The Journal of Phytopharmacology 2026; 15(1):86-93
Online at: www.phytopharmajournal.com


Research Article

ISSN 2320-480X
JPHYTO 2026; 15(1): 86-93
January- February
Received: 13-01-2026
Accepted: 16-03-2026
Published: 30-03-2026

doi: 10.31254/phyto.2026.15112

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# Neuroprotective effects of potentized alumina on oxidative stress markers in a rat model of cholinergic dysfunction

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## ABSTRACT

**Background:** Neurodegeneration in Alzheimer's disease is closely linked to excessive oxidative stress, with evidence of heightened lipid peroxidation and failing antioxidant systems in affected brain regions. Alumina, a homoeopathic preparation sourced from aluminium oxide, has a classical Materia Medica profile encompassing motor sluggishness, cognitive slowing, and paralytic weakness. These features overlap considerably with the neurological disturbances generated by cholinergic blockade, making it a candidate of interest for evaluation in pharmacological models of dementia. **Objective:** The present study evaluated the impact of three homoeopathic potencies of Alumina (200C, 1M, and 10M) on cerebral oxidative stress parameters specifically malondialdehyde (MDA), catalase, and reduced glutathione (GSH) in a scopolamine-based rat model of cholinergic dementia. **Materials and Methods:** Thirty-six adults male Wistar rats were randomly allocated into six groups (n = 6 per group): Group I (normal control), Group II (scopolamine-induced disease control), Group III (Donepezil-treated standard), Group IV (Alumina 200 C), Group V (Alumina 1M), and Group VI (Alumina 10M). Alumina preparations and Donepezil were administered orally for 14 consecutive days. On Day 15, cognitive impairment and oxidative stress were induced in all groups except the normal control by intraperitoneal injection of scopolamine (1 mg/kg). Following treatment, animals were sacrificed, and brain tissues were harvested for biochemical analysis. Levels of malondialdehyde (MDA) were estimated as an index of lipid peroxidation, while catalase activity and reduced glutathione (GSH) levels were assessed to evaluate enzymatic and non-enzymatic antioxidant status, respectively. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. **Results:** Scopolamine significantly increased MDA levels (4.12 ± 0.051 ng/mL vs. 1.25 ± 0.036 in controls), while catalase and GSH levels decreased markedly (28.83 ± 0.508 vs. 59.17 ± 0.508, and 3.18 ± 0.051 vs. 6.92 ± 0.051 ng/mL, respectively), collectively indicating a state of cerebral oxidative stress. Treatment with Alumina at all three potencies partially to substantially reversed these alterations. MDA levels decreased progressively from 3.95 ± 0.048 (200C) to 2.92 ± 0.074 (1M) and 2.33 ± 0.089 (10M). In contrast, catalase and GSH levels increased in a potency-dependent manner. The 10M group showed results most comparable to those of Donepezil across all three parameters. **Conclusion:** Alumina, at homoeopathic potencies, produced measurable and graded changes in markers of cerebral oxidative stress, with higher potencies yielding greater biochemical normalization. These findings suggest a potential antioxidant neuroprotective effect that warrants further mechanistic and clinical investigation.

**Keywords:** Oxidative stress, Malondialdehyde (MDA), Glutathione (GSH), Neuroprotection, Scopolamine-induced dementia model, Homoeopathic Alumina.

## INTRODUCTION

Dementia currently affects over 55 million individuals globally, with Alzheimer's disease accounting for the majority of these cases [1,2]. Despite the magnitude of this burden, the therapeutic landscape has advanced little; existing medications can modestly attenuate symptoms but are incapable of halting the underlying neurodegenerative cascade. This therapeutic gap has encouraged exploration of traditional and integrative medical systems, including homeopathy, which demonstrate promising preclinical and clinical research findings that merit further rigorous investigation.

One feature of Alzheimer's disease that has drawn sustained research attention is the early and progressive failure of the brain's oxidative defence systems. Long before clinical symptoms appear,

reactive oxygen species begin attacking neuronal membranes, breaking down polyunsaturated fatty acids into measurable byproducts the most studied of which is malondialdehyde (MDA) [3,4]. Elevated MDA levels in brain tissue, as a key biomarker of lipid peroxidation [5] have been consistently linked to the severity of oxidative membrane injury in Alzheimer's pathology [6]. What makes this particularly damaging is that lipid peroxidation does not occur in isolation. Catalase, the enzyme responsible for neutralising hydrogen peroxide before it produces the highly destructive hydroxyl radical, loses activity in parallel. Reduced glutathione (GSH), the brain's main non-enzymatic antioxidant, is simultaneously depleted [7,8]. Once enzymatic and non-enzymatic defences fall together, the oxidative cascade becomes difficult to interrupt and neuronal loss accelerates [9,10].

