

Antidepressant- and Anxiolytic-Like Effects of Nutmeg Essential Oil (Myristica Fragrans Houtt.) In A Chronic Unpredictable Mild Stress-Induced Mouse Model

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SUMMARY

Depression and anxiety are among the most prevalent psychiatric disorders worldwide, yet pharmacotherapies are associated with significant adverse effects and delay onset of action. Nutmeg oil has demonstrated central nervous system activity in preclinical models, but its efficacy in chronic stress-induced depression and anxiety remains unexplored. This study was done to evaluate the antidepressant- and anxiolytic-like effects of nutmeg essential oil (NEO) in chronic unpredictable mild stress (CUMS) mouse model. Mice were exposed to CUMS and treated with NEO at 5, 10, or 20 mg/kg , fluoxetine, or vehicle. Body weight was monitored over 14 days. Behavioral outcomes were assessed using the Elevated plus maze (EPM) open field test (OFT), splash test, light and dark box test, forced swimming test (FST) , and tail suspension test (TST). Results demonstrated a significant dose-dependent response, with 5mg/kg identified as the most effective dose across all behavioral metrics. In the FST and TST, the 5mg/kg dose significantly reduced immobility time compared to the 10 and 20 mg/kg cohorts, showing effects comparable to the fluoxetine control. Similarly, in anxiety models (EPM, OFT, and light/dark box test) , the 5mg/kg dose effectively restored behavioral patterns closer to those of non-stressed control group, Notably, higher doses (10 and 20 mg/kg) exhibited diminished efficacy across most parameters, including a non-monotonic, inverted U-shaped dose-response relationship. NEO reveals potent anxiolytic and antidepressant-like activities. The findings indicate that 5mg/kg is the optimal therapeutic dose in this CUMS model, while higher doses may lose efficacy, suggesting a narrow therapeutic window. These findings highlight the critical importance of dose optimization when evaluating the psychopharmacological potential of essential oils.

Keywords: Myristica fragrans, nutmeg essential oil, chronic unpredictable mild stress, depression, monoaminergic system

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INTRODUCTION

Depression and anxiety disorders represent a substantial global health burden, affecting over 280 million people worldwide (Depression, 2017). The monoamine hypothesis posits that dysregulation of

serotonin (5HT), norepinephrine (NE), and dopamine (DA) neurotransmission underlies the pathophysiology of these conditions (Hirschfeld, 2000). Current first-line treatments , including

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selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), suffer from delayed therapeutic onset (2-4 weeks), sexual dysfunction, delayed ejaculation, weight gain and treatment resistance in approximately 30% of patients (Rush *et al.*, 2006). Consequently, there is an urgent need for safer, faster-acting, and multi-target therapeutic alternative.

The chronic unpredictable mild stress paradigm is one of the most validated and translationally relevant animal models of depression (Pryce and Fuchs, 2017; Qiao *et al.*, 2020). By exposing rodents to a series of unpredictable, mild stress over 1-4 weeks, CUMS induces behavioral, neurochemical and neuroendocrine alterations that mimic core features of human depression, including anhedonia, behavioral despair, anxiety-like behavior, and hypothalamic-pituitary-adrenal axis dysregulation (Lu, Christian and Lu, 2008; Chiang *et al.*, 2026). This model has excellent predictive validity, as it responds to chronic—but not acute-administration of clinically effective antidepressants (Moretti *et al.*, 2012). Nutmeg (*Myristica fragrans* Houtt), the seed kernel of an evergreen tree native to the Moluccas (Spice Islands) of Indonesia, has been used traditionally for centuries as a spice, digestive aid, and remedy for nervous disorder (Sultan *et al.*, 2023).

The essential oil nutmeg is a complex mixture of volatile compounds, with myristicin as the major constituent (typically 4-12%), along with sabinene, α -pinene, β -pinene, terpinen-4-ol, safrole, and elemicin (Kapoor *et al.*, 2013). Recent preclinical

studies revealed significant central nervous system activity of nutmeg preparations demonstrating that nutmeg oil act as potent anesthetic adjuvant that synergistically enhances xylazine-ketamine induce central nervous system depression (Abdullah and Naser, 2026). The n-hexane extract of nutmeg seeds demonstrated antidepressant-like effects in mice via the tail suspension and open field tests, with mechanism involving serotonergic (5-HT_{2A/2C}, HT₃) and noradrenergic (α ₂-adrenergic) receptor systems (Dhingra and Sharma, 2006). Soltani *et al.* (2025) reported that nutmeg oil produced antidepressant-like effects through monoaminergic mechanism in mice (Soltani *et al.*, 2025).

Additionally, nutmeg extracts have shown anticonvulsant, neuroprotective and anxiolytic properties in various rodent models (Sonavane *et al.*, 2002; Wahab *et al.*, 2009). The major constituent myristicin has shown to modulate GABA receptors, exhibit MAO inhibitory activity, and interact with serotonergic pathways (Seneme *et al.*, 2021). However, to our knowledge, no study has systemically evaluated the antidepressant and anxiolytic effect of NEO in a validated chronic stress model using a comprehensive battery of behavioral tests. Furthermore, the dose-response relationship and the potential for NEO to reverse CUMS-induced behavioral deficits across multiple domains (despair, anxiety, locomotion) remain unexplored. The present study was designed to address these gaps by investigating the behavioral effects of graded doses of NEO (5, 10 and 20 mg/kg) in a 14-day CUMS mouse model, using fluoxetine as the positive control.

MATERIALS AND METHODS

Animals

48 Male Swiss albino mice (25–35 g, 8-10 weeks old) obtained from the Laboratory Animal House, College of Veterinary Medicine, University of Mosul/Iraq. Animals were housed in common polypropylene cages (5 per cage) with free access to standard pellet feed and water *ad libitum*, under controlled conditions (temperature 21 ± 2 °C, 12:12 h light-dark cycle, humidity 50-60%). They were given at least 7 days for acclimatization before experimentation. All experimental procedures were conducted in strict accordance with the ARRIVE

Guidelines 2.0 (Animal Research: Reporting of In Vivo Experiments) and the International Council for Laboratory Animal Science (ICLAS) ethical guidelines for animal experimentation. The study protocol was reviewed and approved by institutional Animal Ethics Committee (IAEC) of the College of Veterinary Medicine, University of Mosul, Mosul, Iraq (Approval No. UM.VET.2025.105).

