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Centella asiatica Mitigates NMDA Receptor Antagonist-Induced Locomotor Enhancement in Zebrafish (*Danio rerio*)

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ABSTRACT

Introduction: In exploring the complexities of the central nervous system and its disorders, animal models have proven indispensable for replicating human neurological conditions and investigating potential therapeutic interventions. Zebrafish, with their genetic and neurobiological similarities to mammals, have become a valuable model for studying neuroactive substances. This study aimed to assess the effects of *Centella asiatica* on MK-801 induced hyperactivity in zebrafish, bridging the gap between traditional herbal medicine and neuropharmacology.
Methods: An experimental design was employed, utilizing adult zebrafish acclimatized under standard laboratory conditions, divided into three groups: a control group without any treatment, a group exposed to 5 µM of MK-801, and a group treated with 5 µM of MK-801 followed by *Centella asiatica* extract at a concentration of 10 µg/L. Swimming velocity served as the primary measure of locomotor activity, analyzed using Ethovision XT software.
Results: The study found that MK-801 significantly increased swimming velocity in zebrafish, indicative of induced hyperactivity. Conversely, subsequent treatment with Centella asiatica notably reduced this hyperactivity, aligning swimming velocities closer to those observed in the control group.
Conclusion: *Centella asiatica* demonstrated significant potential to mitigate MK-801 induced hyperactivity in zebrafish, underscoring its neuroprotective properties. This research highlights the therapeutic promise of natural compounds in neuropharmacology and the utility of zebrafish as a model organism in neuroscience research. Further investigation into *Centella asiatica*'s mechanisms of action and its application in other models of neurological disorders is warranted.

Keywords: Zebrafish; *Centella asiatica*; MK-801; Neuropharmacology; Hyperactivity

INTRODUCTION

The exploration of the central nervous system's complexities and its associated disorders remains a pivotal challenge in neuroscience. Animal models are essential tools for researchers as they can replicate human neurological conditions, providing valuable insights into the underlying mechanisms and potential therapeutic interventions[1]. Zebrafish (*Danio rerio*) have gained prominence as a valuable model organism for studying the effects of neuroactive substances due to their genetic tractability and neurobiological similarities to mammals. The zebrafish model offers several advantages such as physiological resemblance to mammals, ease of genetic manipulation, sensitivity to pharmacological and genetic factors, robust behavior, low cost, and potential for high-throughput

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screening[2]. These characteristics make zebrafish an attractive model for investigating the impacts of neuroactive substances on the nervous system.

The compound MK-801 (dizocilpine) is a non-competitive antagonist of the N-methyl-D-aspartate (NMDA) receptor, a crucial player in synaptic plasticity and neural communication, with implications in various neurological disorders[3]. MK-801 has been extensively studied in neuropharmacological research due to its impact on synaptic function and its potential therapeutic applications. Studies have shown that MK-801 acts through mechanisms similar to phencyclidine (PCP) in altering phosphorylation patterns in the brain[4]. Additionally, MK-801 has been used as a tool in neuropharmacology due to its ability to modulate NMDA receptors[5].

Research has explored the effects of MK-801 on memory, motor function, and hippocampal plasticity, with prenatal choline supplementation showing potential in mitigating MK-801-induced deficits[6]. Furthermore, MK-801 has been investigated in the context of cognitive dysfunction in schizophrenia models, with studies suggesting that intra-accumbens injections of dopamine aptamers can alleviate MK-801-induced cognitive impairments[7]. MK-801 has also been examined in the context of behavioral changes induced by acute stress, with studies exploring the neuropharmacological differences of various compounds on MK-801-induced neurobehavioral alterations[8]. Furthermore, the compound has been investigated for its effects on visual signal detection task performance and locomotor activity, with vortioxetine showing promise in attenuating MK-801-induced impairments[9].