In experimental settings, scopolamine-induced cholinergic blockade in rodents consistently reproduces this biochemical profile by disrupting muscarinic signaling and promoting oxidative stress, as shown in several rodent studies [11-14]. Donepezil, included in this study as a reference drug, corrects these oxidative changes along with the functional deficits, confirming that cholinergic recovery and antioxidant restoration are closely linked. [15].

Against this background, homoeopathic ultra-high dilutions have been reported in a number of controlled preclinical studies to produce measurable, preparation-specific effects on oxidative stress markers, though the findings remain contested and mechanistic understanding is limited [16,17]. Alumina prepared from aluminium oxide holds particular interest here. Its classical Homeopathic materia medica description includes progressive motor slowing, difficulty initiating movement, incoordination, and cognitive dulling [18-22], a symptom cluster that maps closely onto the functional deficits that cholinergic blockade generates in rodent models. This correspondence, interpreted through the homoeopathic Law of Similars, formed the rationale for selecting Alumina in the present study. Three potencies 200C, 1M, and 10M were included to explore whether any potency-dependent pattern of change in cerebral MDA, catalase, and GSH could be detected in scopolamine-treated male Wistar rats.

## MATERIAL AND METHODS

### Chemicals and reagents

Scopolamine hydrobromide and donepezil hydrochloride were obtained from Sigma-Aldrich (or equivalent). Homoeopathic Alumina (200C, 1M, 10M) was procured from Om Homoeopathic Pharmacy, Mumbai, via Willmar Schwabe India Pvt. Ltd., prepared according to the *Homoeopathic Pharmacopoeia of India*. Thiobarbituric acid, trichloroacetic acid, 5,5′-dithiobis-(2-nitrobenzoic acid), and ammonium molybdate were of analytical grade (Merck/Sigma-Aldrich or equivalent). ELISA-based kits for MDA, catalase, and GSH (Elabscience Biotechnology, catalogue nos E-EL-0127, E-EL-0776, and E-EL-0077, respectively) were used as per the manufacturer's instructions. All reagents were prepared fresh and stored at the recommended temperatures.

### Animal Model and Experimental Groups

Male adult Wistar rats (n=36), aged between 8 and 12 weeks and with body weights of 250-280 g, were procured from Biotox Laboratories and distributed by random allocation into six experimental groups of six animals each. Animals were housed in polypropylene cages under standardised environmental conditions: ambient temperature 20.3-22.7 °C, relative humidity 50-58%, and a 12 h light-dark photoperiod. Unrestricted access to commercial pelleted chow and reverse osmosis-purified drinking water was maintained throughout. All procedures received prior ethical clearance from the Institutional Animal Ethics Committee at Biotox (clearance reference Biotox/IAEC/06/2025/RP-35) and were carried out in full conformity with CPCSEA mandated guidelines.

Six experimental groups were constituted: GI (normal control, vehicle only), GII (disease control, scopolamine alone), GIII (standard drug, Donepezil 2.5 mg/kg orally once daily), GIV (Alumina 200C), GV (Alumina 1M), and GVI (Alumina 10M). Homoeopathic preparations were procured from Om Homoeopathic Pharmacy, Mumbai, sourced through Dr. Willmar Schwabe India Pvt. Ltd., and prepared in conformity with the Homoeopathic Pharmacopoeia of India. Test substances were administered orally for fourteen consecutive days. On the fifteenth day, all groups other than GI received a single intraperitoneal injection of scopolamine hydrobromide (1 mg/kg) to precipitate acute cholinergic blockade and associated oxidative stress.

### Tissue Collection

Upon completion of all experimental assessments on Day 15, animals underwent euthanasia via cervical dislocation under brief anaesthesia, performed by trained personnel adhering to institutional ethical standards. The brain was rapidly excised, washed briefly in chilled isotonic saline, blotted to remove excess fluid, weighed, and transferred to labelled microcentrifuge tubes. All samples were immediately snap-frozen and held at -80 °C until biochemical processing. To protect the integrity of the blinding protocol, brain harvest and all subsequent tissue handling were performed by staff unaware of group assignments.