Drugs and Chemicals

Pure, commercially sourced nutmeg essential oil (*Myristica fragrans*) was obtained from Tiny

Kitchen Soap Co. (USA), a commercial supplier. The oil was derived from *M. fragrans* kernels. The nutmeg essential oil was prepared in varying concentrations in corn oil to deliver the exact doses (5, 10 and 20mg/kg), which served as the vehicle, by mechanical mixing. To ensure uniform dispersion, the oil–vehicle mixture was vortexed for 30 s immediately before loading into the syringe. Fluoxetine hydrochloride (Sigma-Aldrich, St. Louis, MO, USA) was dissolved in sterile saline at concentration of 2 mg/mL to deliver the positive control dose of 10 mg/kg/day. Nutmeg essential oil and fluoxetine were administered intraperitoneally (i.p.) at a standardized volume of 5 mL/kg body weight. All treatments were freshly prepared immediately before administration.

Experimental Design

Mice were randomly divided into six groups (n = 8 per group):

Group 1: Normal Control (NC) - No stress + vehicle

Group 2: CUMS Control (CC) - CUMS + vehicle

Group 3: CUMS + fluoxetine 10 mg/kg/day

Group 4: CUMS + nutmeg essential oil 5 mg/kg/day

Group 5: CUMS + nutmeg essential oil 10 mg/kg/day

Group 6: CUMS + nutmeg essential oil 20 mg/kg/day

Drug treatments were administered once daily, 30 minutes before the daily stressor, throughout the 14-day CUMS protocol.

CUMS Protocol

The CUMS protocol was adapted from Lu et al. (Lu *et al.*, 2006) and Ashraf et al. (Ashraf *et al.*, 2019), consisting of a variety of stressors randomly applied at varying times of day for 14 consecutive days to minimize predictability (Table 1). Control and stressed animals were group-housed and kept in separate rooms to prevent olfactory communication of stress. Control mice were handled daily but otherwise maintained under standard housing conditions.

Table 1. Schedule of stressors used in the 14-day CUMS protocol.

Day	Stressor	Duration	Time
1	Cold swim (12°C)	10 min	9:30 a.m.
2	Wet wood shavings (200 mL/100 g), cage tilted 45°	10 h	11 a.m.
3	Inversion of light/dark cycle	12 h	5 p.m.
4	Tail pinch (clothespin, 1 cm from tail base)	10 min	6:30 p.m.
5	Forced swim (25°C)	5 min	2 p.m.
6	Food and water deprivation	16 h	8 a.m.
7	Stroboscopic illumination (300 flashes/min)	3 h	8 a.m.
8	Forced swim (25°C)	5 min	2 p.m.
9	Wet wood shavings, cage tilted 45°	10 h	11p.m.
10	Cold swim (12°C)	10 min	9.30 p.m.
11	Inversion of light/dark cycle	12 h	5 p.m.
12	Food and water deprivation	16 h	8 p.m.
13	Stroboscopic illumination	3 h	10:30 a.m.
14	Tail pinch	10 min	9:30 a.m.

Body Weight Monitoring

Body weights were recorded on days 0, 7, and 14 of the experiment. The percentage body weight change was calculated as:

$$\text{Weight change (\%)} = [(W_{\text{day}} - W_{\text{day0}}) / W_{\text{day0}}] \times 100$$

Behavioral Tests

All behavioral tests were conducted on day 15, 24 h after the last administration, in a dimly lit, sound-attenuated room. The observer was blind to the treatment groups. The apparatus was cleaned with 70% ethanol between trials.

Tail Suspension Test (TST)

The TST was performed as described by Steru *et al.* (Steru *et al.*, 1985). Mice were suspended 50 cm above the floor using adhesive tape placed approximately 1 cm from the tip of the tail. The total immobility time during a 6-min test period was manually recorded by an experienced observer; the first 1 min was excluded. Mice were considered immobile only when they hung passively and completely motionless.

Forced Swim Test (FST)

The FST was conducted according to Porsolt *et al.* (Porsolt, Bertin and Jalfre, 1977). with minor modifications. Mice were individually placed in a transparent cylindrical container ($25 \times 12 \times 25$ cm, L $\times$ W $\times$ H) filled with water ($23 \pm 2^\circ\text{C}$) to a height of 15 cm. After the initial 2–3 min of vigorous activity, mice exhibited an immobility period characterized by floating with minimum movements. The total immobility time during the 6-min test was recorded. An animal was considered immobile when it remained floating passively in a slightly hunched but upright position with its nose above the water surface.

Elevated Plus Maze (EPM)

The EPM apparatus consisted of two opposite open arms (30×5 cm, L $\times$ W) and two opposite closed arms ($30 \times 5 \times 15$ cm, L $\times$ W $\times$ H) extending from a central square (5×5 cm), elevated 50 cm above the floor (Rodgers and Dalvi, 1997). Each mouse was placed in the central square facing an open arm, and behavior was recorded for 5 min. The following parameters were measured: (1) time spent in open arms (s), (2) time spent in closed arms (s), (3) number of entries into open arms, and (4) number of entries into closed arms.

Light-Dark Box Test (LDB)

The LDB apparatus consisted of two equal-sized compartments ($42 \times 30 \times 20$ cm, L $\times$ W $\times$ H) separated by a partition with a 6×6 cm opening at floor level (Bourin and Hascoët, 2003). One compartment was brightly illuminated (100 W bulb), while the other was kept dark. Each mouse was placed in the center of the dark compartment facing away from the opening, and behavior was recorded for 5 min. Parameters measured included:

(1) time spent in the light compartment (s), (2) time spent in the dark compartment (s), (3) number of transitions between compartments, (4) number of entries into the light compartment, and (5) latency to first entry into the light compartment (s).

Open Field Test (OFT)

The open-field test was performed to assess anxiety-related behavior in mice. The apparatus consisted of a transparent acrylic box measuring $72 \times 72 \times 36$ cm, with the floor divided into 16 equal squares (18×18 cm each) (Prut and Belzung, 2003). The central area was defined as four squares, while the peripheral area consisted of the 12 squares adjacent to the walls. A video camera was used to record the animals' behavior, including the duration of time spent in the central and peripheral areas of the field. After each animal, the apparatus was thoroughly rinsed to remove residual odors and minimize potential olfactory influences on the behavior of subsequent animals.

Splash test.

