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Combined Diazepam and Nutmeg Essential Oil Induces Respiratory Depression in Male Mice

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ABSTRACT

Nutmeg (*Myristica fragrans*) essential oil contains myristicin and safrole, which exhibit sedative, anticonvulsant, and neuroprotective activity through modulation of GABA-A receptors. Its combination with diazepam has been reported to enhance anticonvulsant effects and improve cognitive impairment caused by diazepam. Because both agents act on the central nervous system, co-administration may increase the risk of adverse effects such as respiratory depression, which warrants further investigation. We therefore hypothesized that co-administration of diazepam and nutmeg essential oil would produce a greater reduction in oxygen saturation and heart rate than either agent alone. This study evaluated the effect of combining diazepam and nutmeg essential oil on oxygen saturation and heart rate in male mice. Twenty-five mice were divided into five groups: control, diazepam 10 mg/kg body weight (BW), diazepam 10 mg/kg BW + nutmeg oil 200 µL/kg BW, diazepam 2 mg/kg BW + nutmeg oil 200 µL/kg BW, and nutmeg oil alone. Parameters were recorded at 0, 20, 40, 60, and 120 minutes. The Friedman test indicated significant differences across groups for oxygen saturation ($p = 0.000$) and heart rate ($p = 0.000$). The high-dose combination group produced the lowest mean values, with a minimum oxygen saturation of 72.40% and a minimum heart rate of 127.40 bpm, both observed at 60 minutes. Compared with diazepam alone, the combination showed a trend toward a greater decline in oxygen saturation and heart rate, but the difference did not reach statistical significance at any time point ($p > 0.05$), consistent with an additive rather than a synergistic effect. Notably, the low-dose combination (diazepam 2 mg/kg BW + nutmeg oil) produced depressant effects that were statistically comparable to high-dose diazepam alone at all time points ($p > 0.05$), indicating that nutmeg oil may potentiate diazepam activity even at a sub-therapeutic diazepam dose.

1. INTRODUCTION

Nutmeg (*Myristica fragrans*) essential oil is a traditional herbal remedy known for a broad pharmacological profile that includes sedative, anticonvulsant, anti-inflammatory, and neuroprotective activities [1, 2]. These effects are attributed to bioactive constituents such as myristicin and safrole, which may modulate GABA-A receptors in the central nervous system—the same receptors targeted by conventional anxiolytics and anticonvulsants such as diazepam [3]. Because the phenylpropanoid composition of the oil varies substantially with geographic origin, harvesting season, and distillation protocol, the reported chemotype of each batch is a critical determinant of its biological activity [4].

Previous studies have reported that co-administration of diazepam and nutmeg essential oil enhances anticonvulsant activity compared with either agent alone, delaying seizure onset and shortening seizure duration [5]. The combination has also been associated with attenuation of diazepam-induced cognitive impairment, suggesting a possible protective interaction at therapeutic dose levels [6].

Despite this therapeutic potential, the safety of the combination remains poorly characterised, particularly with respect to respiration and cardiovascular function. Benzodiazepine-induced hypoventilation has been well documented in rodent models, where therapeutic and supratherapeutic doses depress tidal volume, arterial oxygen tension, and heart rate through central GABA-A-mediated suppression of brainstem respiratory output [7-10]. Similarly, myristicin- and safrole-rich nutmeg oil preparations have been shown to exert dose-dependent psychoactive and neurotoxic effects in rodents, with chemotype-dependent variability further complicating safety prediction [11-14]. When such agents are combined with diazepam, these shared depressant pathways may converge on brainstem respiratory centres and predispose the animal to hypoxia.

From a clinical standpoint, the issue is far from theoretical. Nutmeg-containing herbal preparations are widely consumed

in Indonesia and other tropical regions for sleep, anxiety, and pain relief, often without medical supervision and frequently in combination with prescription anxiolytics. Patients receiving benzodiazepines who self-medicate with nutmeg oil may therefore experience pharmacodynamic interactions that are not detected during routine clinical monitoring. Pulse oximetry is a non-invasive, real-time surrogate for alveolar ventilation, and heart rate is a simple readout of autonomic tone; together they provide a sensitive, repeatable, and translationally relevant pair of endpoints with which to detect benzodiazepine–herbal interactions in vivo. In this study, we therefore selected oxygen saturation and heart rate as the primary outcomes and used a fixed-time-point design (0, 20, 40, 60, and 120 minutes) to capture both the onset and the recovery phases of the pharmacodynamic response.