Despite the insights gained from MK-801 induced models, there is a pressing need to explore interventions that can mitigate or reverse the deleterious effects induced by NMDA receptor blockade. *Centella asiatica*, a medicinal herb known for its cognitive-enhancing and neuroprotective properties, emerges as a candidate of interest. The study by Yeo et al. provides insights into the potential cognitive benefits of *Centella asiatica* extract on learning and memory improvement in adolescent rats[10]. The research suggests that Centella asiatica extract may have a positive impact on cognitive function. Additionally, Diniz et al. explore the wound healing effects and therapeutic potential of *Centella asiatica* and its metabolite Asiaticoside[11]. The study indicates the beneficial properties of *Centella asiatica* that could be relevant in addressing neuroprotective strategies. Historically valued in traditional medicine and increasingly recognized in neuropharmacology, *Centella asiatica* represents a bridge between conventional herbal wisdom and contemporary scientific inquiry. However, the herb's efficacy in counteracting the behavioral alterations induced by MK-801, particularly in models of hyperactivity and neurodegeneration, remains underexplored.

The novelty of this study lies in the intersection of traditional herbal medicine and neuropharmacology, utilizing zebrafish as a model organism to explore the potential moderating effects of *Centella asiatica* on MK-801 induced hyperactivity. Previous studies, such as those by Ariani, have demonstrated that *Centella asiatica* extracts can ameliorate neurological dysfunction induced by deoxygenation in zebrafish larvae through the upregulation of Brain-Derived Neurotrophic Factor (BDNF) and Vesicular Glutamate Transporter 1 (VGLUT1)[12]. Additionally, Gray et al. highlighted the neuroprotective effects of *Centella asiatica* against beta-amyloid toxicity in in vitro models, involving increased mitochondrial activity and improved antioxidant status[13]. Furthermore, recent studies by Yuningsih et al. have shown that *Centella asiatica* can suppress reactive oxygen species (ROS) and protect mitochondrial membranes in zebrafish model[14]. This research aims to expand the dialogue between natural compounds and neuropharmacological agents, offering fresh perspectives on therapeutic strategies for neurological disorders. The primary objective of this investigation is to assess the impact of *Centella asiatica* on the swimming velocity of zebrafish exposed to MK-801. Through this approach, the study aims to contribute to the broader understanding of herbal neuropharmacology and its relevance in addressing complex neurological disorders.

METHOD

Research Design

This study adopted an experimental approach to evaluate the effects of MK-801 and *Centella asiatica* on the locomotor activity of zebrafish (*Danio rerio*). Conducted in a controlled environment, the experiment aimed to observe variations in swimming velocity as a measure of the potential neuroprotective impact of *Centella asiatica* against MK-801 induced alterations in activity levels.

Research Location

The experimental procedures were carried out within the Histology Laboratory and Pharmacology Laboratory at the Faculty of Medicine, Universitas Brawijaya. These facilities were chosen for their specialized equipment and environment conducive to conducting detailed pharmacological and behavioral analyses. The zebrafish used in the study were sourced from the Faculty of Fisheries and Marine Sciences, Universitas Brawijaya, ensuring high-quality specimens for research.

Research Subjects and Sampling Techniques

Adult zebrafish, aged between 5-6 months, were obtained from the Faculty of Fisheries and Marine Sciences, Universitas Brawijaya. Before the commencement of the experiment, the fish underwent a two-week acclimatization period in 50 L tanks under standard conditions (27 ± 2 °C water temperature, pH 7.0-7.2), with a density of up to 5 fish per liter. They were fed twice daily with *TetraBits Complete* fish pellets, ensuring optimal health and nutrition. The zebrafish were randomly divided into eight groups, each consisting of 7 individuals, to facilitate a comprehensive analysis across different treatments: 1) Control group without any treatment, 2) Zebrafish exposed to 5 µM of MK-801, 3) Zebrafish treated with MK-801 followed by Centella asiatica extract at a concentration of 10 µg/L.

Preparation of MK-801 and *Centella Asiatica* Extract

MK-801 was precisely diluted in DMSO and then administered by adding 30 µL of the solution to 200 mL of water for the experimental treatments. For the group designated for MK-801 exposure, zebrafish were placed in 600 mL beakers containing the specified solution for 20 minutes.

The *Centella asiatica* extract was prepared by initially drying the plant material, followed by a solvent extraction process using ethanol, as described in previous studies[13-15]. The extract was then concentrated under reduced pressure to remove the solvent. In this study, to enhance bioavailability and ensure effective delivery, the concentrated extract was formulated into a nanoemulsion. This formulation process involves creating a stable mixture of the extract with surfactants and water, which has been shown to improve absorption and therapeutic efficacy in various models.