This test was performed as previously described (Isingrini *et al.*, 2010), with minor modifications. It consists of squirting a 10% sucrose solution on the dorsal coat of an individually-placed mouse in a clear Plexiglas box ($9 \times 7 \times 11$ cm³). Because of its viscosity, the sucrose solution dirties the mouse fur and animals start grooming behavior. After applying sucrose solution, the time spent grooming was manually recorded for a period of 5 min as an index of self-care and motivational behavior, considered to be parallel with apathetic symptoms of depression (Willner, 2005). The apparatus was cleaned with a solution of 10% ethanol between tests in order to hide animal clues.

Statistical Analysis

Data are expressed as mean $\pm$ standard error of the mean (SEM). Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A probability value of $P < 0.05$ was considered statistically significant. Statistical analyses were performed using IBM SPSS Statistics v. 22 (IBM Corp., Armonk, NY, USA).

RESULTS

Body Weight change

Chronic exposure to the CUMS protocol resulted in a significant reduction in body weight gain compared to the non-stressed control group by the end of the 14-day monitoring period. As shown in Figure 1, the CUMS control group exhibited lower

body weight at day 14 compared to the normal control. Treatment with NEO demonstrated a dose-dependent effect on body weight; the 5mg/kg and 10mg/kg treatment showed weight maintenance comparable to the non-stressed controls, whereas the CUMS control group displayed the most pronounced stress-induced deficit.

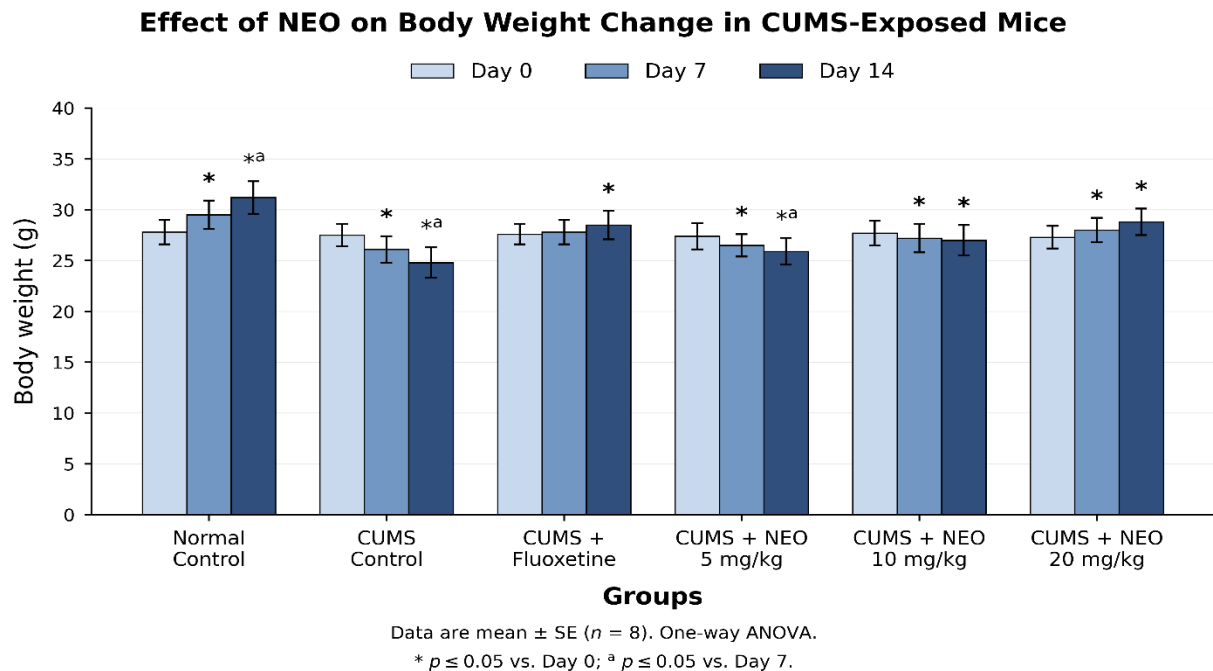


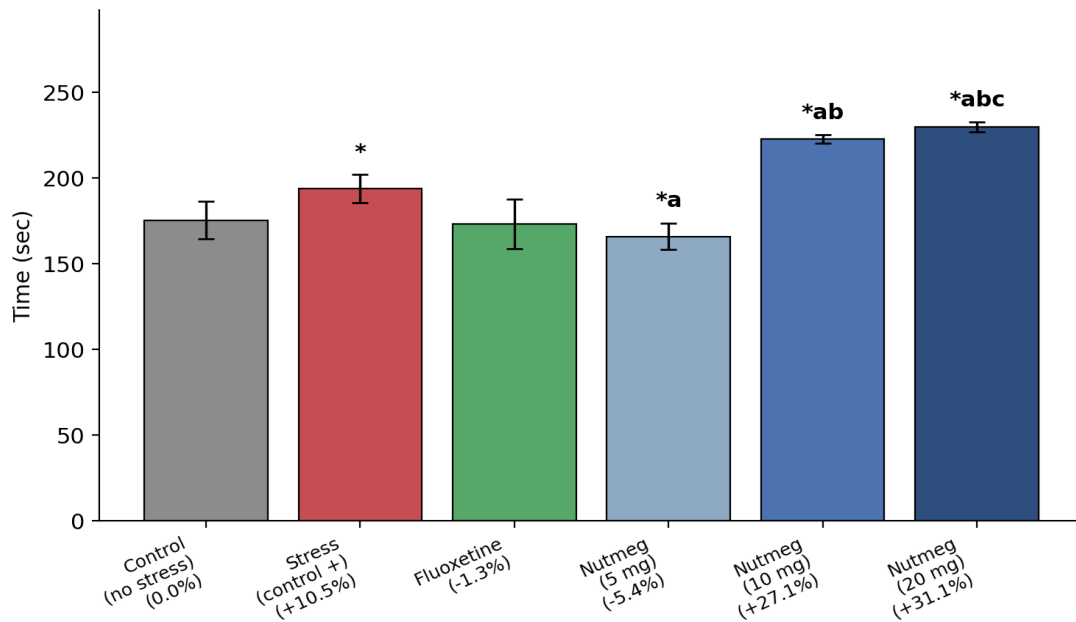
Figure 1. Effect of NEO on body weight change in CUMS-exposed mice