Given this potential risk, it is essential to determine whether co-administration of diazepam and nutmeg essential oil produces enhanced respiratory depression. Such evidence is necessary to support the safe and rational use of herbal–pharmaceutical combinations. We hypothesized that co-administration of diazepam and nutmeg essential oil would produce a greater reduction in oxygen saturation and heart rate than either agent administered alone. Accordingly, the present study evaluated the effect of the combination on oxygen saturation and heart rate in male mice, using pulse oximetry at multiple time points to characterise the temporal profile of the interaction. In addition, we incorporated a low-dose diazepam arm (2 mg/kg body weight) co-administered with nutmeg oil so that any potentiation occurring at sub-sedative diazepam doses—the doses most likely to be encountered in clinical practice—could be detected.

2. MATERIALS AND METHODS

2.1 Materials

The test articles were diazepam injection 5 mg/mL (Meprofarm, Indonesia; BPOM Reg. No. GPL1515623743A1) and nutmeg (*Myristica fragrans*) essential oil (PT. Tarmonanesia, Bogor, West Java, Indonesia; Ther Real Essential Oil Fresh From Distillation). Diazepam stock was diluted with 0.9% sodium chloride (Otsuka, Indonesia) to the working concentrations required for the 10 mg/kg BW and 2 mg/kg BW doses, and the essential oil was administered at 200 µL/kg BW. Aqua pro injection (Ikapharmindo, Indonesia) was used where additional dilution was required.

Physiological parameters were recorded with a veterinary pulse oximeter (CMS60D-Vet, Contec Medical Systems, China). Syringes (1 mL and 5 mL; Terumo, Japan) were used for drug administration, and a digital balance (AND EK-2000i; A&D Company, Japan) was used to weigh each animal prior to dosing.. The nutmeg essential oil was obtained by steam distillation of dried nutmeg seeds and certified as 100% pure and natural, with no carrier oil added. According to the Certificate of Analysis (CoA) dated 25 February 2025, the oil met all specified physicochemical standards, including a specific gravity of 0.896 (range: 0.892-0.898) and a refractive index of 1.479 at 20 °C (range: 1.475-1.492). The major constituents included sabinene (21.78%), α-pinene (15.67%), and myristicin (9.00%), with minor components such as safrole (1.68%) and methyleugenol (0.34%), all within acceptable limits. The oil was described as a mobile liquid, colorless to pale yellow, with a characteristic nutmeg odor, and free from fatty oil contamination.

2.2 Animals

Twenty-five male Swiss Webster mice (*Mus musculus*), aged 8-10 weeks and weighing 25-35 g, were obtained from a licensed laboratory animal supplier. Animals were housed five per polycarbonate cage under controlled conditions (temperature 22 ± 2 °C, relative humidity 55 ± 10%, 12:12 h light/dark cycle, lights on at 06:00) with ad libitum access to standard pellet feed and water. Mice were acclimatised for seven days before any procedure and were randomised to treatment groups using a computer-generated randomisation list (simple randomisation, block size = 5) applied after stratification by body weight. On the morning of dosing, food was withheld for eight hours, while water remained available to prevent dehydration. All procedures were approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Kristen Maranatha (Decree No. 093/KEP/V/2025).

Welfare monitoring was performed every 30 minutes throughout the 120-minute observation window, with predefined humane endpoints (loss of righting reflex sustained beyond 10 minutes, cyanosis, or sustained SpO_2 below 70%) triggering immediate cessation of measurement and warming on a heat pad until recovery; no animal met these criteria during the study, and all twenty-five animals completed the protocol. Investigators conducting drug administration and pulse-oximetry recording were blinded to the treatment-group label by use of opaque coded vials prepared by a third party not otherwise involved in the experiment. After completion of the 120-minute observation, animals were monitored daily for seven days for late-onset adverse effects before euthanasia by cervical dislocation under deep isoflurane anaesthesia.

2.3 Dose selection rationale

The high diazepam dose (10 mg/kg BW, intraperitoneal) was selected because it has been shown in published rodent studies to consistently induce measurable respiratory depression, reflected in reduced partial pressure of oxygen and lowered heart rate within 60 minutes of administration [7, 9]. This dose is above the sedative range and is commonly used as a reference dose for modelling benzodiazepine-induced hypoventilation. The low diazepam dose (2 mg/kg BW, intraperitoneal) was chosen to approximate the therapeutic/anxiolytic-equivalent range in mice, allowing assessment of whether nutmeg essential oil could potentiate a clinically relevant diazepam dose. The nutmeg essential oil dose (200 µL/kg BW) was selected on the basis of prior work from our laboratory demonstrating anticonvulsant activity at this dose without overt sedation [5, 6].

