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Research Article

The potential of kemukus (*Piper cubeba* L.f.) seed essential oil nanoemulsion on behavior and biochemical parameters in a rat model of scopolamine-induced Alzheimer's disease

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Abstract

Piper cubeba L.f. fruit, specifically its seeds, has been used traditionally to strengthen memory. This study aimed to examine the neuroprotective effect of a nanoemulsion of *P. cubeba* essential oil (NPCEO) in a scopolamine-induced rat model of Alzheimer's disease. Male Wistar rats were divided into six groups: negative control, normal control, citicoline 200 mg/kg, NPCEO 100 mg/kg, NPCEO 200 mg/kg, and NPCEO 400 mg/kg. The brain was used for biochemical measurements, including tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-10, acetylcholinesterase (AChE) activity, brain-derived neurotrophic factor (BDNF), nuclear factor erythroid 2-related factor 2 (Nrf2), lipid peroxidase activity, and catalase (CAT) levels. Cognitive performance was examined using water-E maze (WEM), novel object recognition test (NORT), and open field test (OFT) methods. The results showed that NPCEO could reduce neuroinflammation and improve cognitive function impairment caused by scopolamine. In conclusion, *P. cubeba* could improve cognitive function in scopolamine-treated rats, possibly through its antioxidant and anti-inflammatory properties in the hippocampus and cerebral cortex.

Keywords

Alzheimer's disease, essential oil, cognitive function, nanoemulsion, *Piper cubeba*

Introduction

Traditionally, *Piper cubeba* fruits have been utilized for their medicinal properties, including the treatment of headaches through decoctions or nasal formulations (Abolhasanzadeh et al. 2017). Additionally, they have been reported to enhance cognitive function, including memory and brain health (Adams et al. 2007). The neuroprotective potential of *P. cubeba* is also supported by the traditional use of the fruit to relieve headaches, mental fatigue, and other nervous problems, indicating the benefits of this plant for brain and nervous system health (Drissi et al. 2022). The traditional use and studies of *P. cubeba* demonstrate its neuroprotective potential and suggest it could be beneficial in neurodegenerative diseases, such as Alzheimer's disease.

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Alzheimer's disease is characterized by cognitive decline, memory loss, and behavioral changes due to the death of nerve cells (neurons) in the brain (Mariani et al. 2007). The global prevalence of cognitive impairment is 76.8% per 1000 people each year (Pais et al. 2020), while in Indonesia, it has affected more than 4.2 million people in 2021 (Farina et al. 2023). Cognitive impairment is often associated with mild chronic inflammation, which increases oxidative stress (Tan and Norhaizan 2019). Previous research found that elevated levels of inflammatory markers such as tumor necrosis factor (TNF)-α and interleukin (IL)-1β are associated with cognitive impairment (Dimopoulos et al. 2006). Free radicals and oxidative stress can also cause neurodegenerative diseases and accelerate the development of cognitive impairment. Neuroprotective interventions through antioxidants and anti-inflammatories could reduce the loss of neurons and decrease amyloid beta (Aβ) accumulation, which constitutes a significant approach in Alzheimer's research and therapy development (Longo and Massa 2004).

Previous studies have revealed the anti-inflammatory properties of *P. cubeba* in various animal models (Choi and Hwang 2003, 2005; Godoy de Lima et al. 2018; Perazzo et al. 2013; Souza et al. 2004). The anti-inflammatory mechanism of this plant has been demonstrated in vitro through the inhibition of cyclooxygenase (COX)-1, COX-2, and 5-lipoxygenase (LOX) (Lima et al. 2017), as well as through the inhibition of Nuclear Factor Kappa B (NF-κB) activation and of TNF-α and IL-1β (Deme et al. 2019; Qomaladewi et al. 2019). *P. cubeba* extract has also been studied for its potential to prevent brain inflammation in an animal model induced by lipofuscin (Muchandi and Dhawale 2018). The anti-inflammatory and antioxidant potential of *P. cubeba* essential oil has also been studied (Kumar 2021).

In earlier studies, *P. cubeba* has been shown to have neuroprotective capacity by increasing the antioxidant levels in the brain, both in the form of extracts and essential oils (Dwita et al. 2023b, 2023a). Other studies have demonstrated the potential of *P. cubeba* as an anti-Alzheimer's agent in vitro (Somani et al. 2017; Tarbiat et al. 2023). *P. cubeba* fruit has also been tested for improving cognitive function in scopolamine-induced rats by modulating cholinergic function in the hippocampal region, in combination with an Ayurvedic preparation (Abdul-jalil and Nasser 2020).

P. cubeba seeds contain 18% oil (Singh et al. 2007), sabinene, eucalyptol, 4-terpineol, β-pinene, camphor, δ-3-carene (Akbar 2020), and lignans such as yatein, hinokinin, and cubebin (Elfahmi et al. 2007). Gas chromatography-mass spectrometry (GC-MS) analysis of the essential oil showed 48.4% sesquiterpene hydrocarbons and 36.3% monoterpenes, with main compounds such as sabinene, β-cubebene, α-copaene, β-phellandrene, linalool, and cubebol (Singh et al. 2007). Nanoemulsion preparations provide significant advantages, especially in increasing the effectiveness of active lipophilic substances of essential oils that tend to be poorly soluble in water and have low bioavailability when administered in conventional forms (Naqvi et al. 2020). In this study, it is expected that nanoemulsion dosage forms will provide substantial benefits that contribute to the effectiveness and efficacy of *P. cubeba* essential oil as a neuroprotective agent.