Anxiety-like Behavior

Elevated Plus Maze

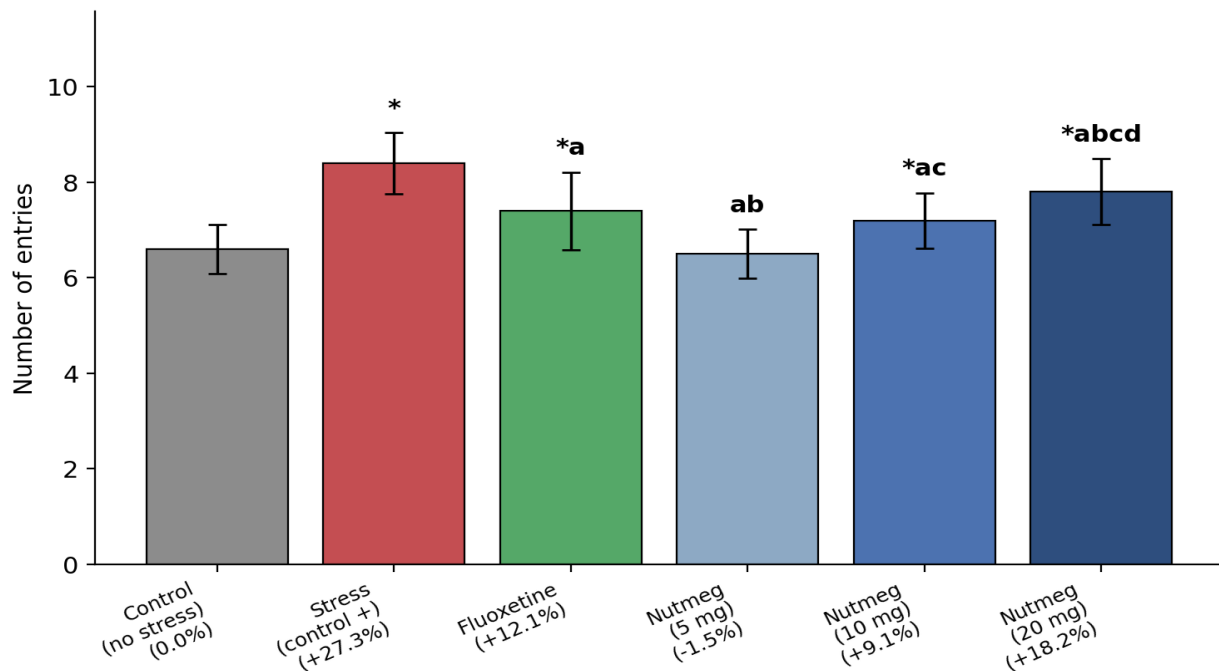
Stress-exposed mice (CUMS control) showed increased time spent in closed arm (Figure 2) and a higher number of entries into the closed arm (Figure 3) compared to normal control (* $P < 0.05$).

Treatment with NEO (5mg/kg) significantly reversed these parameters, with performance level comparable to the Fluoxetine-treated positive control. Notably, the higher doses of NEO (10 mg/kg and 20mg/kg) were significantly less effective than the 5mg/kg dose (Figure 2,3).



* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
 Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, $N = 8$ /group).

**Figure 2. EPM — time spent in closed arm (% change vs. control shown under group labels).
 Number of entries into closed arm**



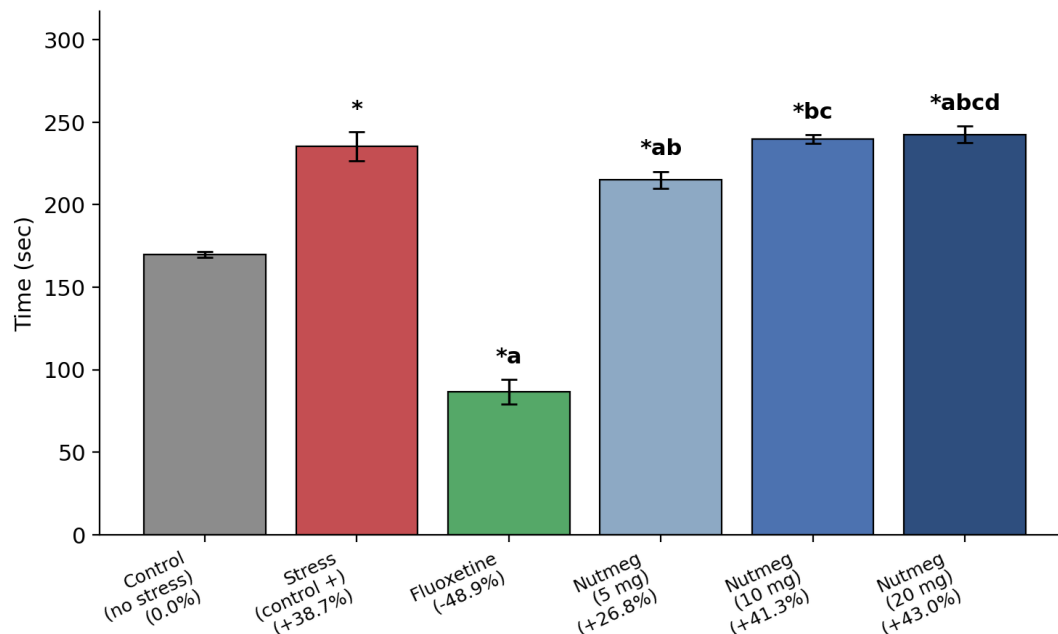
* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
 Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, $N = 8$ /group).

Figure 3. EPM — number of entries into closed arm (% change vs. control shown under group labels).

Open Field Test (OFT)

CUMS exposure significantly increased the time spent in the peripheral area of the open field (Figure 4), indicating heightened anxiety. NEO 5 mg/kg

treatment effectively reduced this peripheral avoidance, mirroring the effect of fluoxetine. Higher doses of NEO (10 and 20 mg/kg) resulted in increased peripheral time compared to the 5 mg/kg dose (Figure 4).



* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, N = 8/group).

Figure 4. OFT — time spent in peripheral area (% change vs. control shown under group labels).