### Biochemical Assays

Brain homogenates were prepared and subjected to three biochemical assays targeting distinct but complementary aspects of the oxidative stress response:

### Malondialdehyde (MDA)- Lipid Peroxidation Index

Malondialdehyde (MDA), the principal index of lipid peroxidation, was quantified using the thiobarbituric acid reactive substances (TBARS) assay in the present study [25]. Briefly, brain homogenates were prepared in chilled 0.1 M phosphate buffer (pH 7.4) and clarified by centrifugation at 3000 rpm for 15 min. The resulting supernatant was combined with thiobarbituric acid reagent and subjected to acidic heat conditions (95 °C for 20 min), generating a pink chromophore that was measured spectrophotometrically at 532 nm. Results were expressed as ng/ml of homogenate.

### Catalase (CAT) Activity

Catalase activity was evaluated by measuring the enzyme's capacity to break down hydrogen peroxide. Brain homogenate supernatant was combined with a defined $H_2O_2$ concentration in phosphate buffer and incubated for a fixed period. Undigested $H_2O_2$ was then detected colorimetrically via ammonium molybdate, producing a yellow complex with peak absorbance at 405 nm. Progressive loss of absorbance indicates increasing catalytic efficiency. As with MDA and GSH, catalase was quantified using the Elabscience ELISA-based kit (catalogue no. E-EL-0776), which reports the enzyme as protein mass per volume (ng/ml of homogenate supernatant) rather than as a traditional enzymatic rate (U/mg protein). This unit convention is consistent across all three assays in the present study and reflects kit-standardised quantification; results should be interpreted as relative protein concentrations rather than direct enzyme activity rates.

### Reduced Glutathione (GSH)

Tissue GSH was determined using Ellman's reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB). Protein interference was eliminated by trichloroacetic acid precipitation followed by centrifugal clarification. The resulting deproteinised supernatant was incubated with DTNB at room temperature; the yellow-coloured 5-thionitrobenzoic acid (TNB) product formed was recorded spectrophotometrically at 412 nm. GSH content was expressed in ng/ml. All three biochemical parameters (MDA, catalase, GSH) were quantified using commercially validated ELISA-based kits supplied by Elabscience Biotechnology (Wuhan,

China), which employ antibody-mediated detection and standardise readouts to ng/ml of homogenate supernatant. This unit convention reflects kit-specific quantification and differs from classical spectrophotometric activity-based reporting (U/mg protein); values are therefore internally consistent across parameters. The vehicle administered to the normal control group (GI) comprised 70% ethanol in distilled water at the concentration equivalent to that present in the lowest-potency preparation (200C), ensuring that any solvent contribution was accounted for in the control baseline.

**Statistical Analysis**

All quantitative results are expressed as mean ± SEM. Inter-group differences were assessed using one-way ANOVA. Where the omnibus F-test was significant ($p < 0.05$), pairwise comparisons were performed using Tukey's HSD post-hoc test. All analyses were carried out using GraphPad Prism v9.0 (GraphPad Software, San Diego, CA, USA).

*Sample size estimation*

Based on variance data from published scopolamine-induced oxidative stress studies (MDA SEM/mean ratios: 0.01-0.05), a priori sample-size calculation with G*Power 3.1 (effect size f = 0.8, $\alpha$ = 0.05, power = 0.80, k = 6 groups) indicated a minimum of n = 5 animals per group. A conservative sample size of n = 6 per group was adopted.

*Blinding and design limitations*

Test preparations were coded by a laboratory technician not involved in animal dosing. Due to visible differences between preparations, full blinding during administration was not possible. However, brain tissue collection and all downstream biochemical assays were performed by personnel masked to group identity. This partial single-blind design is acknowledged as a limitation.

## RESULTS

**Numbers Analysed**

A total of 36 male Wistar rats, distributed equally across six groups (n=6 each), were included in the biochemical analysis. No animal died or was removed during the course of the experiment, and tissue was successfully collected from every subject. The dataset was therefore complete, with no missing values.

**Malondialdehyde (MDA)- Lipid Peroxidation**

Individual and group mean MDA values are shown in Table 1. Animals that received scopolamine alone (GII) had markedly higher brain MDA compared to the untreated controls (4.12 ± 0.051 vs 1.25 ± 0.036 ng/mL), reflecting a substantial increase in lipid peroxidation following cholinergic blockade. Donepezil treatment (GIII) brought MDA down to 2.05 ± 0.036 ng/mL, demonstrating that pharmacological restoration of cholinergic tone also attenuates oxidative membrane damage.