The nanoemulsified extract was then diluted to the desired concentration of 10 µg/L in water, a dosage chosen based on previous findings demonstrating its efficacy at lower doses due to improved absorption and distribution[12,16]. For the group receiving *Centella asiatica* post-MK-801 exposure, zebrafish were transferred to a solution of the nanoemulsified extract for an additional 20 minutes before locomotor testing. This approach leverages the enhanced bioavailability provided by the nanoemulsion to maximize the neuroprotective effects *of Centella asiatica*.

Locomotor Activity Data Collection

Locomotor activity was assessed in a 24x8x20 cm aquarium, with a water depth of 15 cm, situated in a darkened enclosure to minimize external visual stimuli. Adequate lighting was provided to facilitate clear video recording. After undergoing treatment, each zebrafish was placed in the testing aquarium. Following a 5-minute acclimatization, their activity was recorded for 45 minutes using a high-resolution webcam connected to a laptop. The Ethovision XT software was employed to analyze the video recordings for locomotor activity.

Ethical Clearance

This research was approved by the Health Research Ethic Committee of the Faculty of Medicine, Universitas Brawijaya (approval no. 107/EC/KEPK/05/2023), ensuring compliance with ethical standards for animal welfare throughout the study's duration.

RESULT

Swimming Velocity Analysis

The study aimed to investigate the effects of MK-801 and *Centella asiatica* on the swimming velocity of zebrafish. Three groups were analyzed: a control group, a group exposed to MK-801, and a group treated with MK-801 followed by *Centella asiatica*. The mean swimming velocities for each group were recorded and statistically analyzed to understand the impact of these treatments on zebrafish locomotion. Table 1 showed the velocity swimming of each group.

Table 1. The swimming velocity of zebrafish (cm/s)

Control	MK-801	MK-801 + *Centella asiatica*
7,08	7,74	5,79
5,84	8,9	4,27
5,64	8,3	3,18
7,42	9,9	2,31
4,39	11,59	6,54
3,24	6,43	5,46
5,9	8,3	5,93

The control group exhibited a baseline swimming velocity, with a mean value indicating typical zebrafish activity levels under normal conditions. Upon exposure to MK-801, a significant increase in swimming velocity was observed, suggesting a hyperactive state induced by the treatment. This effect was notably reversed in the group treated with *Centella asiatica* after MK-801 exposure, where the swimming velocity decreased towards the baseline levels observed in the control group. This reduction indicates a potential mitigating effect of *Centella asiatica* on MK-801 induced hyperactivity.

Statistical Analysis

The Shapiro-Wilk test confirmed that the velocity data for all groups passed the normality test, allowing for further analysis through ANOVA. The One-Way ANOVA revealed a significant difference among the mean velocities of the groups, with an F-value of 12.46 and a p-value of 0.0004, indicating significant disparities in swimming velocities due to the treatments.

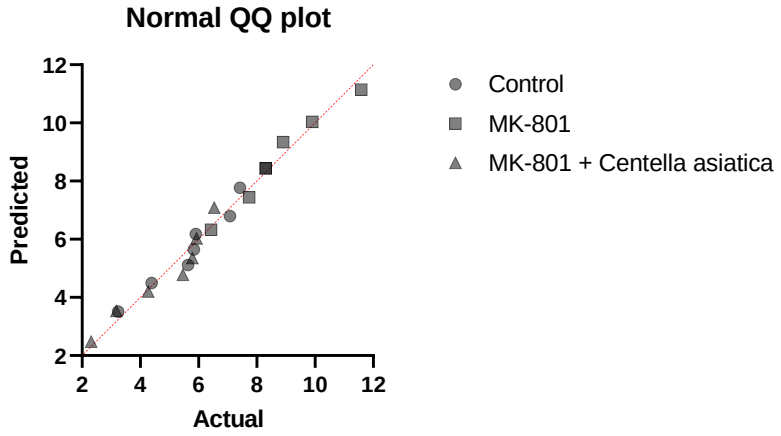

Figure 1. The normality plot of samples

Table 2. One-Way ANOVA Summary

F	12,46
P value	0,0004
P value summary	***
Significant diff. among means (P < 0.05)?	Yes
R squared	0,5807