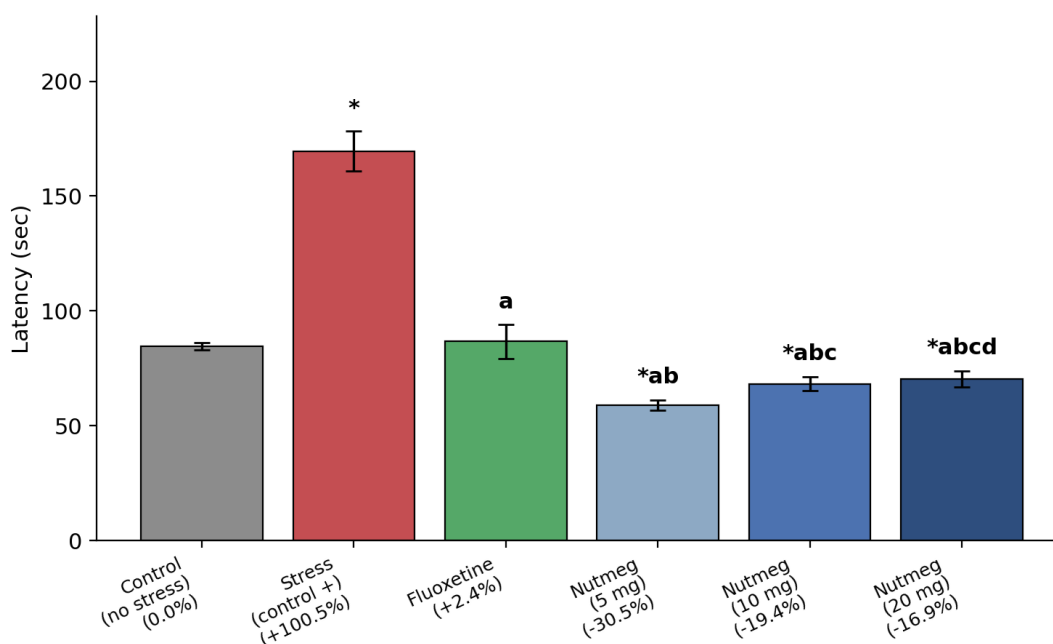
Light and Dark Box test

Consistent with the EPM and OFT results, CUMS mice spent significantly less time in the light area (Figure 6) and displayed fewer entries (Figure 7) compared to control (*** $P < 0.05$**). Administration of 5 mg/kg NEO significantly increased time spent in the light compartment and the frequency of entries, demonstrating an anxiolytic effect. The efficacy of the 5mg/kg dose was superior to both 10 mg/kg and 20 mg/kg treatments, which showed significant deviations from the 5mg/kg group (Figure 6,7).

Depressive-like behavior

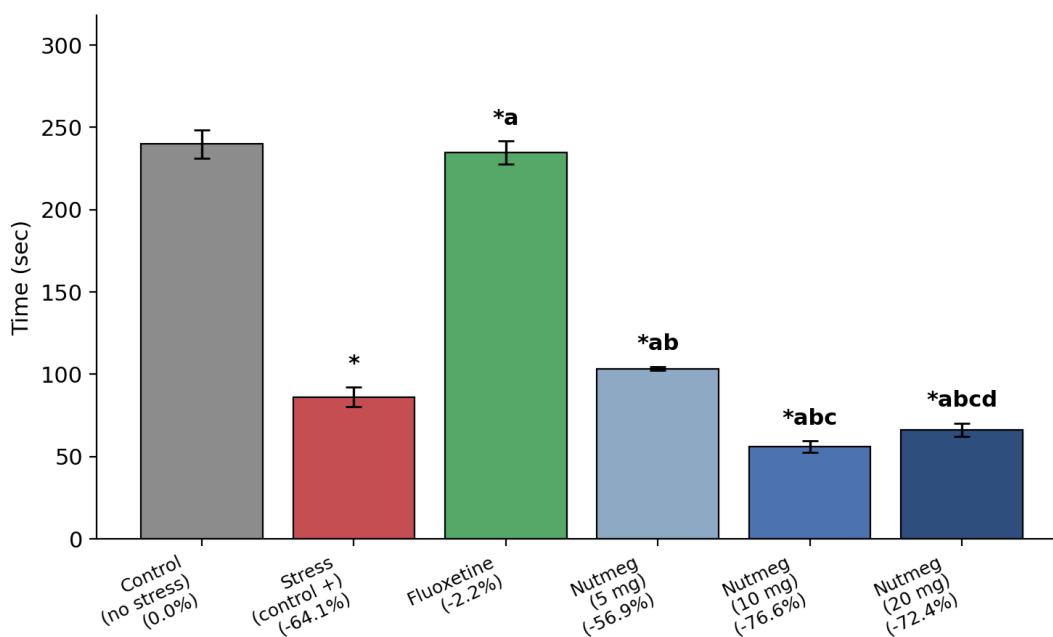
Splash Test

CUMS-exposed mice exhibited an increased latency to initiate grooming behavior compared to non-stressed controls (Figure 5). NEO 5 mg/kg treatment significantly reduced this latency, indicating an antidepressant-like effect. The 10 and 20 mg/kg doses were significantly less effective in reducing grooming latency than the 5 mg/kg dose (Figure 5).



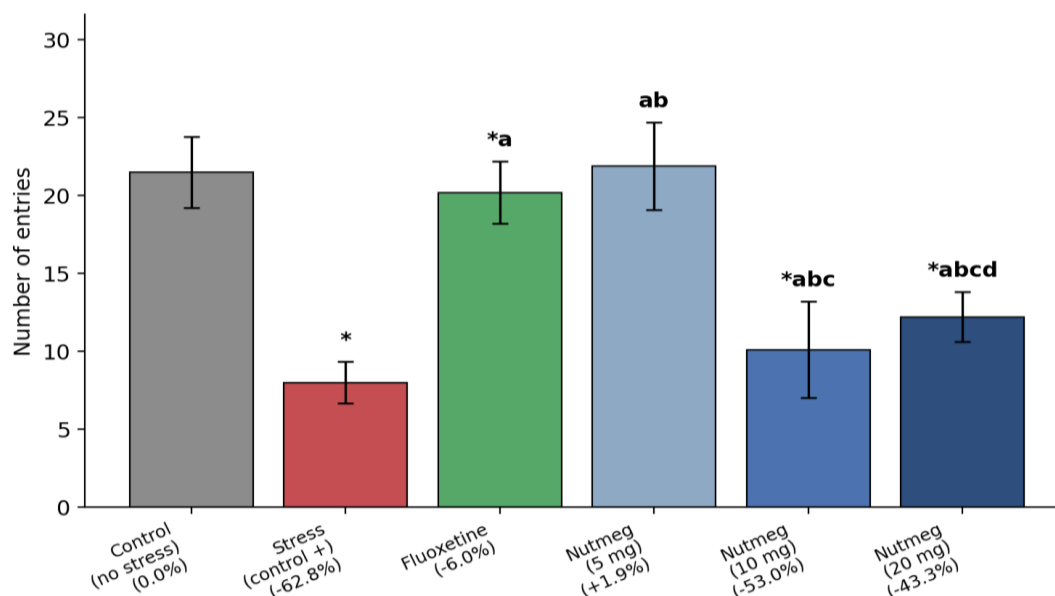
* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
 Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, N = 8/group).