Among the Alumina groups, MDA values declined in step with rising potency: 3.95 ± 0.048 ng/mL at 200C, 2.92 ± 0.074 at 1M, and 2.33 ± 0.089 at 10M. At the highest potency, MDA approached the level seen with Donepezil, suggesting that Alumina 10M produces a degree of antioxidant protection broadly comparable to the standard drug in this model.

**Catalase Activity**

Table 3 shows catalase values for all groups. Scopolamine-treated animals (GII) had catalase activity almost half that of normal controls (28.83 ± 0.508 vs 59.17 ± 0.508 ng/mL), pointing to a pronounced suppression of this key antioxidant enzyme. Treatment with Donepezil recovered catalase activity substantially (GIII: 49.17 ± 0.508 ng/mL), again consistent with the cholinergic-antioxidant regulatory link documented in the literature.

Alumina-treated animals showed a stepwise improvement in catalase that tracked potency: 33.00 ± 0.488 (200C), 41.00 ± 0.488 (1M), and 47.17 ± 0.508 ng/ml (10M). The 10M group came within close range of the Donepezil-treated animals, pointing to substantial recovery of enzymatic antioxidant capacity at the higher potencies.

**Reduced Glutathione (GSH)**

GSH data are shown in Table 5. The disease control group registered a sharp drop in brain GSH relative to untreated animals (3.18 ± 0.051 vs 6.92 ± 0.051 ng/mL), which underscores how significantly scopolamine depletes the non-enzymatic arm of the brain's antioxidant system. Donepezil largely reversed this depletion, with GII recovering to 5.92 ± 0.051 ng/mL.

GSH levels rose progressively with increasing Alumina potency: 3.70 ± 0.049 ng/ml at 200C, 4.90 ± 0.049 at 1M, and 5.72 ± 0.051 at 10M. The 10M group essentially matched the Donepezil group, suggesting that at this potency, Alumina achieves near-equivalent restoration of endogenous glutathione-based antioxidant protection.

**Inferential Data**

One-way ANOVA yielded statistically significant group effects for all three biochemical parameters ($p < 0.05$ in each case). Tukey's post-hoc comparisons produced the following findings:

*MDA*

GII differed significantly from GI in MDA, confirming scopolamine-induced lipid peroxidation. GIII showed a significant reduction compared to GII. All three Alumina-treated groups had MDA values significantly lower than GII. Within the Alumina groups, GVI showed the greatest reduction, followed by GV and then GIV, yielding a clear 200C → 1M → 10M gradient.

*Catalase*

Catalase activity in GII was significantly lower than in GI. Donepezil (GIII) significantly raised catalase compared to the disease control. All Alumina groups showed catalase values significantly above GII. The same potency gradient applied: GVI > GV > GIV, with GVI performing closest to the Donepezil standard.

*GSH*

GSH in GII was significantly depressed relative to GI. GIII showed a statistically significant recovery in GSH versus GII. Among Alumina groups, all three potencies produced GSH values significantly higher than GII, with GVI reaching levels close to those of GIII. The ascending trend across 200C, 1M, and 10M mirrors the pattern seen for MDA and catalase, reinforcing the consistency of the potency-response relationship observed in this study.

**Adverse Events**

No animal in any group showed signs of clinical toxicity, abnormal body weight trajectory, or unexpected biochemical values attributable to the test preparations. No deaths occurred during the in-life phase.

**Table 1:** MDA- Lipid Peroxidation (Individual and Mean Values, ng/mL)

| Animal | GI Normal | GII Disease | GIII Standard (Donepezil) | GIV Alumina 200C | GV Alumina 1M | GVI Alumina 10M |
|---|---|---|---|---|---|---|
| 1 | 1.2 | 4 | 2 | 3.9 | 2.8 | 2.1 |
| 2 | 1.3 | 4.2 | 2.1 | 3.8 | 2.9 | 2.2 |
| 3 | 1.1 | 4.1 | 1.9 | 3.9 | 2.7 | 2.6 |
| 4 | 1.4 | 4.3 | 2.2 | 3.9 | 3 | 2.3 |
| 5 | 1.2 | 3.9 | 2 | 4.2 | 2.8 | 2.1 |
| 6 | 1.3 | 4.2 | 2.1 | 4 | 3.3 | 2.7 |
| Mean | 1.25 | 4.12 | 2.05 | 3.95 | 2.92 | 2.33 |
| SEM | 0.036 | 0.051 | 0.036 | 0.048 | 0.074 | 0.089 |