Material and methods

P. cubeba essential oil GC-MS analysis

The *P. cubeba* essential oil was provided by local industry (Pavettia, Indonesia). The essential oil content of *P. cubeba* was determined using an Agilent Technologies 7890 gas chromatograph. The instrument includes an autosampler, a 5975 mass selective detector, a ChemStation data system, and an INNOWAX column. One of the essential oil substances of *P. cubeba* that was determined in this study was eugenol. Eugenol is one of the major chemical constituents of *P. cubeba* essential oil and has shown antioxidant potential (Alminderej et al. 2020). A eugenol standard (Mark Herb, Indonesia) was used to determine the eugenol content in *P. cubeba* essential oil. The percentage was calculated using the formula:

$$\text{Eugenol content}(\%) = \frac{\text{eugenol concentration} \times \frac{P.cubeba\text{area}}{\text{eugenol area}} \times P.cubeba\text{volume} \times \text{dilution factor}}{P.cubeba\text{weight}}$$

Nanoemulsion preparation

The nanoemulsion contained *P. cubeba* essential oil and Capryol 90 as the oil phase. Tween 20 and propylene glycol were used as the surfactant and co-surfactant, respectively. The preparation was carried out using an Ultra-Turrax homogenizer at 500 rpm.

Evaluation of nanoemulsion preparations

The evaluation of nanoemulsion included globule size, polydispersity index, zeta potential, pH, and viscosity (Gharbavi et al. 2018).

Experiment design

Wistar rats (200–230 g, 2–3 months old) were divided into six groups: NPCEO 400 mg/kg, 200 mg/kg, and 100 mg/kg; citicoline 200 mg/kg; scopolamine control (negative control); and normal control. The experiment was conducted based on a previous study with modifications. The animals were given the test material for one week, followed by 20 mg/kg scopolamine administered intraperitoneally (i.p.) for 14 days (Jafarian et al. 2019). Cognitive function and behavioral tests were conducted 30 minutes after the scopolamine injection (Fig. 1). The experiment was approved by Prof. Dr. Hamka's ethics committee (No: 02/24/01/03034).

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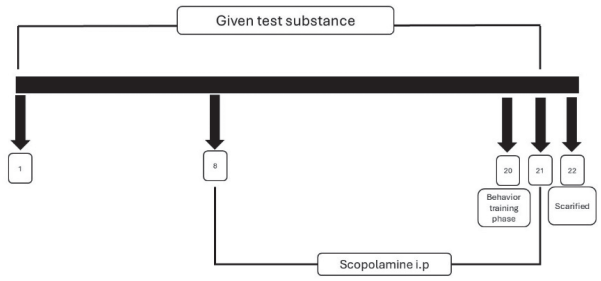

Figure 1. Cognitive test procedure flowchart.

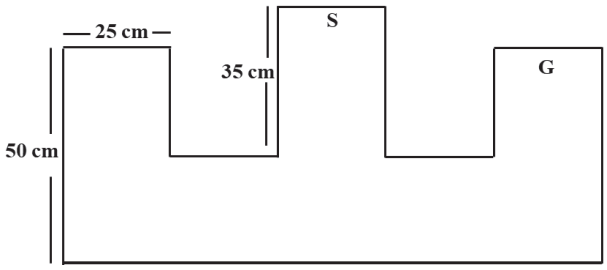

Figure 2. Water-E maze scheme. S: Start; G: Goal.

Water-E maze (WEM) test

Learning and memory tests were carried out using the WEM equipment (Kartinah et al. 2019). The pool contained water to a depth of 26.5 cm, and the temperature was maintained at 24 ± 2 °C. The rats were placed at point S and allowed to swim for 120 seconds. If a rat could not find the ladder (point G) within the allotted time, it was guided to point G. The training phase was conducted three times, with a 30-minute rest between sessions. The next day, the test was conducted 30 minutes after scopolamine induction. The time taken by each rat to reach point G was recorded as travel time.

Open field test (OFT)

This test used a 50 cm × 50 cm × 50 cm box with opaque walls, divided into 25 imaginary squares. The movement of the rats across the imaginary lines in both the inner and outer zones of the square was recorded. In addition, addition, grooming duration, number of fecal pellets, and instances of rearing were recorded. Each rat was tested for 5 minutes (Haider et al. 2016).

Novel object recognition test (NORT)

This test was conducted using the same apparatus as the OFT. In this case, two objects were placed in the box, each 10 cm from the wall. During the training phase, two identical objects (yellow triangles) were placed in opposite corners of the box, and the rats were allowed to explore them for 5 minutes. The testing phase was conducted after 24 hours using familiar objects (yellow triangles) and novel objects (blue blocks). Rats were allowed to explore the objects for 3 minutes, and their behavior was recorded (Yadang et al. 2020).

Hole board (HB) test

Each animal was placed in the hole board apparatus for 5 minutes, and the number of head dips was recorded.

Brain homogenate preparation

Immediately after sacrifice, the brain was isolated, washed with cold phosphate-buffered saline (PBS), and blotted dry with absorbent paper. Then the hippocampus and cortex were separated and weighed. Each part was made into a 10% homogenate in PBS (pH 7.4) and centrifuged at 5000 × g for 10 minutes at 4 °C. The homogenate was stored at −20 °C, and biochemical tests were performed within one week.

Catalase (CAT) activity test

The catalase test was performed according to Hadwan (2018). A total of 100 µL of homogenate was mixed with 200 µL of 10 mM hydrogen peroxide (H_2O_2) and incubated at 37 °C for 2 minutes. After that, 1.2 mL of working solution, a mixture of cobalt (II) nitrate hexahydrate, Graham salt $(NaPO_3)_6$, and sodium bicarbonate (1:1:18), was added. Finally, the mixture was measured at 450 nm. Catalase activity = (2.303/2) × log (standard absorbance / test absorbance).

