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ORIGINAL ARTICLE

Therapeutic Potential of Alkaloid-rich *Mitragyna speciosa* Extract in MPTP-Induced Zebrafish Parkinson's Disease Model

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ABSTRAK

Penyakit parkinson adalah keadaan saraf neurologi yang mempengaruhi fungsi motor otak yang dicirikan oleh degenerasi neuron dopaminergik pada bahagian substantia nigra pars compacta. Gejala utama penyakit ini termasuklah diskinesia, bradikinesia dan gegaran berehat. Kajian ini dijalankan untuk menyiasat potensi kesan kratom terhadap ikan zebra dengan penyakit parkinson yang dihasilkan. 1-methyl-1,2,3,6-tetrahydropyridine (MPTP) diberikan secara intraperitoneal kepada ikan zebra untuk mewujudkan gejala parkinson dalam tempoh 2 hingga 3 hari, diikuti dengan rawatan tiga kepekatan Mitragyna speciosa yang berbeza selama 28 hari. Pada hari ke-28, tingkah laku lokomotor dinilai untuk menentukan tempoh masa yang dihabiskan di tiga zon yang berbeza iaitu zon atas, tengah dan bawah, jumlah jarak perjalanan dan kelajuan berenang. Kemudian, ikan zebra dimatikan dan diawet dalam larutan formalin 10%. Seterusnya, ikan zebra diproses dan dibenamkan dalam blok parafin untuk pewarnaan hematoksilin & eosin dan Cresyl Violet. Keputusan kumpulan rawatan menunjukkan bahawa kratom mempunyai kesan neuroprotektif, meningkatkan masa yang dihabiskan dari zon bawah ke zon atas, jarak perjalanan dan kelajuan berenang berbanding kumpulan negatif. Selain itu, kumpulan rawatan mengalami peningkatan dalam penghasilan semula neuron dan peningkatan dalam penampilan neuron berikutan rawatan selama 28 hari menggunakan kratom. Kesimpulannya, kratom menunjukkan potensi besar sebagai

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rawatan alternatif untuk penyakit parkinson dengan mengurangkan gejala secara berkesan dan meningkatkan pergerakan lokomotor.
Kata kunci: *Anti-radang; ikan zebra; Mitragyna speciosa; MPTP; penyakit parkinson*

ABSTRACT

Parkinson's disease (PD) is a neurological condition affecting the motor functions of the brain, characterised by the degeneration of dopaminergic neurons in the substantia nigra pars compacta region. The primary symptoms include dyskinesia, bradykinesia and resting tremors. This study investigated the potential impacts of *Mitragyna speciosa* or known as kratom on zebrafish with PD. 1-methyl-1,2,3,6-tetrahydropyridine (MPTP) solution was administered intraperitoneally to zebrafish to develop Parkinson's symptoms in 2 to 3 days, followed by three concentrations treatment of kratom, for 28 days. On day 28, locomotor behaviour was evaluated to determine the duration spent in the top, middle, and bottom zones, total distance travelled and swimming speed. Then, the zebrafish were euthanised and preserved in a 10% formalin solution. Fixed zebrafish were processed and embedded in paraffin blocks for haematoxylin and eosin, and cresyl violet staining. The results of treatment groups showed that kratom had a neuroprotective impact, increasing time spent from bottom to top zone, distance travelled and swimming speed compared to the negative group. Moreover, the treatment groups experienced a rise in neuron regeneration and an enhancement in neuron appearance following a 28-day exposure to kratom. In conclusion, kratom shows promise as a potential treatment for PD by effectively reducing symptoms and improving movement.
Keywords: Anti-inflammatory; *Mitragyna speciosa*; MPTP; parkinson's disease; zebrafish

INTRODUCTION

Parkinson's disease (PD) is a progressive neurological disorder that primarily affects movement. It occurs when dopaminergic neurons in the brain, particularly those in the substantia nigra region, degenerate and die. These neurons produce dopamine, a neurotransmitter crucial for regulating movement and coordination. As dopamine levels decrease, symptoms such as tremors, rigidity, bradykinesia (slowness of movement), and postural instability emerge (Armstrong et. al. 2020; Danial Muhammad et. al. 2022). PD is recognised as the second most common neurodegenerative diseases. This disease involves the accumulation of alpha synuclein in the brain, which leads to the progressive loss of neurons and brain function (Shajahan et al. 2024).