Figure 5. Splash test — latency to grooming (% change vs. control shown under group labels).



* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
 Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, N = 8/group).

Figure 6. Light & dark box — time spent in light area (% change vs. control shown under group labels).



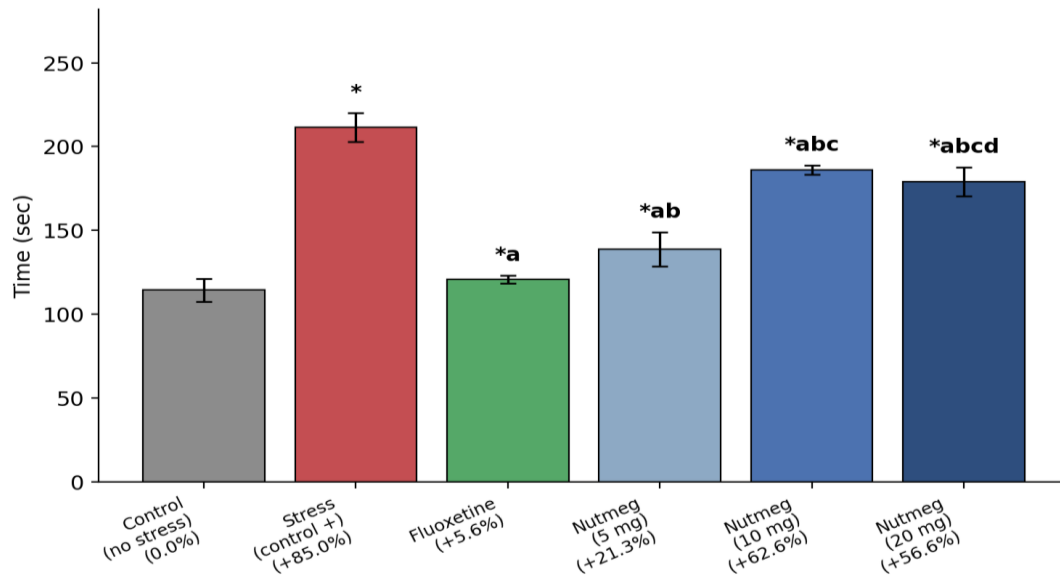
* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
 Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, N = 8/group).

Figure 7. Light & dark box — number of entries (% change vs. control shown under group labels).

Forced Swimming Test (FST) and Tail Suspension Test (TST)

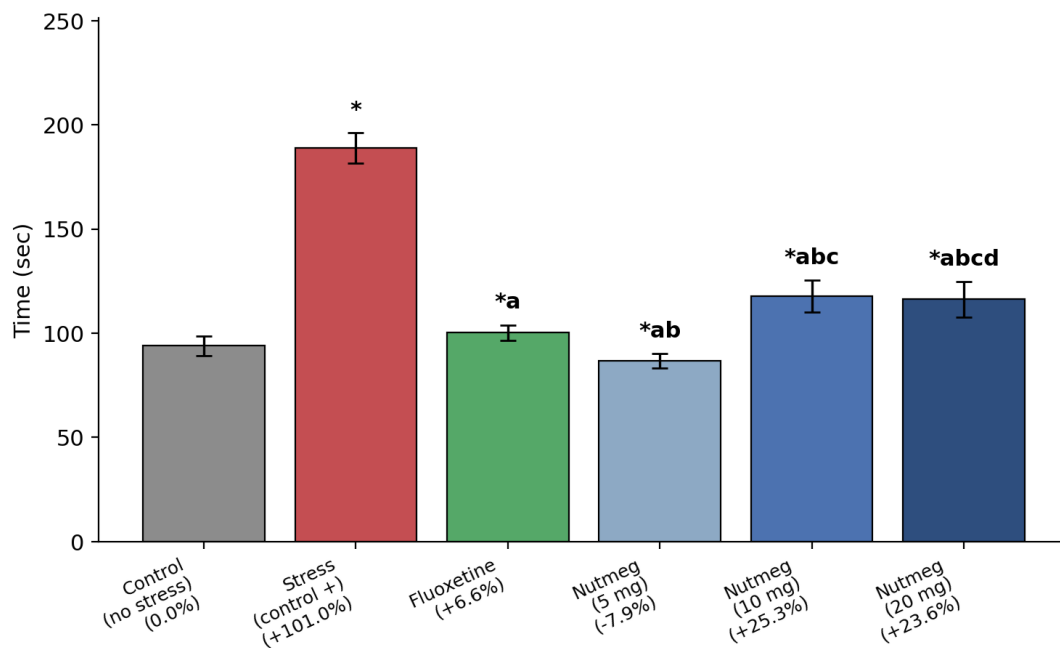
In both tests, CUMS control mice demonstrated significantly increased immobility time compared to normal controls (Figure 8, 9). Treatment with NEO (5 mg/kg) significantly attenuated this

immobility, yielding results comparable to the Fluoxetine group. In both FST and TST, the antidepressant efficacy observed at 5 mg/kg was statistically superior to the outcomes produced by the 10 mg/kg and 20 mg/kg doses, which showed significantly higher immobility times (Figure 8, 9).



* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
 Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, N = 8/group).

Figure 8. FST — immobility time (% change vs. control shown under group labels).



* $P < 0.05$ vs. Control (no stress); a $P < 0.05$ vs. stress (positive control); b $P < 0.05$ vs. Fluoxetine; c $P < 0.05$ vs. Nutmeg 5 mg; d $P < 0.05$ vs. Nutmeg 10 mg.
 Symbols and values reproduced exactly as reported in the source table (Data represent Mean $\pm$ SEM, N = 8/group).

Figure 9. TST — immobility time (% change vs. control shown under group labels).

DISCUSSION

The present study provides the first systematic evaluation of nutmeg essential oil (NEO) in a chronic unpredictable mild stress (CUMS) mouse model using a comprehensive battery of behavioral tests spanning both anxiety-like and depression-like domains. The principal finding is that NEO at 5 mg/kg produced robust antidepressant- and anxiolytic-like effects, reversing CUMS-induced deficits in body weight, behavioral despair (FST and TST), and anxiety-like behavior (EPM, OFT, LDB, and splash test). Strikingly, higher doses (10 and 20 mg/kg) not only failed to replicate the efficacy of the 5 mg/kg dose but, in several anxiety-related parameters, produced behavioral profiles that deviated further from the non-stressed control group. This non-monotonic, inverted U-shaped dose-response relationship carries significant implications for the therapeutic application of NEO and aligns with emerging evidence that essential oils often exhibit narrow therapeutic windows governed by multi-target receptor interactions (Willner, 2005; Sharma *et al.*, 2024). These findings are consistent with the established validity of the CUMS model, which produces neurochemical and neuroendocrine alterations that closely mirror the pathophysiology of human stress-related affective disorders, including monoamine depletion, HPA axis hyperactivation, and hippocampal neuroplasticity deficits (Panjwani *et al.*, 2025; Ren *et al.*, 2026).