Lipid peroxidase test

Approximately 0.5 mL of homogenate was mixed with 0.5 mL of 20% trichloroacetic acid (TCA) and 1 mL of 0.67% thiobarbituric acid (TBA). The mixture was heated at 95 °C for 45 minutes, and the absorbance was measured at 532 nm.

Acetylcholinesterase (AChE) activity assay

The AChE activity of hippocampal homogenates was determined using an AChE assay kit (BT Lab) and performed according to the procedures in the brochure.

Enzyme-linked immunosorbent assay (ELISA) test

The ELISA test was carried out using TNF-α, IL-1β, nuclear factor erythroid 2-related factor 2 (Nrf2), and brain-derived neurotrophic factor (BDNF) ELISA kits (BT Lab). The hippocampal homogenates were used to determine cytokine levels, following the procedures listed in the product brochures.

Statistical analysis

Data were tested for normality and homogeneity, followed by ANOVA (Kobayashi and Pillai 2013).

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Results

GS-MS analysis results of *P. cubeba* essential oil

Based on the GC-MS analysis results (Table 1), *P. cubeba* essential oil contains various compounds with potential neuroprotective activity. The compounds that might contribute to this potential are eugenol (Barot and Saxena 2021), linalool (Farooqui and Farooqui 2017), and caryophyllene (Postu et al. 2022). The GC-MS results were then further used to determine the eugenol content in *P. cubeba* essential oil. The chromatogram of *P. cubeba* essential oil and eugenol is shown in Fig. 3.

The eugenol content *of P. cubeba* essential oil used in this study was 44.33%. Eugenol is a phenolic compound known to have various biological activities, including neuroprotective activity (Barot and Saxena 2021). Eugenol's capacity to reduce inflammation and oxidative stress, two leading causes of dementia, might be responsible for P. cubeba essential oil's neuroprotective properties.

Evaluation results of *P. cubeba* essential oil nanoemulsion (NPCEO)

The pH of NPCEO was 5.02 ± 0.01, which met the criteria for oral pH preparations. The viscosity of the nanoemulsion was 29.97 ± 0.21 mPa.s. Evaluation of globule size, polydispersity index, and zeta potential is presented in Table 2.

Based on the evaluation results, the NPCEO globule size has met the requirements of nano preparations, which is less than 100 nm. The PDI indicates the uniformity of globule size.

Table 1. Analysis results of *P. cubeba* essential oil content using GC-MS.

Retention time (min)	Quality	Compounds	Content (%)
3.358	96	Alpha-Pinene	2.24
4.467	91	Beta-Pinene	1.02
4.695	91	Beta-Phellandrene	1.54
6.641	95	Eucalyptol	1.51
16.261	98	Alpha-Cubene	5.71
17.776	99	Copaene	6.32
20.204	97	Beta-Cubene	2.83
21.693	96	Linalool	2.27
22.985	99	Caryophyllene	5.32
25.419	99	Alloaromadendrene	4.26
26.695	98	Humulene	1.81
27.909	99	Gamma-Muurolene	3.49
28.942	96	Cis-Muurola-4 (15), 5-diene	1.91
29.845	99	Alpha-Muurelene	2.13
31.630	94	Cis-Muurola-3,5-diene	14.58
35.365	97	Trans-Calamenene	7.16
38.489	70	Epicubebol	2.80
41.088	70	Cubebol	4.31
44.907	99	Ledol	6.03
46.355	62	Gamma-Muurolene	1.99
46.640	93	Di-epi-1,10-Cubebol	8.86
48.171	98	(-)-Spathulenol	2.33
48.991	98	Eugenol	1.26
49.094	94	Alpha-Cadinol	1.21
49.670	98	Alpha-Cadinol	1.45
50.002	99	Isospathulenol	1.11
51.435	91	Apiol	1.18
53.090	98	Asarone	1.49

Effect of nanoemulsion *P. cubeba* essential oil on cognitive function

In this study, a 14-day administration of scopolamine caused a significant decrease in cognitive function ($p < 0.001$) in the negative control group compared to the treatment groups (Fig. 4a). On the other hand, the normal control group demonstrated optimal cognitive per-