2.4 Experimental design and groups

A post-test-only control time-series design was used. The twenty-five mice were allocated equally (n = 5 per group) to the following groups: (i) Control-received the vehicle (0.9% saline, volume-matched to the diazepam arms) by intraperitoneal injection to control for injection stress; (ii) Diazepam 10 mg/kg BW; (iii) Diazepam 10 mg/kg BW + nutmeg essential oil 200 µL/kg BW; (iv) Diazepam 2 mg/kg BW + nutmeg essential oil 200 µL/kg BW; and (v) Nutmeg

essential oil 200 µL/kg BW. Diazepam was administered intraperitoneally, and nutmeg essential oil was administered orally by gavage immediately after diazepam.

2.5 Pulse oximetry protocol

Oxygen saturation (SpO_2) and heart rate were measured with the CMS60D-Vet pulse oximeter at 0 (baseline, pre-dose), 20, 40, 60, and 120 minutes after treatment. At each time point, a single investigator (blinded to treatment where feasible) placed the clip-on sensor on the same anatomical site (base of the tail) for every animal to minimise inter-site variability. Mice were gently restrained in a soft cloth wrap for approximately 90 seconds per measurement. After a 30-second stabilisation period to allow the waveform to settle, three consecutive 20-second readings with an acceptable perfusion index and a stable waveform were recorded; the mean of the three readings was used as the value for that time point. Measurements showing motion artefact or low perfusion were rejected and repeated. Ambient temperature and handling conditions were kept constant across groups.

Tail-clip pulse oximetry has been validated in mice against arterial co-oximetry [11], with the principal sources of error being motion artefact, low peripheral perfusion, and ambient-light contamination. To minimise these confounders, the recording room was kept at a stable 22 ± 1 °C and shielded from direct overhead light, and only readings with a perfusion index ≥ 0.6 and a stable plethysmographic waveform across the full 20-second window were retained. The investigator confirmed pulse synchrony with the displayed waveform before each measurement, and a maximum of two repeat attempts was permitted at any single time point; in practice, fewer than 5% of readings required repetition, and no animal contributed missing data.

2.6 Statistical analysis

Because of the small sample size ($n < 30$ per group), non-parametric statistics were applied. The Friedman test was used to evaluate within-group differences across repeated time points for oxygen saturation and heart rate. Where the Friedman test reached significance ($p < 0.05$), pairwise comparisons between groups at each time point were performed using the Mann–Whitney U test. A p-value < 0.05 was considered statistically significant. All analyses were performed in IBM SPSS Statistics version 26 (IBM Corp., Armonk, NY, USA). Descriptive statistics are reported as mean ± standard deviation (SD). For each Mann–Whitney comparison, both the U statistic and the exact two-tailed p-value were computed; only the p-values are tabulated for clarity. Because the study was hypothesis-driven and the number of pre-planned pairwise comparisons was limited (Group 2 vs Group 3, Group 3 vs Group 4, and the low-dose-combination arm versus each of the other groups), no formal correction for multiple comparisons was applied; the corresponding inflation of the family-wise error rate is acknowledged as a limitation in the Discussion.

An a posteriori sensitivity analysis indicated that, with $n = 5$ per group and $\alpha = 0.05$ (two-tailed), the design had approximately 80% power to detect a between-group difference of about 8 percentage points in SpO_2 or 18 bpm in heart rate, given the within-group standard deviations actually observed; smaller effects, such as the ≤ 6-percentage-point gap between high-dose diazepam and the high-dose combination at 60 minutes, would therefore be expected to remain statistically undetected at this sample size. Effect sizes were also estimated as the percent change from each animal's own baseline (time 0) to the 60-minute nadir, providing a within-animal index that is less sensitive to between-cage variability than absolute group means.

3. RESULT AND DISCUSSION

3.1 Oxygen saturation activity test

Oxygen saturation values for each group at each time point are shown in Table 1 and plotted in Figure 1.

3.2 Heart rate activity test

Heart rate values for each group at each time point are shown in Table 2 and plotted in Figure 2.

Table 1. Mean oxygen saturation (%) of mice per treatment group (n = 5 per group; mean ± SD)

Treatment Group	0 min	20 min	40 min	60 min	120 min
Control	90.40 ± 1.67	91.60 ± 1.52	90.60 ± 1.34	91.00 ± 1.58	89.60 ± 0.55
Diazepam 10 mg/kg BW	90.20 ± 1.92	84.60 ± 1.14	79.60 ± 1.34	78.20 ± 1.79	81.80 ± 1.10
Diazepam 10 mg/kg BW + Nutmeg EO 200 µL/kg BW	89.80 ± 1.92	79.80 ± 3.03	72.80 ± 2.59	72.40 ± 3.29	77.80 ± 2.05
Diazepam 2 mg/kg BW + Nutmeg EO 200 µL/kg BW	91.20 ± 0.84	84.40 ± 2.70	80.00 ± 4.06	77.00 ± 1.22	80.60 ± 1.82
Nutmeg EO 200 µL/kg BW	89.60 ± 2.07	88.48 ± 1.78	87.00 ± 1.87	85.80 ± 1.10	85.28 ± 0.94

BW: Body weight, EO: Essential oil.