Treatment with NEO (5 mg/kg) significantly attenuated these stress-induced behavioral abnormalities. In the FST and TST, the 5 mg/kg dose reduced immobility time to levels comparable to fluoxetine, suggesting a genuine antidepressant-like effect rather than a confounding change in locomotor activity. This is corroborated by the EPM and OFT data, where the 5 mg/kg dose normalized closed-arm time and peripheral zone exploration, respectively. Importantly, the splash test which assesses motivational behavior and self-care, constructs with strong face validity for the psychomotor retardation and anhedonia seen in clinical depression showed a marked reduction in grooming latency following 5 mg/kg NEO treatment. Collectively, these behavioral outcomes indicate that low-dose NEO exerts broad-spectrum psychotropic activity against both the despair and

anxiety dimensions of the CUMS phenotype (Becker, Pinhasov and Ornoy, 2021).

The antidepressant- and anxiolytic-like properties of nutmeg preparations reported here are consistent with, and extend, earlier preclinical investigations. Dhingra and Sharma demonstrated that an n-hexane extract of *M. fragrans* seeds produced antidepressant-like effects in mice via serotonergic (5-HT_{2A/2C} and 5-HT₃) and noradrenergic (α ₂-adrenergic) receptor mechanisms (Dhingra and Sharma, 2006). More recently, Soltani and colleagues reported that NEO produced acute antidepressant-like effects through monoaminergic mechanisms in mice (Soltani *et al.*, 2025). The present study advances this literature by demonstrating that these effects are reproducible in a chronic stress model, a paradigm with superior translational validity compared to acute screening tests and that they extend to anxiolytic-like activity when administered at an optimized dose.

Furthermore, prior work has documented the anticonvulsant and neuroprotective properties of nutmeg extracts in rodent models. The methanolic extract of *M. fragrans* has been shown to protect against scopolamine-induced oxidative damage and inflammation in cortical tissue, while the ethanolic extract attenuated neuronal loss and glial activation in a pentylenetetrazol kindling model (Wahab *et al.*, 2009). These neuroprotective and anti-inflammatory actions are particularly relevant to the CUMS model, given that chronic stress is known to induce neuroinflammatory cascades, microglial activation, and hippocampal atrophy. Although the present study did not assess neurochemical endpoints directly, the behavioral restoration observed with 5 mg/kg NEO is consistent with a mechanism involving the suppression of stress-induced neuroinflammation and the preservation of neuronal integrity (Al-Quraishy *et al.*, 2020; Le *et al.*, 2025).

Perhaps the most consequential finding of this study is the inverted U-shaped dose-response curve exhibited by NEO. While 5 mg/kg was clearly optimal, the 10 and 20 mg/kg doses showed significantly diminished efficacy across nearly all behavioral parameters. In the EPM, OFT, and LDB, the higher doses not only failed to reverse stress-induced anxiety but, in some cases, appeared to exacerbate anxiety-like behavior relative to the

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CUMS control group. This pattern is not unprecedented in the psychopharmacology of essential oils.

A systematic review of anxiolytic-like effects of essential oils in animal models documented inverted U-shaped curves for multiple species, including *Angelica sinensis*, *Citrus aurantium* subsp. *bergamia* (bergamot), *Citrus limon*, *Citrus latifolia*, *Foeniculum vulgare* (fennel), and *Ocimum gratissimum* (De Sousa *et al.*, 2015). Similarly, citronellol, a constituent of rose oil, exhibited an inverted U curve in the Vogel conflict test (Umezuru *et al.*, 2002). In humans, cannabidiol another plant-derived multi-target compound has demonstrated a comparable inverted U-shaped anxiolytic dose-response during public speaking tasks (Zbozinek, Khalsa and Craske, 2026).

The major constituent of NEO, myristicin (4–12% of oil composition), is a weak, non-selective inhibitor of monoamine oxidase (MAO) (Rajput, 2010; Seneme *et al.*, 2021). At low concentrations, this MAO inhibition may produce a modest, physiologically beneficial increase in synaptic serotonin, norepinephrine, and dopamine—consistent with the antidepressant and anxiolytic profile observed at 5 mg/kg. However, at higher doses, excessive MAO inhibition combined with possible direct serotonergic agonism (via myristicin metabolites such as MDMA-like amphetamine derivatives acting at 5-HT_{2A} receptors) could lead to serotonergic overstimulation, receptor desensitization, or downstream ex-cytotoxicity, paradoxically worsening anxiety-like behavior (van Lennep *et al.*, 2015; Ziaja *et al.*, 2025). Also, myristicin has been shown to modulate GABA-A receptors, with evidence suggesting it may act as a weak antagonist at higher concentrations (Thomas, 2011).

GABA-A antagonism would disinhibit limbic circuits, producing an anxiogenic rather than anxiolytic phenotype precisely what was observed in the EPM, OFT, and LDB at 10 and 20 mg/kg (Myers *et al.*, 2016). At 5 mg/kg, the net effect may favor GABAergic facilitation or allosteric modulation by other NEO constituents (e.g., elemicin, eugenol), whereas at supraoptimal doses, myristicin-mediated GABA-A antagonism may dominate (Rajput, 2010).

Furthermore, hepatic metabolism of myristicin via cytochrome P450 enzymes (particularly CYP1A2 and CYP2E1) generates active metabolites including 1'-hydroxymyristicin and potentially MDMA-like amphetamine derivatives (Yang *et al.*, 2015). At higher NEO doses, metabolic saturation or the accumulation of these psychoactive metabolites could shift the pharmacodynamic profile from anxiolytic to psychostimulant or even hallucinogenic, explaining the loss of behavioral normalization.