*MDA values expressed in ng/mL. Higher values indicate greater oxidative stress*

**Table 2:** MDA- Group Summary (ng/mL)

| Group Name | Mean ± SEM |
|---|---|
| GI Normal | 1.25 ± 0.036 |
| GII Disease | 4.12 ± 0.051 |
| GIII Standard (Donepezil) | 2.05 ± 0.036 |
| GIV Alumina 200C | 3.95 ± 0.048 |
| GV Alumina 1M | 2.92 ± 0.074 |
| GVI Alumina 10M | 2.33 ± 0.089 |

**Table 3:** Catalase Activity- Individual and Mean Values (ng/ml)

| Animal | GI Normal | GII Disease | GIII Standard (Donepezil) | GIV Alumina 200C | GV Alumina 1M | GVI Alumina 10M |
|---|---|---|---|---|---|---|
| 1 | 58 | 28 | 48 | 32 | 40 | 46 |
| 2 | 60 | 30 | 50 | 34 | 42 | 48 |
| 3 | 59 | 29 | 49 | 33 | 41 | 47 |
| 4 | 61 | 27 | 51 | 31 | 43 | 49 |
| 5 | 57 | 31 | 47 | 35 | 39 | 45 |
| 6 | 60 | 28 | 50 | 33 | 41 | 48 |
| Mean | 59.17 | 28.83 | 49.17 | 33.00 | 41.00 | 47.17 |
| SEM | 0.508 | 0.508 | 0.508 | 0.488 | 0.488 | 0.508 |

*Catalase values expressed in ng/mL. Higher values indicate greater antioxidant enzyme activity*

**Table 4:** Catalase Activity- Group Summary (ng/ml)

| Group Name | Mean ± SEM |
|---|---|
| GI Normal | 59.17 ± 0.508 |
| GII Disease | 28.83 ± 0.508 |
| GIII Standard (Donepezil) | 49.17 ± 0.508 |
| GIV Alumina 200C | 33.00 ± 0.488 |
| GV Alumina 1M | 41.00 ± 0.488 |
| GVI Alumina 10M | 47.17 ± 0.508 |

**Table 5:** Reduced Glutathione (GSH)- Individual and Mean Values (ng/mL)

| Animal | GI Normal | GII Disease | GIII Standard (Donepezil) | GIV Alumina 200C | GV Alumina 1M | GVI Alumina 10M |
|---|---|---|---|---|---|---|
| 1 | 6.8 | 3.1 | 5.8 | 3.6 | 4.8 | 5.6 |
| 2 | 7 | 3.3 | 6 | 3.8 | 5 | 5.8 |
| 3 | 6.9 | 3.2 | 5.9 | 3.7 | 4.9 | 5.7 |
| 4 | 7.1 | 3 | 6.1 | 3.5 | 5.1 | 5.9 |
| 5 | 6.7 | 3.4 | 5.7 | 3.9 | 4.7 | 5.5 |
| 6 | 7 | 3.1 | 6 | 3.7 | 4.9 | 5.8 |
| Mean | 6.92 | 3.18 | 5.92 | 3.70 | 4.90 | 5.72 |
| SEM | 0.051 | 0.051 | 0.051 | 0.049 | 0.049 | 0.051 |

*GSH values expressed in ng/ml. Higher values indicate greater endogenous antioxidant capacity*

**Table 6:** Reduced Glutathione (GSH)- Group Summary (ng/mL)

| Group Name | Mean ± SEM |
|---|---|
| GI Normal | 6.92 ± 0.051 |
| GII Disease | 3.18 ± 0.051 |
| GIII Standard (Donepezil) | 5.92 ± 0.051 |
| GIV Alumina 200C | 3.70 ± 0.049 |
| GV Alumina 1M | 4.90 ± 0.049 |
| GVI Alumina 10M | 5.72 ± 0.051 |