Table 2. Mean heart rate (bpm) of mice per treatment group (n = 5 per group; mean ± SD)

Treatment Group	0 min	20 min	40 min	60 min	120 min
Control	179.40 ± 6.07	184.00 ± 16.31	186.80 ± 10.92	185.00 ± 11.05	191.00 ± 9.82
Diazepam 10 mg/kg BW	183.60 ± 6.73	149.60 ± 7.47	149.20 ± 12.68	129.60 ± 5.55	157.00 ± 3.94
Diazepam 10 mg/kg BW + Nutmeg EO 200 µL/kg BW	182.60 ± 5.22	143.80 ± 4.97	136.60 ± 5.13	127.40 ± 5.94	148.00 ± 5.70
Diazepam 2 mg/kg BW + Nutmeg EO 200 µL/kg BW	178.40 ± 4.34	151.80 ± 4.60	147.60 ± 5.22	134.60 ± 2.61	151.20 ± 5.59
Nutmeg EO 200 µL/kg BW	182.60 ± 4.98	168.00 ± 6.28	160.20 ± 7.01	160.80 ± 5.40	162.60 ± 6.84

BW: Body weight, EO: Essential oil.

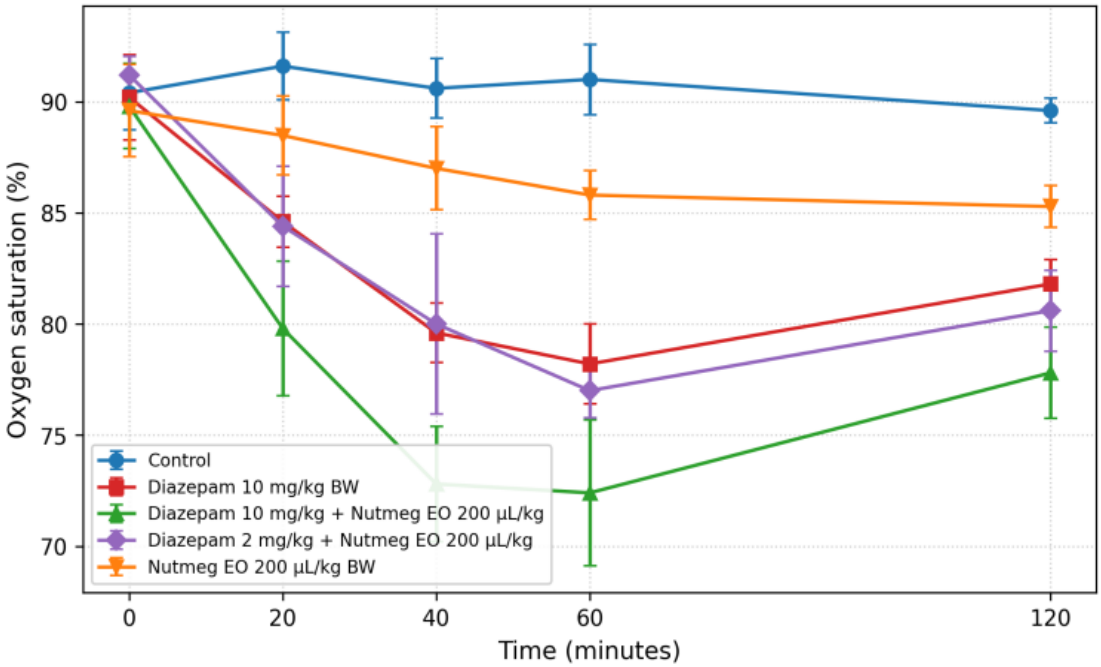

Figure 1. Mean oxygen saturation (± SD) over time across treatment groups
BW: Body weight, EO: Essential oil.

