
Co-administration with valproate	Benefit in seizure models with less valproate liver injury	[28]
Aqueous solubility and stability	Poorly soluble; degrades with light and alkaline pH	[19]
Oral pharmacokinetics rabbits	Measurable exposure but variable bioavailability	[20]

Reading the reports together, the strongest statement that can be made is that several independent laboratories, using different routes and models, have described protective effects of thymoquinone or *Nigella sativa* preparations against chemically or pharmacologically induced seizures. The same laboratories often supply the data, though, and replication by groups with no stake in the compound is scarce. Effect sizes are seldom accompanied by confidence intervals, and dose-response curves are often limited to two or three points, so the potency of thymoquinone relative to valproate or diazepam is poorly defined [25, 28, 33]. This is not unusual for natural-product pharmacology, but it is a reminder that apparent consistency can reflect common methods and a shared origin of the data rather than independent confirmation.

How would a protective effect in the PTZ test be positioned against established treatment? The test picks up drugs such as valproate and ethosuximide, and a compound active in it is conventionally assumed to have potential against generalised seizures, including absence and myoclonic types [13, 17]. That assumption is only partly justified. Absence seizures are better modelled by genetic strains such as the GAERS and WAG/Rij rats, in which spike-wave discharges occur spontaneously, and thymoquinone has not, as far as published reports show, been examined in those strains. A result in the PTZ test is therefore a reason to test further in more specific models, not a statement about which seizure type the compound might help [8, 11].

A few cautions are worth stating plainly. Protection against PTZ lethality can arise from nonspecific sedation or motor impairment, because a mouse that is too drowsy to display a full convulsion will score as protected. Rotarod or open-field testing alongside the seizure test helps separate anticonvulsant action from motor depression, but not all studies include these tests [9, 13]. Where they were absent, claims of anticonvulsant action should be considered provisional. The same applies to anxiolytic or sedative effects of the compound itself, which have been described in separate behavioural studies [32]. A sedative compound would be expected to look protective in the PTZ test whether or not it has any specific anticonvulsant property, and the literature does not always make that distinction clear for thymoquinone, so the point is worth testing explicitly in every new study.

5. PROPOSED MECHANISMS

The leading hypothesis links thymoquinone to enhanced GABAergic inhibition. PTZ acts at the GABA_A receptor, and anything that supports inhibitory neurotransmission in this system will tend to raise the seizure threshold [12, 34]. Evidence for a GABA-related action in thymoquinone comes mainly from the behavioural study in mice in which effects were altered by GABAergic modulators [32], and from the PTZ study, in which the anticonvulsant effect was explored using antagonists. The hypothesis is plausible, but direct receptor binding or electrophysiology

data for thymoquinone appear to be limited. Experiments such as patch-clamp recordings from neurons and binding assays with labelled ligands would give a more direct answer than behavioural antagonism alone.

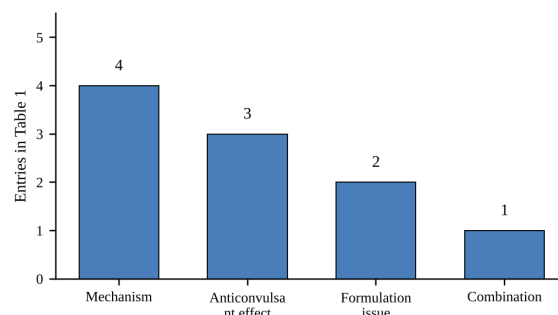


Fig. 1: Number of entries in table 1 by type of finding

An opioid contribution has been proposed on the basis of antagonism experiments. In the original PTZ studies, the anticonvulsant effect of thymoquinone was reported to be reduced by the opioid antagonist naloxone, which led the authors to suggest involvement of opioid receptors [25, 26]. Endogenous opioid peptides can modulate seizure threshold in rodents, so the idea has some precedent. At the same time, antagonist experiments of this kind are indirect, naloxone itself has effects on seizure susceptibility at some doses, and the finding has not been widely replicated. Studies using more selective antagonists, knockout animals or direct receptor binding would help to decide whether the effect is genuine.