Further post-hoc analysis using Tukey's multiple comparisons test elucidated the relationships between groups. A significant negative difference was observed between the control and MK-801 groups, highlighting the substantial impact of MK-801 on zebrafish activity levels. Conversely, no significant difference was found between the control group and the group treated with MK-801 followed by *Centella asiatica*, suggesting that *Centella asiatica* treatment effectively neutralizes the hyperactive effect of MK-801. A significant positive difference was noted when comparing MK-801 and MK-801 plus Centella asiatica groups directly, further supporting the moderating role of *Centella asiatica* on MK-801 induced changes.

Table 3. Post-Hoc Tukey HSD Result

Tukey's multiple comparisons test	Mean Diff,	95,00% CI of diff,	Summary	Adjusted P Value
Control vs. MK-801	-3,093	-5,219 to -0,9670	**	0,0043
Control vs. MK-801 + *Centella asiatica*	0,8614	-1,264 to 2,987	ns	0,5656
MK-801 vs. MK-801 + *Centella asiatica*	3,954	1,828 to 6,080	***	0,0005

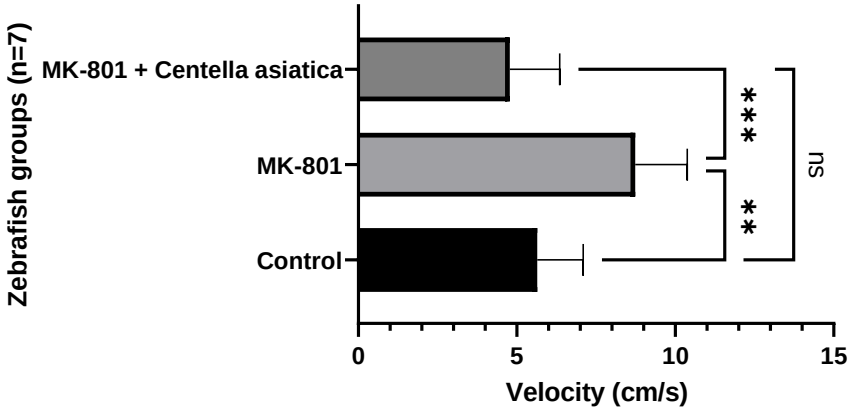

Figure 2. The comparisons of control, MK-801 treated, and MK-801 plus Centella asiatica treated groups.

DISCUSSION

The current study provides valuable insights into the neuropharmacological effects of *Centella asiatica* in mitigating hyperactivity induced by MK-801, a known NMDA receptor antagonist, in zebrafish. Our findings demonstrated that while MK-801 significantly elevated the swimming velocities of zebrafish—an indicator of induced hyperactivity—the subsequent administration of *Centella asiatica* extract notably reduced this hyperactivity towards baseline levels observed in the control group. This effect underscores the potential of *Centella asiatica* as a modulatory agent against disturbances in neural activity caused by NMDA receptor antagonism.

Centella asiatica, a medicinal herb known for its cognitive-enhancing and neuroprotective properties, has been studied for its potential neuropharmacological effects in mitigating hyperactivity induced by MK-801, an NMDA receptor antagonist. Previous research has extensively documented the

cognitive-enhancing and neuroprotective effects of *Centella asiatic*a in mammalian models, suggesting its influence on central nervous system pathways. The study of Gray et al. delve into the phytochemistry and mechanisms of neuroprotection and cognitive enhancement associated with *Centella asiatica*[13]. The study highlights the neurotropic and neuroprotective properties of *Centella asiatica*, which have been linked to triterpene compounds such as asiatic acid, asiaticoside, and madecassoside. These compounds play a crucial role in the cognitive-enhancing and neuroprotective effects of *Centella asiatica*. Furthermore, Matthews et al. investigate the contribution of caffeoylquinic acids in *Centella asiatica* to reversing cognitive deficits in an Alzheimer's disease model[17]. The study sheds light on the potential cognitive benefits of *Centella asiatica* and its constituents in mitigating cognitive dysfunction, emphasizing its neuroprotective properties. Moreover, Hussin et al. explore the regenerative potential of *Centella asiatica* in promoting peripheral nerve regeneration[18]. The study underscores the neuroregenerative properties of *Centella asiatica*, suggesting its efficacy in nerve regeneration and repair. Our observations resonate with these findings, indicating that *Centella asiatica* may exert similar neuromodulatory effects in zebrafish, potentially through mechanisms that restore neurotransmitter balance or receptor function disrupted by MK-801.