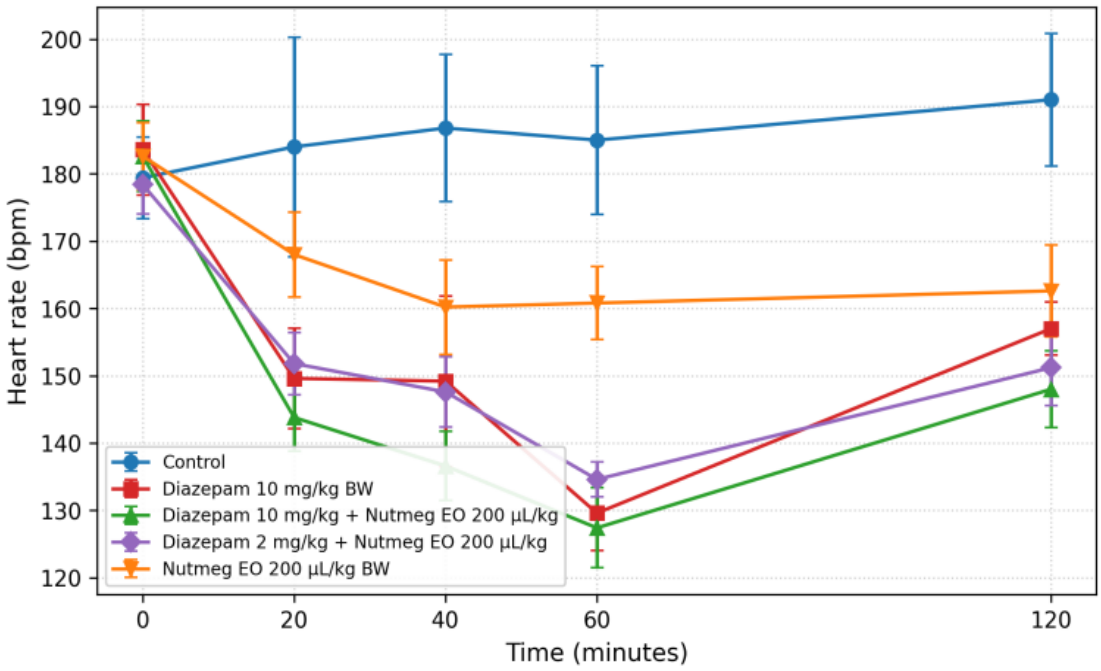

Figure 2. Mean heart rate (± SD) over time across treatment groups
BW: Body weight, EO: Essential oil.

Expressed as percent change from each animal's own baseline, mean SpO_2 at the 60-minute nadir fell by approximately 13.3% in the diazepam 10 mg/kg BW group, 19.4% in the high-dose combination, 15.6% in the low-dose combination, and only 4.2% in the nutmeg-oil-only group, while the control group remained within 1% of baseline. The depth of desaturation, therefore, tracked the presence of diazepam in the regimen rather than the diazepam dose alone, with the addition of nutmeg essential oil contributing a further numerical decrement of approximately 6 percentage points at the high diazepam dose.

Heart rate followed a similar pattern: relative to baseline, the 60-minute nadir corresponded to falls of approximately 29.4% in the diazepam 10 mg/kg BW group, 30.2% in the high-dose combination, 24.5% in the low-dose combination, and 11.9% in the nutmeg-essential-oil-alone group. The two diazepam-containing arms produced near-identical bradycardia at 60 minutes, supporting the inference that diazepam, rather than nutmeg essential oil, is the primary cardiovascular depressant in this combination. Importantly, the percent-change framework also makes clear that the low-dose combination produced ≥ 80% of the heart-rate depression seen with five-fold-higher diazepam alone, an effect that would not be obvious from absolute group means alone.

3.3 Hypothesis test

The Friedman test yielded $p = 0.000$ ($p < 0.05$) for both oxygen saturation and heart rate, so the null hypothesis of no difference between groups was rejected for both endpoints. Post-hoc Mann–Whitney comparisons were therefore conducted to isolate the pairwise differences driving this effect. Two sets of comparisons were prioritised: (i) diazepam 10 mg/kg BW alone versus the high-dose combination, to test for an additive or synergistic effect of nutmeg oil on high-dose diazepam; and (ii) the low-dose combination (diazepam 2 mg/kg BW + nutmeg oil) versus every other group, to determine whether nutmeg oil can potentiate a clinically relevant low diazepam dose.

This pre-specified two-tier comparison structure was chosen because it isolates two distinct mechanistic questions—whether nutmeg essential oil amplifies a maximally sedative diazepam dose, and whether nutmeg essential oil can substitute for the missing 8 mg/kg BW of diazepam at the low end of the dose range—rather than performing every possible pairwise contrast, which would otherwise have inflated the family-wise error rate beyond what could be reliably interpreted at this sample size. Time-point-specific U statistics ranged from 0.0 to 11.5, with the lowest U values (and therefore the most clearly separated distributions) consistently observed at 60 minutes, the same time point at which both endpoints reached their pharmacodynamic nadir.