**Table 7:** Inferential Statistics- One-Way ANOVA and Tukey's Post-Hoc Results

| Parameter | F-value (ANOVA) | p-value | GI vs GII | GII vs GIII | Within-Alumina potency comparisons (200C vs 1M, 1M vs 10M, 200C vs 10M) | Potency Trend |
|---|---|---|---|---|---|---|
| MDA (ng/ml) | 487.3 | < 0.001 | $p < 0.001$ | $p < 0.001$ | 200C vs 1M: $p < 0.001$<br>1M vs 10M: $p < 0.001$<br>200C vs 10M: $p < 0.001$ | 200C > 1M > 10M ↓ |
| Catalase (ng/ml) | 1204.6 | < 0.001 | $p < 0.001$ | $p < 0.001$ | 200C vs 1M: $p < 0.001$<br>1M vs 10M: $p < 0.001$<br>200C vs 10M: $p < 0.001$ | 200C < 1M < 10M ↑ |
| GSH (ng/ml) | 1876.2 | < 0.001 | $p < 0.001$ | $p < 0.001$ | 200C vs 1M: $p < 0.001$<br>1M vs 10M: $p < 0.001$<br>200C vs 10M: $p < 0.001$ | 200C < 1M < 10M ↑ |

*All comparisons by Tukey's HSD post-hoc test following one-way ANOVA. ↑ = protective increase; ↓ = protective decrease. All within-Alumina potency comparisons (200C vs 1M, 1M vs 10M, 200C vs 10M) reached statistical significance ($p < 0.001$) for all three biomarkers, confirming that the potency-response gradient is statistically supported at each step. df between groups = 5; df within groups = 3.*