The monoamine hypothesis remains the most robust framework for understanding the antidepressant-like effects observed with 5 mg/kg NEO. CUMS is well-documented to depress central monoaminergic tone, particularly in the hippocampus and prefrontal cortex. Essential oils with antidepressant activity, including saffron oil, *Zanthoxylum bungeanum* oil, and *Perilla frutescens* oil, have been shown to restore hippocampal 5-HT, dopamine, and norepinephrine levels in CUMS-exposed rodents. Given that myristicin inhibits MAO and that NEO has been shown to engage monoaminergic mechanisms, it is highly probable that the 5 mg/kg dose restored synaptic monoamine availability in stress-vulnerable brain regions.

Beyond monoaminergic modulation, chronic stress induces neuroinflammation characterized by microglial activation, astrogliosis, and elevated pro-inflammatory cytokines (IL-1 β , IL-6, TNF- α) in the hippocampus and prefrontal cortex. These inflammatory mediators impair neurogenesis, synaptic plasticity, and BDNF signaling. Nutmeg extract and its constituents, particularly myristicin, elemicin, and myristic acid have demonstrated significant anti-inflammatory activity, inhibiting nitric oxide (NO), COX-2, IL-1 β , and IL-6 production in LPS-stimulated macrophages and in carrageenan-induced edema models (Lee and Park, 2011). By attenuating neuroinflammatory cascades, NEO may have protected against stress-induced hippocampal atrophy and synaptic loss, thereby facilitating the behavioral recovery observed. Neurotrophin signaling, particularly the BDNF–TrkB pathway, represents another convergent mechanism (Grønli *et al.*, 2006). CUMS reliably downregulates hippocampal BDNF, which in turn suppresses downstream

survival signaling through PI3K/Akt, ERK/CREB, and PLC- γ pathways (Pryce and Fuchs, 2017).

Essential oils such as *Syzygium aromaticum* and *Toona ciliata* have been shown to restore hippocampal BDNF, p-ERK, and p-CREB expression in CUMS rats. Although BDNF was not measured in the present study, the robust reversal of behavioral despair and anxiety by 5 mg/kg NEO is biologically consistent with enhanced BDNF–TrkB signaling and subsequent neuroplasticity recovery. The HPA axis represents an additional locus of action. CUMS induces hyperactivity of the HPA axis, elevating circulating corticosterone and central corticotropin-releasing factor (CRF). Essential oils with antidepressant efficacy in CUMS models, such as *Zanthoxylum bungeanum* oil, have been shown to reduce hippocampal CRF and serum corticosterone while simultaneously restoring monoamine levels. Given the traditional use of nutmeg as a nervine tonic and its modulatory effects on stress-responsive circuits, it is plausible that 5 mg/kg NEO exerted partial normalization of HPA axis tone, although direct neuroendocrine measurements are required to confirm this hypothesis.

An interesting observation from present findings is that the dose dependent dissociation between anxiolytic and antidepressant effects was not uniform. In the forced swimming test and tail suspension test, the 10 and 20 mg/kg doses produced partial attenuation of immobility, suboptimal relative to 5 mg/kg, but not clearly worse than the CUMS control. In contrast in the elevated plus maze, open field test and light /dark box test, the higher doses appeared to actively worsen anxiety-like indices. This dissociation suggests that neuronal substrates underlying NEOs antidepressant and anxiolytic effects may have distinct dose response relationships or receptor thresholds.

The FST and TST primarily recruit descending serotonergic and noradrenergic pathways from the

raphe nuclei and locus coeruleus to hippocampus and prefrontal cortex. Even moderate monoaminergic enhancement (as might occur at 10-20 mg/kg via MAO inhibition) may suffice to reduce behavioral despair (Detke, Rickels and Lucki, 1995; Cryan, Mombereau and Vassout, 2005). In contrast, anxiety-like behavior in the EPM, OFT and LDB is more tightly regulated by GABAergic interneurons within the amygdala, bed nucleus of the stria terminalis, and hippocampal CA1/subiculum. If higher NEO doses produce net GABA_A receptor antagonism in these limbic structures, the resulting disinhibition would manifest as increased avoidance behavior, exactly as observed. This neuroanatomical and pharmacological dissociation underscores the importance of multi-test batteries rather than single behavioral endpoints when characterizing the psychopharmacology of complex natural products.

The identification of 5 mg/kg as the sole effective dose- with 10 and 20 mg/kg showing diminished or paradoxical efficacy establishes a narrow therapeutic window for NEO in this CUMS model. This result carries direct clinical relevance. In traditional medicine, nutmeg has been used at low doses as a calming agent and digestive tonic, whereas high dose consumption is associated with toxicity, tachycardia, agitation and hallucinations. The present preclinical data provide a mechanistic rationale for empirical observation: the transition from anxiolytic to anxiogenic activity occurs within a relatively small dose range, likely driven by the multi-target pharmacology of myristicin and its metabolites. For translational development, these findings emphasize that dose optimization must precede any clinical evaluation of NEO. The “more is better” assumption that often pervades herbal medicine is clearly inapplicable here. Future formulation strategies such as microemulsion, nanoencapsulation or transdermal delivery systems should aim to achieve stable plasma concentrations within the narrow therapeutic range rather than maximizing dose (De Sousa *et al.*, 2017).

CONCLUSION

This study demonstrates that nutmeg essential oil exerts potent antidepressant and anxiolytic-like effects in a CUMS mouse model, but only at a low, optimally defined dose of 5 mg/kg. Higher doses (10 and 20 mg/kg) exhibited a marked loss of

efficacy and in anxiety-related paradigms, paradoxical anxiogenic –like behavior. These findings establish NEO as a promising candidate for the pharmacotherapy of stress-related affective disorder, while simultaneously highlighted the

critical importance of dose precision. The non-monotonic dose response likely reflects the multi-target pharmacology of myristicin and related compounds, involving monoaminergic enhancement, GABAergic modulation and anti-inflammatory neuroprotection at low doses, transitioning toward GABA-A antagonism and serotonergic overstimulation at supraoptimal doses.

Study limitations

Several limitations of this study should be acknowledged. First, the absence of neurochemical, neuroendocrine and molecular endpoint measurements preclude definitive mechanistic conclusions. While the behavioral data are highly suggestive of monoaminergic, GABAergic and anti-inflammatory mechanisms, direct quantification of hippocampal 5HT, DA, NE,

BDNF, TrkB, CRF, corticosterone and pro-inflammatory cytokines is essential to validate these hypotheses. Second, the study utilized only male mice; given well-documented sex differences in stress responsivity and antidepressant pharmacokinetic. Third, the 14-day CUMS protocol, while sufficient to induce robust behavioral deficits, is relatively short compared to the 4–6-week protocols used in some laboratories.

Competing Interests

The authors declare that they have no competing interests.

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