Table 3. Mann–Whitney test results between treatment groups on oxygen saturation (per time point)

Time (min)	Group 2 vs 3 (P-Value)	Group 3 vs 4 (P-Value)
0	0.287*	0.008
20	0.395*	0.045
40	0.710*	0.162*
60	0.334*	0.008
120	0.277*	0.008

Note: *not significantly different. Group 2 = Diazepam 10 mg/kg BW; Group 3 = Diazepam 10 mg/kg BW + Nutmeg EO 200 µL/kg BW; Group 4 = Nutmeg EO 200 µL/kg BW.

As shown in Table 3, no statistically significant difference in oxygen saturation was detected between diazepam alone (Group 2) and the high-dose combination (Group 3) at any time point (all $p > 0.05$). This indicates that adding nutmeg essential oil to diazepam 10 mg/kg BW did not produce a statistically greater reduction in SpO_2 than diazepam alone. In contrast, the high-dose combination was significantly more depressant than nutmeg oil alone at 0, 20, 60, and 120 minutes ($p < 0.05$), with only the 40-minute comparison failing to reach significance ($p = 0.162$). These findings suggest that diazepam drives the respiratory depression, while the contribution of nutmeg oil, although numerically present, is statistically additive rather than synergistic.

Table 4. Mann–Whitney test results between treatment groups on heart rate (per time point)

Time (min)	Group 2 vs 3 (P-Value)	Group 3 vs 4 (P-Value)
0	0.207*	0.207*
20	0.753*	0.009
40	0.902*	0.134*
60	0.115*	0.009
120	0.094*	0.036

Note: *not significantly different. Groups defined as in Table 3.

Table 4 shows the same pattern for heart rate: no statistically significant difference between diazepam alone and the high-dose combination at any time point (all $p > 0.05$). Significant differences between the high-dose combination and nutmeg oil alone emerged at 20, 60, and 120 minutes ($p < 0.05$). Taken together, Tables 3 and 4 indicate that, while nutmeg essential oil alone produced mild respiratory and cardiovascular depression, it did not reach a statistically greater depression when added to high-dose diazepam.

As reported in Table 5, the low-dose combination (diazepam 2 mg/kg BW + nutmeg oil) produced an oxygen-saturation profile that was not statistically different from that of high-dose diazepam (10 mg/kg BW) alone at any time point (all $p > 0.05$). At the same time, it produced a significantly greater reduction than nutmeg essential oil alone from 20 to 120 minutes, and a significantly greater reduction than the high-dose combination at 20, 40, 60, and 120 minutes. This is an important finding: reducing the diazepam dose fivefold did not reduce the depressant effect on SpO_2 when nutmeg essential oil was co-administered.

Table 5. Mann–Whitney test results: Diazepam 2 mg/kg BW + nutmeg EO vs other groups on oxygen saturation

Time (min)	Diazepam 10 mg/kg BW	Diazepam 10 mg/kg BW + Nutmeg EO	Nutmeg EO Only
0	0.287*	0.197*	0.139*
20	0.395*	0.020	0.045
40	0.456*	0.009	0.045
60	0.334*	0.015	0.008
120	0.277*	0.045	0.008

Note: *not significantly different. EO: Essential oil.

Table 6. Mann–Whitney test results: Diazepam 2 mg/kg BW + nutmeg essential oil vs other groups on heart rate

Time (min)	Diazepam 10 mg/kg BW	Diazepam 10 mg/kg BW + Nutmeg EO	Nutmeg EO Only
0	0.207*	0.293*	0.207*
20	0.753*	0.027	0.009
40	0.834*	0.016	0.028
60	0.115*	0.036	0.009
120	0.094*	0.530*	0.036

Note: *not significantly different. EO: Essential oil.

As shown in Table 6, the low-dose combination produced significant reductions in heart rate relative to nutmeg essential oil alone and relative to the high-dose combination between 20 and 60 minutes, yet remained statistically comparable to diazepam 10 mg/kg BW alone at every time point (all $p > 0.05$). This reinforces the finding that the combination of a low diazepam dose with nutmeg oil can reproduce the cardiovascular depressant profile of a five-fold higher diazepam dose, pointing to a clinically meaningful herbal–drug interaction at therapeutic dose levels.

3.4 Interpretation of temporal profiles

Across the observation window, the control group maintained stable oxygen saturation (89-92%) consistent with the normal physiological range reported for healthy unanaesthetised mice by Polšek et al. [12] and with the steady regional oxygen consumption described by Zhou et al. [13]. Heart rate in the control group drifted mildly upward from

179.40 to 191.00 bpm over 120 minutes; this pattern is within the published resting range for conscious mice (approximately 190-220 bpm [15, 16]) and likely reflects a modest rise in sympathetic tone in response to repeated handling and restraint required for pulse oximetry rather than a true pharmacological effect.