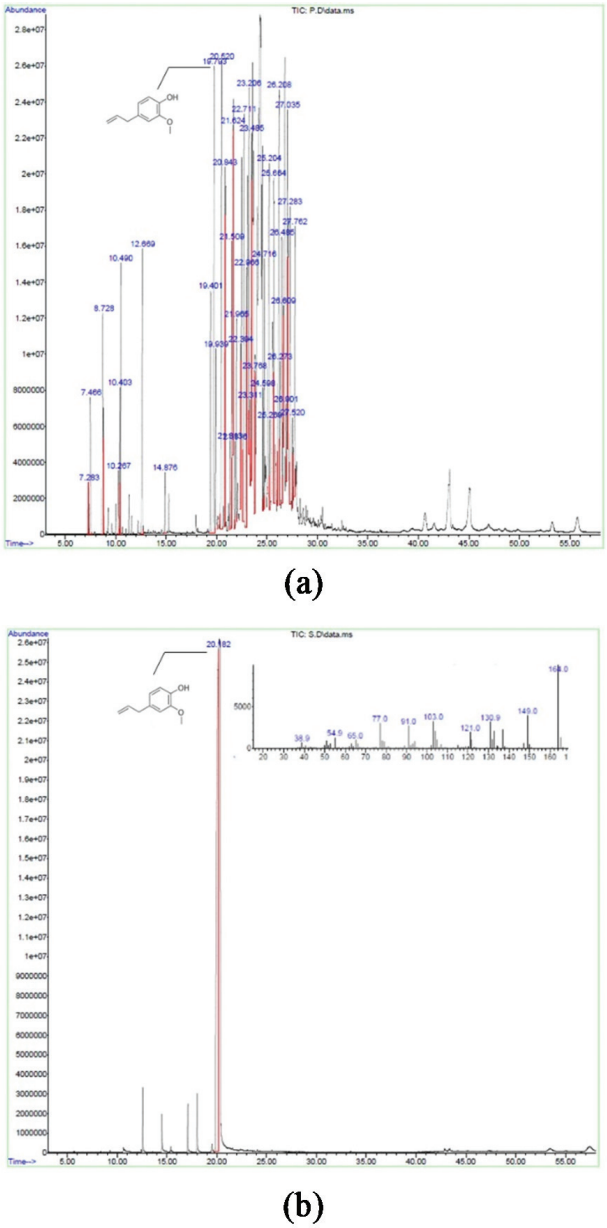

Figure 3. The chromatogram shows eugenol in *P. cubeba* essential oil at 19.99 min (**a**), compared to the eugenol standard, which shows a peak at 20.18 min (**b**), based on GC-MS analysis.

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Table 2. Nanoemulsion characterization results.

Characters	Results
Globule size (nm)	13.3 ± 0.45
Polydispersity index (PDI)	0.234 ± 0.01
Zeta Potential (mV)	-8.92 ± 0.80

formance without any impairment. The travel time of the NPCEO groups was equivalent to that of the citicoline and normal control groups, showing that NPCEO administration effectively restored the rats' memory to near-normal levels and improved cognitive function impaired by scopolamine. The NPCEO groups showed significantly shorter travel times ($p < 0.0001$) compared to the negative control, especially at a dose of 100 mg/kg.

The NORT test yielded a negative score for the scopolamine-induced control rats (Fig. 4b). Scopolamine causes impairments in memory and cognition, which prevent animals from exhibiting a preference for new objects that differ from their familiar surroundings. Consistent with the findings for the normal control and citicoline groups, the animals in the NPCEO group interacted with new objects longer than with familiar ones. The 100 mg/kg NPCEO group had the highest NORT score, demonstrating the ability to prevent scopolamine-induced memory impairment.

Effect of nanoemulsion *P. cubeba* essential oil on motor activity and anxiety

According to the results of the OFT and HB assessments, anxiety and exploratory behavior were also affected by scopolamine-induced cognitive impairments. Increased grooming indicates an elevated stress response in negative controls. This group also displayed significant anxiety behavior, with a low number of rearings and a low frequency of crossings (Fig. 5a). Administration of NPCEO showed reduced anxiety, as indicated by shorter grooming time than the negative control ($p<0.0001$). Meanwhile, the increase in rearing and head-dip frequencies in the group receiving NPCEO indicated healthy exploratory behavior (Fig. 5b). These results suggest that NPCEO has a positive effect on overcoming behavioral disorders caused by scopolamine. Brain biochemistry tests were carried out to further understand the mechanism of action underlying the behavioral improvements.

Effect of nanoemulsion *P. cubeba* essential oil on brain biochemical parameters

Based on the study's findings, the administration of NPCEO to scopolamine-induced rats had a significant neuroprotective impact on neuroinflammation. The treat-

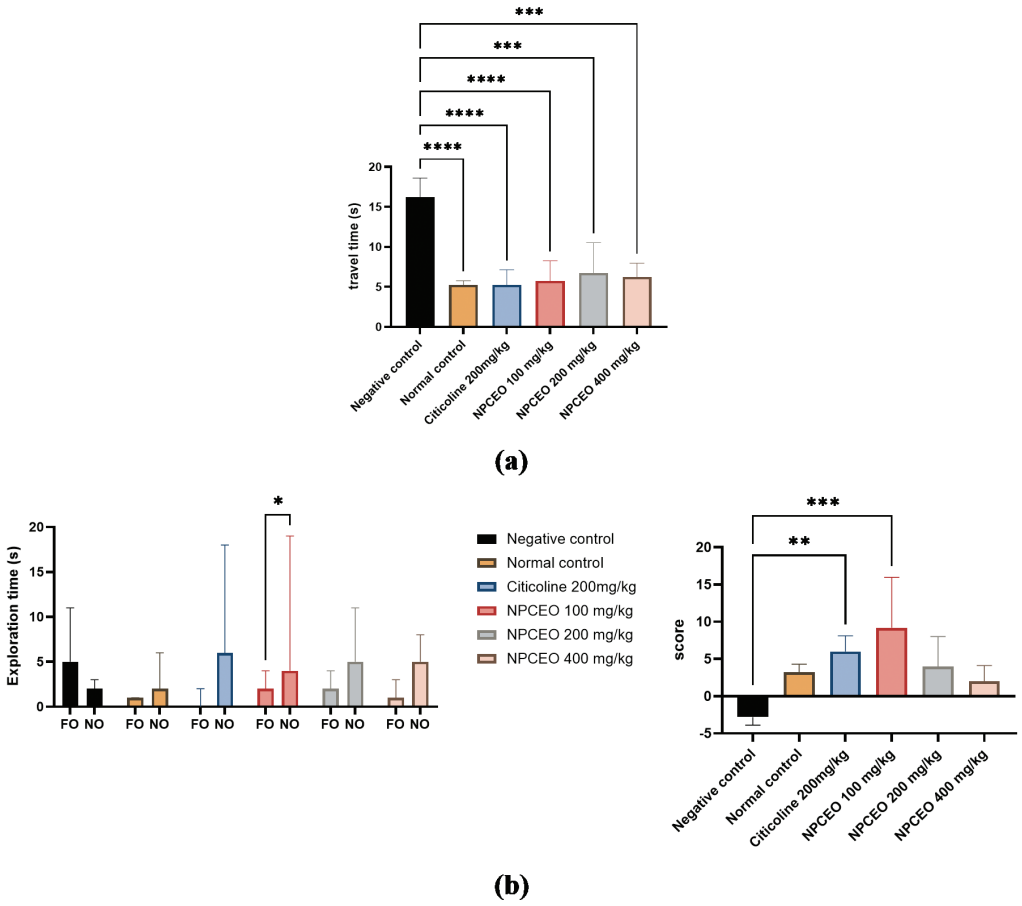

Figure 4. Effect of nanoemulsion P. cubeba essential oil (NPCEO) administration on: (**a**) travel time in scopolamine-induced animals using the WEM method, and (**b**) Novel Object Recognition Test (NORT), including familiar object (FO) and novel object (NO). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