The exact mechanisms through which *Centella asiatica* mitigates MK-801 induced hyperactivity remain to be fully elucidated. It is plausible that the active compounds in *Centella asiatica* modulate glutamatergic neurotransmission, counteracting the NMDA receptor blockade by MK-801. This hypothesis is supported by studies indicating that *Centella asiatica* can influence synaptic plasticity and protect neuronal cells from excitotoxic damage[13,17-18]. Puttarak et al. discuss the cognitive-enhancing and neuroprotective effects of *Centella asiatica* in mammalian models, suggesting its influence on central nervous system pathways[19]. The study highlights the potential of *Centella asiatica* to modulate glutamatergic neurotransmission and protect against excitotoxicity, supporting its role in mitigating hyperactivity induced by NMDA receptor antagonism. Moreover, Sbrini et al. demonstrate that *Centella asiatica* phytosome improves cognitive performance by promoting BDNF expression in the rat prefrontal cortex[20]. This finding suggests that *Centella asiatica* may exert its neuromodulatory actions through the upregulation of neurotrophic factors, contributing to its neuroprotective effects. Further research focusing on the molecular and cellular targets of *Centella asiatica* in the context of NMDA receptor antagonism will be crucial in elucidating its precise mechanisms of action. Investigating the specific pathways through which *Centella asiatica* interacts with glutamatergic neurotransmission and synaptic plasticity can provide valuable insights into its potential as a neuromodulatory agent in counteracting hyperactivity induced by NMDA receptor blockade.

Limitations and Directions for Future Research

Our study, while informative, has several limitations that warrant consideration. The use of a single dose for both MK-801 and *Centella asiatica* treatment restricts our understanding of their dose-dependent effects on zebrafish behavior. Future studies employing a range of doses could provide a more nuanced understanding of the therapeutic window and efficacy of *Centella asiatica* in counteracting NMDA receptor antagonist-induced disturbances. Moreover, the study's scope was limited to assessing changes in locomotor activity as a measure of hyperactivity, without delving into the underlying neurobiological changes. Investigating the biochemical and molecular changes associated with *Centella asiatica* treatment, such as alterations in neurotransmitter levels, receptor expression, and signaling pathways, would offer a more comprehensive understanding of its neuroprotective mechanisms.

Given the complexity of neurological disorders involving NMDA receptor dysfunction, extending this line of research to include models of other related conditions, such as Alzheimer's disease, epilepsy, and mood disorders, could elucidate the broader therapeutic potential of *Centella*

asiatica. Additionally, longitudinal studies assessing the long-term effects of *Centella asiatica* treatment on neurobehavioral outcomes would provide valuable insights into its efficacy and safety profile.

CONCLUSION

In this study, we explored the effects of *Centella asiatica* on MK-801 induced hyperactivity in zebrafish, revealing that *Centella asiatica* significantly mitigates this hyperactivity, aligning the treated group's behavior closer to the untreated control group. This finding aligns with existing research on the neuroprotective and cognitive-enhancing effects of *Centella asiatica*, yet extends its implications by demonstrating its potential in counteracting NMDA receptor antagonist-induced disturbances. Despite limitations related to dosing and the focus on locomotor activity, this study underscores the therapeutic promise of *Centella asiatica* against neuropharmacological challenges and highlights the value of the zebrafish model in neuroscience research. These results not only pave the way for more detailed investigations into the dose-response relationship and underlying mechanisms of *Centella asiatica* but also solidify its position as a pivotal agent in the advancement of herbal neuropharmacology. Future studies should delve deeper into optimizing dosing strategies and elucidating the molecular pathways involved, thereby enriching the broader dialogue between herbal medicine and modern neuropharmacological approaches.

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CONFLICT OF INTEREST

In this study, the author discloses no conflicts of interest.

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