The high-dose diazepam group showed a decline in oxygen saturation that began at 20 minutes and reached a nadir of 78.20% at 60 minutes. This time course is consistent with Mohammed et al. [7], who reported that intraperitoneal diazepam caused a significant decrease in arterial oxygen tension and heart rate within the first 60 minutes. The selection of 10 mg/kg BW as a reference dose was deliberate: it lies above the sedative range and within the range previously reported to induce reliable hypoventilation in mice, and the observed SpO_2 of approximately 72-78% across the diazepam-containing groups is consistent with the expected pharmacodynamic response at this dose. The subsequent partial recovery at 120 minutes is compatible with redistribution and metabolism of the drug as plasma concentrations fall below the threshold required to suppress medullary respiratory output [9].

The high-dose combination produced the greatest numerical reduction, with a mean SpO_2 of 72.40% at 60 minutes. However, this numerical gap over diazepam alone did not reach statistical significance (Tables 3 and 4). We therefore describe the interaction as an additive or non-significant trend rather than a synergistic effect: although the mean values were consistently lower in the combination arm, the variance was such that the difference could not be distinguished from diazepam monotherapy. Mechanistically, the combination may still represent a clinically relevant convergence of pathways: nutmeg essential oil contains myristicin and safrole, which can depress neuronal activity via modulation of GABA-A receptors and sodium-channel inhibition, overlapping the GABA-A activity of diazepam [3, 17-19] and potentially summating at brainstem respiratory centres.

A key limitation of the mechanistic interpretation is that the batch used in this study was not profiled by gas chromatography–mass spectrometry (GC–MS). Consequently, the relative proportions of myristicin, safrole, and other phenylpropanoids in this particular batch cannot be quoted. Because chemotype varies with provenance and distillation, future work from our laboratory will include GC–MS characterisation of each batch so that activity can be correlated with composition. Earlier work has nonetheless shown that high-dose or repeated exposure to myristicin can be neurotoxic, slowing reflexes and reducing consciousness in test animals [14], and that bioactive compounds from *Myristica fragrans* exhibit monoamine oxidase (MAO) inhibitory activity that could amplify sedative effects [15].

The low-dose combination (diazepam 2 mg/kg BW + nutmeg oil) produced an oxygen-saturation nadir of 77.00% at 60 minutes—numerically similar to diazepam 10 mg/kg BW alone (78.20%). All treatment groups showed a decline from 20 to 60 minutes with partial recovery at 120 minutes, differing only in magnitude. Nutmeg essential oil alone produced the mildest reduction, consistent with its reported mild sedative activity, but the absence of clinically significant respiratory depression at this dose.

The heart-rate data mirror the oxygen-saturation findings. High-dose diazepam reduced heart rate from 183.60 to a nadir of 129.60 bpm at 60 minutes, consistent with the expected cardiovascular consequences of central nervous system depression mediated by GABA-A receptor agonism. The high-dose combination produced a slightly deeper nadir (127.40 bpm), and the low-dose combination followed the same downward trajectory before partially recovering. These observations support the interpretation that the dominant pharmacological driver of both endpoints is diazepam, with nutmeg essential oil contributing an additional, mechanistically plausible depressant effect that becomes detectable when low-dose diazepam is used but does not reach statistical significance against high-dose diazepam.

Importantly, the temporal alignment of the three diazepam-containing arms—initial decline at 20 minutes, nadir at 60 minutes, and partial recovery by 120 minutes—mirrors the published plasma pharmacokinetics of intraperitoneal diazepam in mice, in which peak brain concentrations are typically reached within 30-60 minutes and decline through redistribution and hepatic metabolism over the following 60-90 minutes. The recovery phase observed in the combination arms is therefore unlikely to reflect tolerance to the herbal component; rather, it is most parsimoniously explained by clearance of diazepam below the threshold required for brainstem GABA-A-mediated suppression of respiratory output, with nutmeg essential oil providing a smaller, more sustained background of depression that does not reverse over the same 120-minute window. Mechanistically, the combination is therefore best modelled as the superimposition of two depressant inputs operating on partly overlapping but kinetically distinct timescales, which is also why the two combination arms re-converge with diazepam monotherapy by 120 minutes once the dominant benzodiazepine driver has been redistributed.