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ment of scopolamine resulted in a considerable increase in TNF-α and IL-1β levels in the hippocampus of the negative control group, suggesting neuroinflammation in this area. On the other hand, NPCEO treatment reduced inflammatory cytokine levels in the hippocampal areas, particularly TNF-α levels (Fig. 6a), while IL-1β was only significantly reduced in the 100 mg/kg NPCEO group (Fig. 6b). There was a slight increase in IL-10 levels in the NPCEO 200 mg/kg group, indicating an anti-inflammatory effect, although the difference was not statistically significant (Fig. 6c).

Fig. 7a shows that scopolamine administration significantly increased AChE activity in the negative control group. On the other hand, NPCEO groups showed lower AChE activity, especially at a dose of 400 mg/kg ($P < 0.05$). The decreased AChE activity indicates that NPCEO could increase acetylcholine levels in the brain, contributing to improved cognitive function. However, NPCEO did not show satisfactory results regarding BDNF and Nrf2 levels. The 400 mg/kg NPCEO group showed slightly higher BDNF and Nrf2 levels in the hippocampus than the negative control, and these were comparable to the normal and citicoline groups (Fig.7b, c).

Fig. 8a showed that NPCEO could increase CAT activity in the hippocampus and cortex. Increased CAT activity due to NPCEO administration was mainly observed in the hippocampus, with the best results in the 400 mg/kg dose group. On the other hand, CAT activity in the cortex increased significantly at a dose of 100 mg/kg. This result

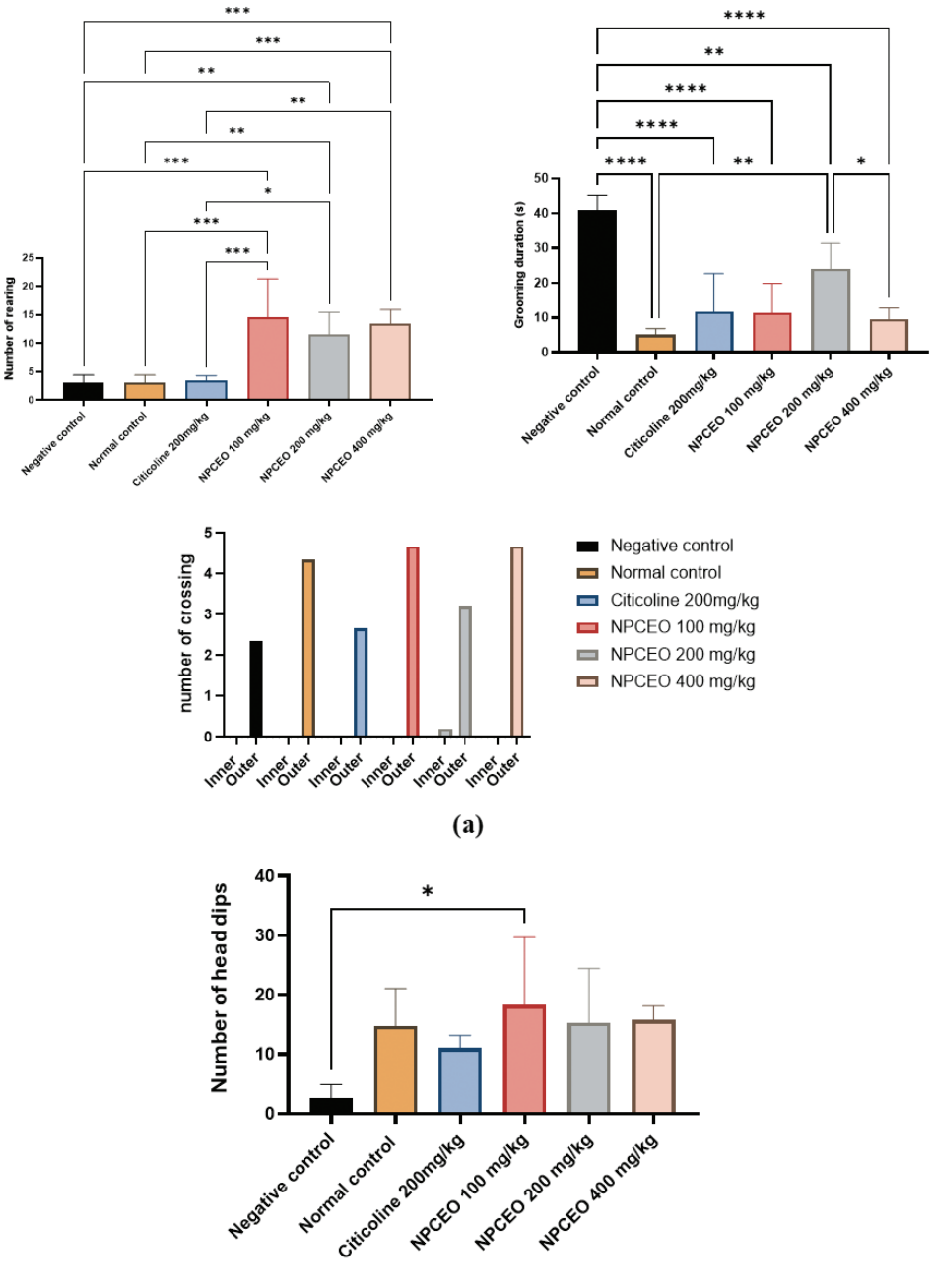

Figure 5. Effect of administering *P. cubeba* essential oil nanoemulsion (NPCEO) on scopolamine-induced rat anxiety using (**a**) open field test (OFT) and (**b**) hole board (HB) test methods. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