Other targets have been floated, though with less support. Voltage-gated sodium and calcium channels, glutamate receptors and potassium channels are all plausible sites for a small lipophilic molecule, and several established antiseizure drugs act on them [11, 34]. Thymoquinone has been reported to affect calcium handling and membrane properties in non-neuronal cells, which makes such effects conceivable in neurons. Nobody has yet shown them in seizure-relevant preparations, and they should be considered hypotheses suggested by the compound's general pharmacology. Patch-clamp work on cultured or slice neurons would be the natural way to test whether any of them is real [11, 35].

Oxidative stress offers a third route. Seizures are accompanied by excessive production of reactive oxygen species, mitochondrial dysfunction and lipid peroxidation, and these changes in turn make neurons more excitable and more vulnerable to death [36, 37]. Thymoquinone is a recognised antioxidant that raises endogenous defences in several tissues, and in the hippocampal ischaemia study it lowered lipid peroxidation markedly [29]. Likewise, *Nigella sativa* oil reduced oxidative stress markers in the pilocarpine model [27]. Antioxidant action could therefore contribute to neuroprotection even if it does little to the acute threshold in PTZ testing. Whether lowering oxidative stress changes seizure threshold acutely, or mainly limits the damage that follows repeated seizures, is a distinction the existing studies do not settle [36, 37]. Whether the antioxidant effect is direct radical scavenging or induction of endogenous enzymes such as superoxide dismutase and glutathione peroxidase also remains uncertain.

Neuroinflammation is related. Cytokines such as interleukin-1 beta and tumour necrosis factor are released in the brain during and after seizures, and experimental work suggests that they can lower seizure threshold and contribute to epileptogenesis [35]. Thymoquinone suppresses inflammatory signalling, including nuclear factor kappa B, in non-neuronal models [5, 21]. Whether the same mechanism operates in the brain in seizure models is not established, though it would fit with the compound's effect on the more chronic kindling and pilocarpine paradigms, as opposed to only on acute chemoconvulsion [27, 35]. Measuring brain cytokines after thymoquinone treatment in a kindling paradigm would be a direct test and appears to be missing from the literature.

A final hypothesis involves nitric oxide, which has dual effects in seizure models, being proconvulsant in some settings and anticonvulsant in others. Interaction with nitric oxide signalling was suggested in the behavioural study of thymoquinone in mice [32]. Mechanisms are not mutually exclusive, and a compound as chemically reactive as a quinone is likely to act on several targets. This multi-target profile might be an

advantage in a complex disease, yet it makes it harder to define a dose-response relationship, to predict adverse effects and to develop the compound along conventional lines [11, 34]. Experiments with inhibitors of nitric oxide synthase, run in parallel with thymoquinone, have not been reported in PTZ models.

6. LIMITATIONS AND FUTURE DIRECTIONS

The first limitation concerns study quality. Reports on thymoquinone in seizure models seldom state whether animals were randomly allocated or observers blinded, and groups are commonly small. Systematic reviews of animal studies have shown that omission of these safeguards is associated with inflated effect sizes, and tools to assess the risk of bias in animal experiments now exist for exactly this purpose [18, 33, 38]. The ARRIVE guidelines, updated in 2020, list the information that every animal paper should provide, and applying them retrospectively to the thymoquinone literature would be a useful exercise, especially as journal uptake of the standard has been uneven [39]. Heterogeneity in dose, route, timing and strain makes a quantitative synthesis impractical, so narrative review is the only honest option at present.