Taken together, these data indicate that each treatment produced a distinct respiratory and cardiovascular profile. High-dose diazepam caused the most pronounced fall in both endpoints between 20 and 60 minutes, consistent with its facilitation of GABA binding at GABA-A receptors, the resulting chloride influx, and hyperpolarisation of neurons in the brainstem respiratory centre [5, 17, 18]. Nutmeg essential oil alone produced a mild, stable reduction compatible with GABAergic modulation and sodium-channel inhibition [19-21]. The low-dose combination reproduced the depressant profile of a much higher diazepam dose, suggesting that nutmeg oil potentiates diazepam activity at therapeutically realistic diazepam doses [22, 23].

3.5 Clinical implications and limitations

Two clinical implications emerge from these data. First, because the low-dose combination produced a depressant profile that was statistically indistinguishable from that of high-dose diazepam alone, patients taking benzodiazepines should be counselled against concomitant use of nutmeg-containing herbal preparations. Second, because the magnitude of the additional depression contributed by nutmeg oil at high diazepam doses was numerical rather than statistical, the most likely real-world setting for a clinically important interaction is the therapeutic-dose range, where ceiling effects of diazepam alone are absent, and any additional depressant input has greater room to operate. These observations are mechanistically congruent with prior reports that benzodiazepines and centrally acting herbal preparations can converge on the same brainstem networks [9], and they reinforce the long-standing recommendation to elicit a complete history of complementary and alternative medicine

use during anxiolytic prescribing.

From a translational standpoint, the 2 mg/kg BW dose used in the low-dose combination corresponds approximately to a human-equivalent dose of 0.16 mg/kg by allometric (Km) scaling, which is well within the routine adult anxiolytic range (2-10 mg orally, ≈ 0.03-0.14 mg/kg). The observation that this clinically realistic diazepam dose, when combined with nutmeg essential oil, reproduced the depressant signature of a five-fold higher diazepam dose, therefore cannot be dismissed as a high-dose laboratory artefact and is most relevant to outpatients who self-medicate with nutmeg-containing herbal preparations while taking prescribed benzodiazepines. The pattern is also consistent with case-series and pharmacovigilance data on benzodiazepine–herbal interactions [22, 23], in which the most clinically meaningful events have similarly arisen at therapeutic rather than supratherapeutic benzodiazepine doses.

Several limitations should nonetheless be acknowledged. First, the sample size (n = 5 per group) limits statistical power and is the most plausible reason that the numerical gap between diazepam alone and the high-dose combination did not reach statistical significance; an a priori power calculation for future studies, based on the means and SDs reported here, would call for approximately 10-12 animals per group to detect a 5-percentage-point difference in SpO_2 at 60 minutes with 80% power. Second, the use of pulse oximetry alone—although translationally relevant—does not provide direct measurements of arterial CO_2, respiratory rate, or tidal volume; arterial blood gas analysis and whole-body plethysmography would refine the mechanistic interpretation. Third, the chemical composition of the essential oil batch was not verified by GC–MS in this study, and the relative contributions of myristicin, safrole, and other phenylpropanoids therefore remain inferred from the literature rather than measured directly. Fourth, because the study used only male mice, sex-specific differences in benzodiazepine pharmacokinetics and pulmonary mechanics could not be assessed and should be addressed in future work. Finally, no formal correction for multiple Mann–Whitney comparisons was applied; while this is partly mitigated by the pre-specified, hypothesis-driven structure of the comparisons, replication studies should consider applying a Bonferroni or Holm correction.

Future studies will (i) characterise each batch of nutmeg essential oil by GC–MS so that activity can be linked to a defined chemotype; (ii) include both sexes and a wider range of diazepam doses to construct full dose–response curves and to examine sex-specific risk; (iii) supplement pulse oximetry with arterial blood gas analysis and plethysmography; and (iv) explore whether the interaction can be reversed by flumazenil or by selective myristicin-pathway antagonists, which would help to establish causality between specific molecular targets and the observed respiratory phenotype.

4. CONCLUSIONS

In male mice, the combination of diazepam 10 mg/kg BW and nutmeg (*Myristica fragrans*) essential oil 200 µL/kg BW produced the greatest numerical reduction in oxygen saturation and heart rate across all groups. However, the difference between the combination and diazepam alone did not reach statistical significance at any time point, so the interaction at a high diazepam dose is best described as additive rather than synergistic. Importantly, the low-dose combination (diazepam 2 mg/kg BW + nutmeg essential oil) produced respiratory and cardiovascular depression that was statistically comparable to high-dose diazepam alone, indicating that nutmeg essential oil may potentiate diazepam activity even at sub-therapeutic diazepam doses. These findings support caution when combining benzodiazepines with nutmeg-containing herbal preparations. Future studies should incorporate GC–MS characterisation of the essential oil, larger sample sizes, arterial blood gas analysis, and full dose–response curves to confirm and extend these observations.

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