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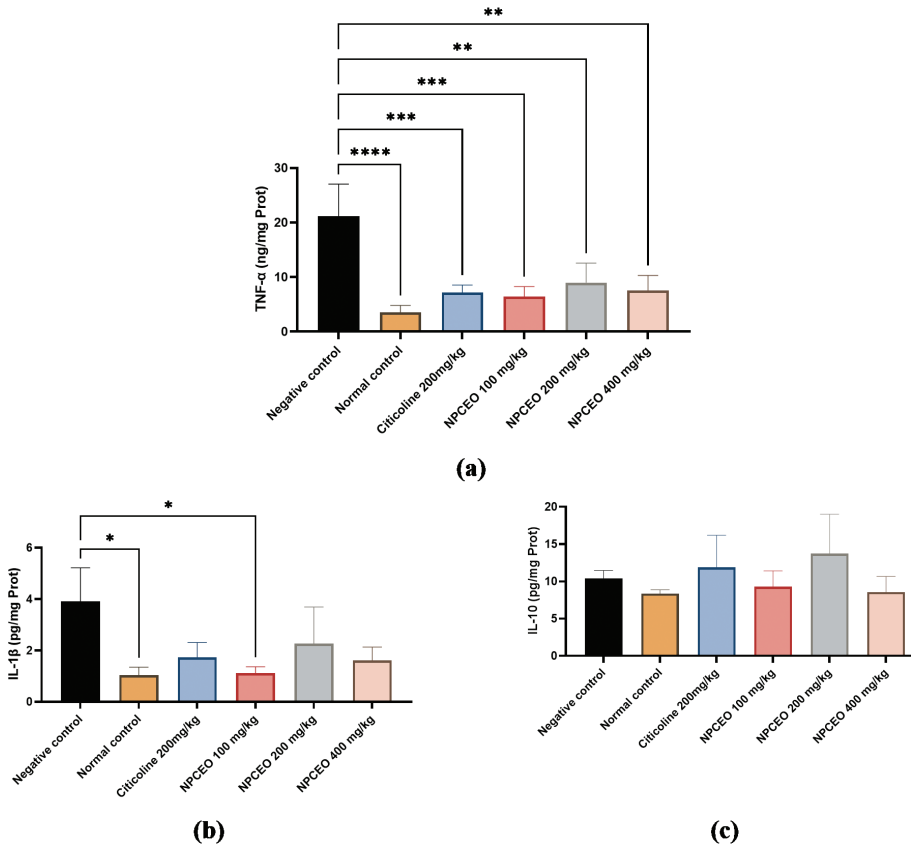

Figure 6. Effect of nanoemulsion *P. cubeba* essential oil (NPCEO) on levels of (**a**) TNF-α, (**b**) IL-1β, and (**c**) IL-10 in the hippocampus of scopolamine-induced rats. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