The second concerns the compound itself. Thymoquinone is unstable, poorly soluble and variably bioavailable, so doses reported as milligrams per kilogram may translate into very different exposures between laboratories [19, 20]. Studies should describe the source and purity of the compound, the vehicle and how long the preparation stood before use. Plasma and brain concentrations measured at the time of seizure testing would be extremely informative. Dose conversion to human equivalents on a body surface area basis, not on weight alone, would also help readers judge whether the doses are realistic [40]. Such measures are inexpensive compared with the cost of repeating an uninterpretable experiment. Reporting the number of animals excluded, and why, completes the picture.

Third, the choice of model needs to broaden. Evidence from acute PTZ tests cannot show that thymoquinone would help in

drug-resistant epilepsy. Models such as the 6 Hz psychomotor seizure test, amygdala kindling, and chronic spontaneous seizure models after status epilepticus would test activity against drug-resistant seizures and disease modification more directly [7, 11]. Chronic dosing studies should also assess cognition and behaviour, because both are affected in epilepsy and by many antiseizure drugs [11, 17]. Positive results in such models are far more persuasive to clinicians because they come closer to the conditions in which patients actually receive treatment. Equally useful would be tests of thymoquinone as an add-on to a standard drug in animals that do not respond to that drug alone.

Future work might usefully combine formulation science with pharmacology. A brain-targeted delivery system, together with pharmacokinetic and pharmacodynamic modelling, could separate the intrinsic activity of thymoquinone from the effects of poor exposure [19, 23]. Studies on interactions with established antiseizure drugs should be done carefully, with attention to pharmacokinetic as well as pharmacodynamic interplay [24, 28]. If these steps give a consistent result, the case for early clinical studies of safety and tolerability in adults with epilepsy could be made on a stronger footing. Collaboration between phytochemists, pharmacologists and clinical epileptologists would suit this kind of question, with shared protocols and open data.

A broader point concerns how natural products are evaluated. Compounds with a long traditional history attract enthusiasm that can run ahead of data. Careful reporting, independent replication and willingness to publish negative results guard against that tendency, and funders could support replication studies as a distinct category of work. In epilepsy, where patients and families may be tempted to try herbal remedies in place of proven medicines, the stakes of getting this right are not merely academic [16]. Reviews such as this one can help by pointing out what has and has not been shown, in language that does not overstate the case. Patient groups and clinicians can also help, by being straightforward that traditional use does not equal proof of benefit.

7. CONCLUSION

Thymoquinone has been reported by several research groups to delay or reduce chemically induced seizures in rodents, and the PTZ test in mice gave the original and most often cited results [25, 26]. Proposed mechanisms include enhancement of GABAergic inhibition, a possible opioid component, antioxidant defence and suppression of neuroinflammation, each supported by indirect rather than conclusive evidence [29, 32, 35]. As a lead from a long-used food and medicinal plant, it is of genuine interest. In a field short of effective new options, such leads deserve careful study, but careful study means being honest about what the present evidence can bear.

The limits of the evidence are equally clear. The PTZ test is a screening assay that predicts activity against generalised seizures but not drug-resistant epilepsy; reporting often omits randomisation, blinding and exposure measurements; and thymoquinone itself is unstable and poorly soluble [4, 19, 33]. Properly controlled studies in chronic and drug-resistant models, with formulation and brain-exposure data, are the sensible next step before any suggestion of clinical use. Reporting standards that are routine in clinical research, such as pre-specified endpoints and transparent handling of exclusions, would raise the value of every future experiment. Until then, thymoquinone is best described as an interesting preclinical lead and not as a candidate treatment.

8. ABBREVIATIONS

ARRIVE-Animal Research: Reporting of In Vivo Experiments, GABA-Gamma-aminobutyric acid, GABA_A-Gamma-aminobutyric acid type A receptor, ILAE-International League Against Epilepsy, NO-Nitric oxide, PTZ-Pentylentetrazole

9. FUNDING

Nil

10. AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

11. CONFLICT OF INTERESTS

Declared none

12. REFERENCES

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