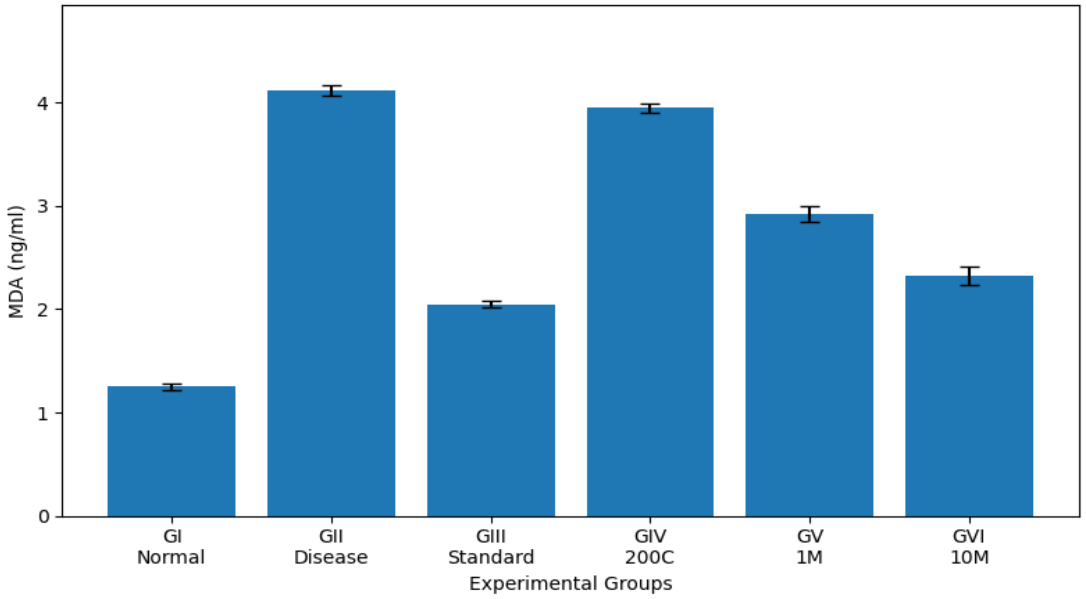


**Figure 1:** Bar diagram of mean MDA levels across all six groups

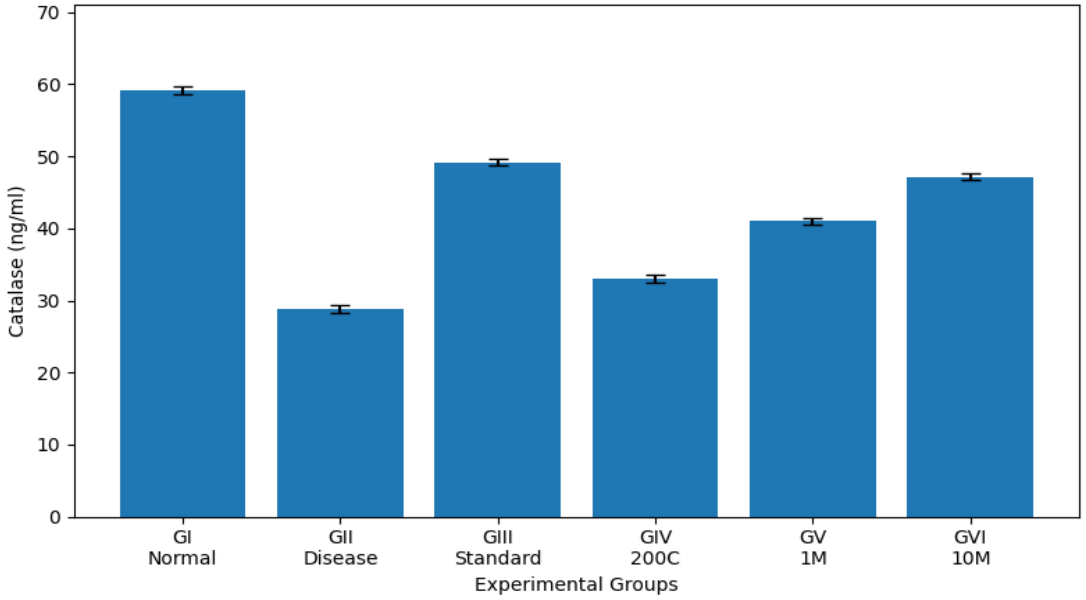


**Figure 2:** Bar diagram of mean catalase activity across all six groups

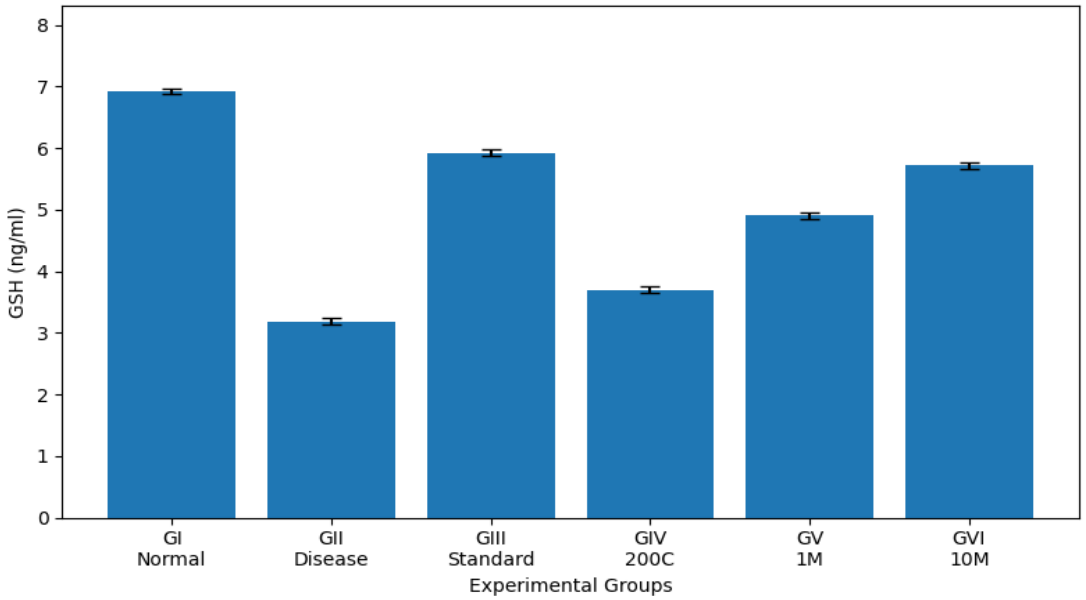


**Figure 3:** Bar diagram of mean GSH levels across all six groups

## DISCUSSION

Scopolamine-induced cholinergic blockade produced a characteristic oxidative stress profile in our rat model, with brain MDA levels more than tripling in the disease control group compared to untreated animals, catalase activity falling to roughly half its baseline, and GSH declining by over 50%. This pattern of elevated lipid peroxidation alongside compromised enzymatic and non-enzymatic antioxidant defences closely matches prior descriptions of muscarinic receptor blockade, donezepil associated redox modulation and scopolamine induced oxidative stress in rodent models [11,14,24], confirming the validity of the model for studying oxidative aspects of cholinergic dysfunction. As a pharmacological reference, donepezil produced the anticipated normalization of all three markers, which concurs with previous findings that cholinergic enhancement is linked with improved redox balance in scopolamine-treated animals [15].