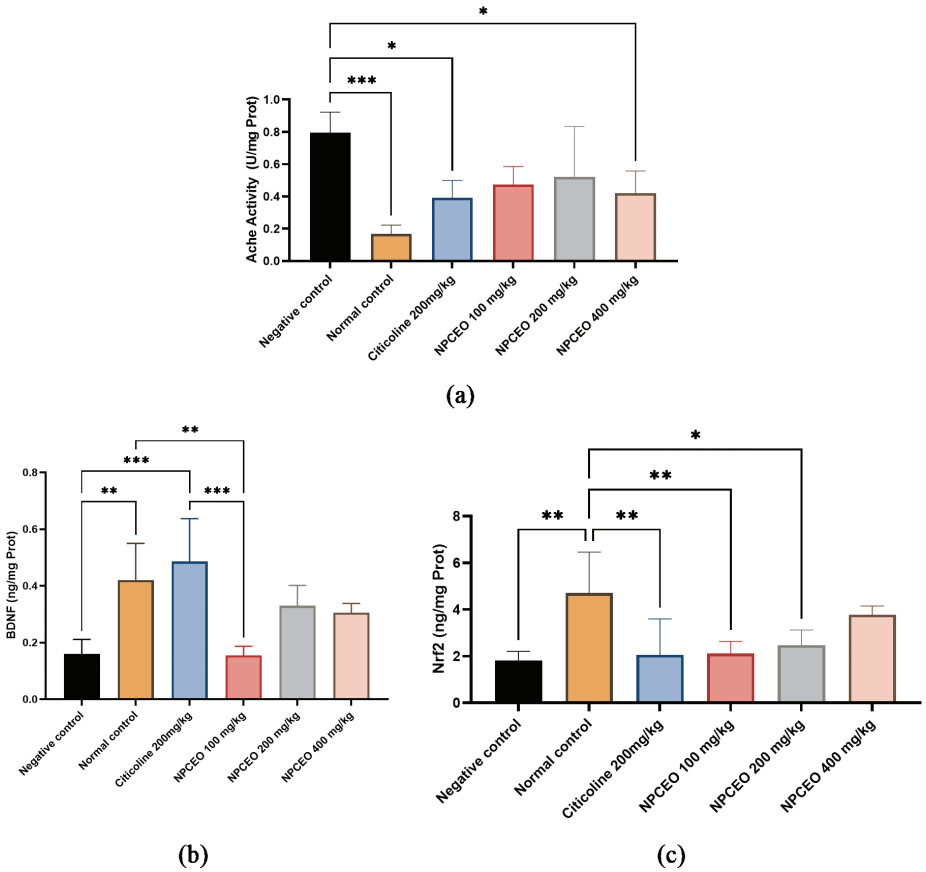

Figure 7. Effect of *P. cubeba* essential oil nanoemulsion administration on (**a**) AChE activity, (**b**) BDNF levels, and (**c**) Nrf2 levels in the hippocampus of scopolamine-induced rats. *p < 0.05, **p < 0.01, ***p < 0.001.

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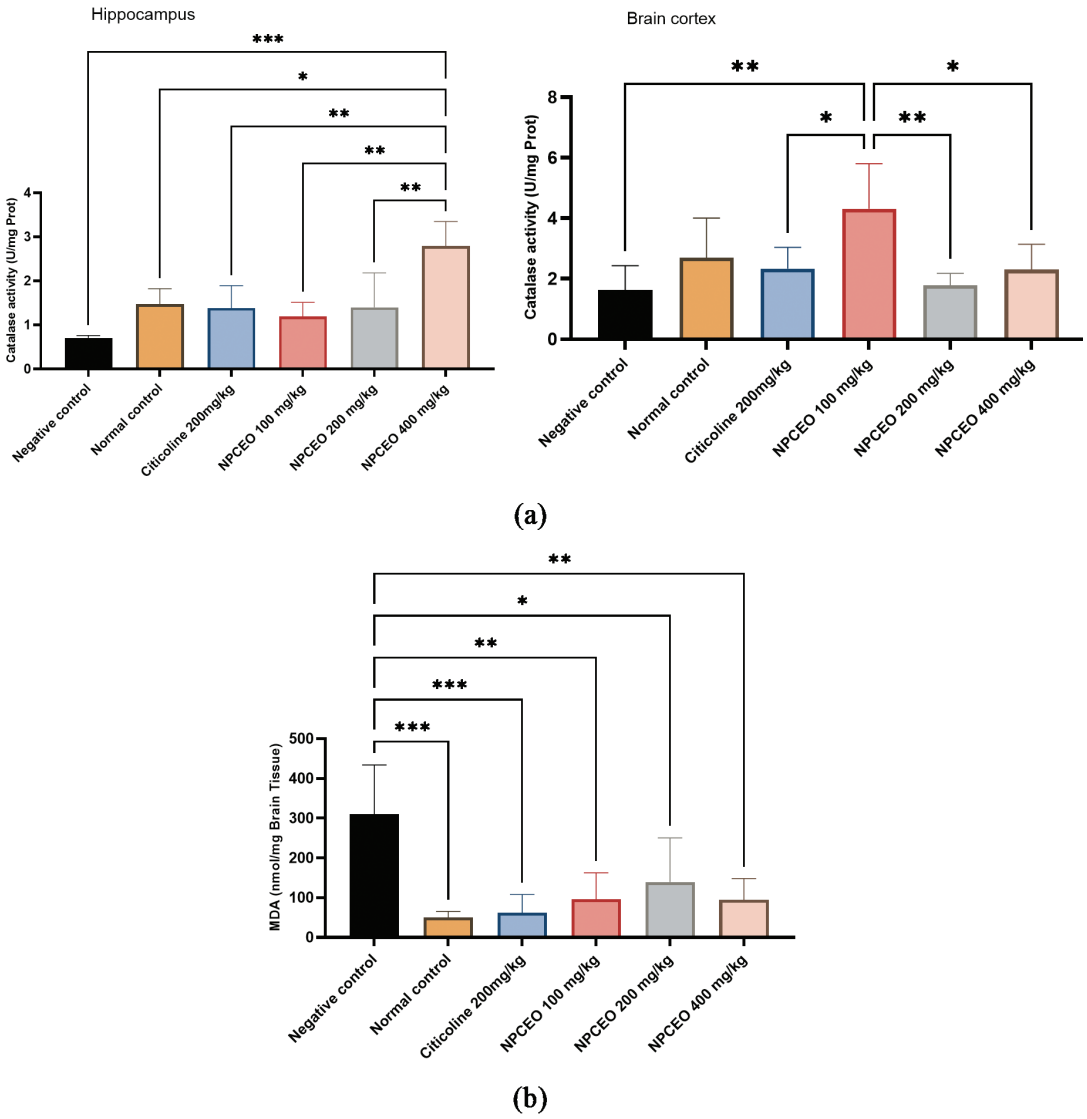

Figure 8. Effect of administering *P. cubeba* essential oil nanoemulsion on (**a**) catalase activity in the hippocampus and cortex, and (**b**) inhibition of cortical lipid peroxidase in scopolamine-induced rats. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

was confirmed by a significant decrease in MDA levels in the brain (Fig. 8b), indicating that all three doses of NPCEO could inhibit lipid peroxidation in the brain.

Discussion

There is great interest in studying natural compounds as possible treatments for Alzheimer's disease (AD). The hallmark cognitive impairment of AD is frequently linked to oxidative stress and chronic low-grade inflammation, which has led researchers to investigate these two targets as potential preventive or therapeutic measures (Behl and Moosmann 2002). *P. cubeba* is a natural ingredient with potent antioxidant and anti-inflammatory properties (Choi and Hwang 2003). Our initial findings suggest that *P. cubeba* essential oil may have neuroprotective properties, as evidenced by elevated antioxidant activity in rats' brains (Dwita et al. 2023a). In this study, *P. cubeba* essential oil was developed as a nanoemulsion preparation and then tested on a scopolamine-induced AD animal model.