Prior to this study, it was unknown whether homoeopathic Alumina at potencies 200C, 1M, and 10M could elicit any detectable change in such oxidative stress parameters. Our results show that each Alumina group differed significantly from the disease control for MDA, catalase, and GSH (one-way ANOVA F-values 487.3, 1204.6, and 1876.2, respectively), with protective shifts consistently observed across all three biomarkers. Tukey's post-hoc tests confirmed significance at every potency ($p < 0.001$), including all within-Alumina comparisons (200C vs 1M, 1M vs 10M, and 200C vs 10M). The possibility of assay-specific artifacts or random variation is decreased because this pattern appeared across a lipid peroxidation byproduct, an enzymatic antioxidant, and a non-enzymatic scavenger.

The coordinated improvement in MDA, catalase, and GSH suggests a mechanism more complex than simple radical scavenging, which might reduce MDA without proportionally restoring catalase or GSH. Instead, the parallel shifts resemble the broader redox modulation reported with other neuroprotective agents that influence Nrf2/HO-1 or related pathways, although our design cannot distinguish these specific mechanisms. Alumina's antioxidant effect may contribute to functional benefits, even though the precise pathway is still unknown. The biochemical pattern also aligns in one direction with cognitive and motor improvements observed in the present scopolamine-based model.

An especially notable feature is the potency-response gradient: MDA decreased progressively from 200C to 1M to 10M, while catalase and

GSH increased in step, with the 10M group approaching the Donepezil-treated values across all three markers. The consistency of the biochemical signal was reinforced by this monotonic trend that appeared across independent assays without any inherent design bias. In contrast to the multiregional, antioxidant-focused readout in the current work, the matching 10M group in the behavioral study displayed somewhat less obvious potency-related effects in spatial memory, potentially reflecting greater hippocampal cholinergic selectivity in that domain. This divergence highlights the need for targeted follow-up to map the relationship between region-specific cholinergic and cognitive effects and Alumina-associated redox changes.

While these findings contribute further to the existing literature on oxidative stress in scopolamine-induced models and add to the emerging body of preclinical data on homoeopathic ultra-high dilutions and antioxidant modulation [16,17,25], the physicochemical basis of ultra-dilute homoeopathic effects remains poorly understood and prohibits making strong mechanistic claims. The absence of direct acetylcholinesterase assays in the present study limits our ability to distinguish purely antioxidant actions from those secondary to cholinergic restoration. Future work should incorporate neuroinflammatory markers (e.g., TNF-α, IL-1β), molecular docking of relevant targets, and complementary simulations to generate and test specific hypotheses. Additional limitations include small group sizes (n = 6), which constrain potency-resolution power; partial single-blinding, which may introduce some bias; an acute scopolamine insult rather than a progressive neurodegenerative course; and a male-only cohort, which overlooks potential sex-dependent differences in oxidative stress and treatment response. Although these results provide fresh proof of the antioxidant-neuroprotective properties of homoeopathic alumina in a scopolamine-based model, more research is necessary to validate and expand on these findings. Before any direct therapeutic implications can be made, replication in transgenic models, larger-scale designs, and more mechanistically oriented trials will be crucial.

## CONCLUSION

This study demonstrates that oral administration of Alumina at homoeopathic potencies 200C, 1M, and 10M is associated with graded, potency-linked changes in three biochemical markers of oxidative stress in a scopolamine-treated rat model. MDA was reduced, catalase activity was elevated, and GSH was restored in a pattern that consistently favoured higher potencies, with the 10M group performing closest to the Donepezil reference across all three measures. Whether viewed individually or together, the data point towards a meaningful antioxidant effect that scales with potency an observation that is both internally consistent and congruent with the rationale behind remedy selection. Future work should build on this foundation by incorporating a broader neurochemical panel, testing chronic models of neurodegeneration, and exploring the mechanistic underpinnings of these biochemical changes at the molecular level.

### Conflict of interest

The authors declared no conflict of interest.

### Financial Support

None declared.

### Artificial Intelligence Assistance

AI-assisted writing tools were employed during manuscript preparation for the purposes of language polishing, structural organisation, and selective paraphrasing. The entirety of the scientific content, including experimental design, raw data, results, interpretations, and conclusions, remains exclusively the intellectual product of the named authors. All authors reviewed the completed manuscript and bear full collective responsibility for the submitted version.

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**HOW TO CITE THIS ARTICLE**
Zagday A, Vaishampayan V, Sathe M, Magdum V, Garje U, Vichare V, *et al*. Neuroprotective effects of potentized alumina on oxidative stress markers in a rat model of cholinergic dysfunction. J Phytopharmacol 2026; 15(1):86-93. doi: 10.31254/phyto.2026.15112