The findings demonstrated that NPCEO nanoemulsions could potentially prevent cognitive impairment from scopolamine administration. Rats' cognitive function was evaluated through learning tasks involving spatial memory, memory that involved remembering places, navigating in a directed manner, and orienting in the environment (Pearson-Leary and McNay 2017). The study showed that rats' travel times were significantly high in negative controls, indicating a decline in cognitive function. It is suggested that the rats had poor learning capacities, which may be due to brain tissue damage following scopolamine induction (Klinkenberg and Blokland 2010). Scopolamine acts as an antagonist of muscarinic receptors located in the hippocampus. The hippocampus is a crucial area in processing spatial memory and environmental localization. Acetylcholine plays a critical role in these processes by enhancing synaptic plasticity and the formation of memory traces. When muscarinic receptors in the hippocampus are blocked by scopolamine, communication between neurons is disrupted, affecting long-term potentiation (LTP), which is necessary for memory consolidation. It disrupts the brain's ability

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to form and store new spatial memories (Klinkenberg and Blokland 2010). NPCEO at doses of 100 mg/kg and 200 mg/kg revealed a significant improvement compared to negative controls, showing the potential benefits of NPCEO in improving cognitive function. On the other hand, the NPCEO dose of 400 mg/kg did not show greater improvement than lower doses, indicating that higher doses may not provide additional benefits or may have limited effectiveness.

In addition to the WEM method, animal memory function was assessed using the Novel Object Recognition Test (NORT) (Fig. 4b). The time rats spent with a new object in the NORT test indicates exploratory and object recognition abilities. Normally, rats tend to spend more time exploring new objects than familiar ones. In this study, scopolamine administration caused a negative NORT score in the negative control group, indicating a lower preference for new objects. The result might correlate with the effect of scopolamine that interferes with memory and the ability to recognize previously seen objects (Zhang et al. 2012). In contrast, the NORT score was positive in the treatment group. The administration of NPCEO showed results comparable to those of citicoline and normal controls, where the animals showed a higher preference towards novel objects. NPCEO 100 mg/kg showed the highest NORT score, indicating its cognitive improvement ability.

Furthermore, the administration of NPCEO demonstrated a reduction in anxiety as assessed by the OFT and Hole Board. NPCEO has been shown to enhance motor activity and decrease anxiety in rats, as shown by increased rearing behavior and decreased grooming duration. All groups displayed a strong preference for the outer area and were statistically insignificant between the groups, even though animals receiving NPCEO showed a higher number of crossings than the negative controls. A similar result was observed in the hole board test, with the best activity shown by the NPCEO 100 mg/kg group. Though its efficacy varies with dose, NPCEO appears to be able to ameliorate scopolamine-induced behavioral disorders based on a notable reduction in anxiety symptoms.

Behavioral test results confirmed brain biochemistry. Based on the cytokine examination, NPCEO treatment could reduce neuroinflammation, particularly at a dose of 100 mg/kg. NPCEO markedly decreased TNF-α and IL-1β levels in the hippocampal regions of rats induced by scopolamine. This result is consistent with earlier research demonstrating the anti-inflammatory properties of the essential oil of *P. cubeba* (Shakeel et al. 2019).

In addition, an increase in CAT activity and decreased MDA levels in brain tissue of rats receiving NPCEO indicated that this formulation was effective in reducing oxidative stress. High oxidative stress in the negative control group might be related to the role of Nrf2 as a key regulator of antioxidant responses (Fão et al. 2019). Nrf2 is one of the potential targets for AD since it is the core of maintaining homeostasis in redox balance and inflammation (Murphy and Park 2020). However, in this study, NPCEO administration did not significantly increase Nrf2. A similar result was observed in the BDNF level parameters. Previous research showed a positive correlation between high BDNF levels and cognitive improvement (Lee et al. 2014). Although NPCEO at a higher dose showed increased BDNF levels compared to the negative control, the results were not significant. Nevertheless, NPCEO at a dose of 400 mg/kg could maintain Nrf2 and BDNF levels comparable to normal levels. Linear results were seen in the acetylcholinesterase (AChE) activity. In this study, NPCEO showed a decrease in AChE activity, especially at a dose of 400 mg/kg.

The GC-MS analysis of *P. cubeba* essential oil showed 44.33% eugenol, which is slightly higher than other studies (33.95%) (Alminderej et al. 2020). Eugenol is a phenolic compound with various biological activities, including neuroprotective activity (Barot and Saxena 2021). In addition to the high eugenol content, the GC-MS results of *P. cubeba* essential oil also showed the presence of copaene, linalool, eucalyptol, and caryophyllene, which also have antioxidant and anti-inflammatory potential (Zahin et al. 2018).

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statements

The authors declared that no clinical trials were used in the present study.

The authors declared that no experiments on humans or human tissues were performed for the present study.

The authors declared that no informed consent was obtained from the humans, donors or donors' representatives participating in the study.

Experiments on animals: The experiment has been approved by Prof Dr. Hamka's ethics committee (No: 02/24/01/03034)

The authors declared that no commercially available immortalised human and animal cell lines were used in the present study.

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Author contributions

LPD: Conceptualization, Methodology, Investigation, Writing-Original draft preparation. FKN: Data curation, Supervision, Validation, Visualization. S: Reviewing and Supervision. KR: Reviewing and Supervision. SRA: Investigation, DAP: Investigation, TA: Investigation, SA: Investigation, GSL: Investigation.

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Data availability

All of the data that support the findings of this study are available in the main text.

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