In 2020, PD accounted for 1,106 deaths in Malaysia, representing 0.66% of all deaths, with an age-adjusted death rate of 4.52 per 100,000 people (World Life Expectancy 2020). By 2040, PD cases are expected to rise fivefold from 20,000 to 120,000 due to the aging population, posing significant challenges to the healthcare system (Malay Mail 2019). PD is commonly treated with levodopa, which remains the most effective therapeutic option for managing the motor symptoms associated with the disease. However, despite its efficacy in alleviating symptoms, there are concerns regarding its long-term use. Specifically, the oxidative metabolism of levodopa may lead

to the production of reactive oxygen species (ROS), which can potentially exacerbate neuronal degeneration and contribute to the progression of PD. This possibility raises concerns that while levodopa improves symptoms, it may simultaneously accelerate neurodegeneration by increasing oxidative stress (Olanow 2015). Researchers emphasise the need for increased awareness, early diagnosis, and improved treatment to mitigate PD's impacts on individuals and healthcare system.

Although the exact causes of PD are unknown, genetic background and mitochondrial dysfunction are thought to be crucial factors in the disease's pathogenesis through oxidative stress and inflammation (Belarbi et al. 2017). Through a variety of biological pathways, such as elevated glial cell activation and oxidative stress, neuroinflammation is connected to neuronal damage and cell death (Niranjan 2014). In these circumstances, pro-inflammatory and neurotoxic factors are released by activated glial cells, leading to neuronal damage and neurodegeneration (Harry & Kraft 2008).

To resemble PD, neurotoxic drugs such as 6-hydroxydopamine (6-OHDA) and 1-methyl-1,2,3,6-tetrahydropyridine (MPTP) are utilised. MPTP exposure in animal models has been associated to PD-like behaviour, molecular and proteomic features (Omar et al. 2023a). These neurotoxins have been researched for their effects on apoptosis, inflammation, mitochondrial dysfunction, protein breakdown and oxidative stress (Bové & Prier 2012). *Mitragyna speciosa*, sometimes called "ketum" in Malaysia and "kratom" in Thailand, is a Southeast Asian natural tree. It includes around thirty different indole alkaloids, the predominant one being mitragynine (Kruegel & Grundmann 2018). Mitragynine has been shown to inhibit the production of prostaglandin E2 and cyclooxygenase-2 (COX-2), which are typically induced by the pro-inflammatory cytokine interleukin-1 (IL-1). This inhibitory effect suggests that mitragynine may play a role in reducing inflammation by targeting key mediators involved in inflammatory pathways, specifically through the suppression of IL-1-induced pathways (George et al. 2019; Utar et al. 2011). Hence, the aqueous kratom extract has shown promise in reducing pro-inflammatory cytokines and free radicals (Sornsenee et al. 2023).

MATERIALS AND METHODS

Instrumentation

An intraperitoneal injection of MPTP was administered to zebrafish by using a 0.5 ml insulin syringe with a short needle (BD Ultra-Fine™, Gurugram/Gurgaon, Haryana, India). An adult zebrafish's swimming activity was recorded using a digital camera (Canon G7X, Tokyo, Japan). The analysis of the captured videos was performed at Monash University, Malaysia. The statistical analysis was conducted using GraphPad Prism 9.00 for Windows, developed by GraphPad Software in California, USA.

Reagents and Materials

The housing and experimental tanks for zebrafish were filled with facility water that had been filtered. Zebrafish were fed (Sanyu Guppy) as their daily food. MPTP hydrochloride (TargetMol®, Boston, Massachusetts, United States) was obtained from the Institute for Medical Research (IMR), National Institute of Health (NIH). Extracted kratom was obtained from the Forest Research Institute Malaysia (FRIM, Kuala Lumpur, Malaysia).

Kratom Extract Preparation

Leaves of kratom (green-vein var.) were collected from Teluk Intan, Perak, Malaysia. The harvested kratom leaves (508.30 g) were washed with distilled water and oven-dried at 55˚C for 48 hours. The dried leaves were milled into fine powder (77.70 g) using Waring blender and macerated in hot 70% ethanol solution (1500 mL) with continuous stirring (250 rpm) for 20 minutes. After filtration, the aqueous ethanol extract was collected and evaporated until dryness in vacuo at 50˚C to obtain an extract designated as KG-E7 (10.09 g). Then, the chemical compositions (Table 1) of the extract were determined using UV-Vis spectrophotometry methods.

TABLE 1: The chemical compositions of KG-E7

Sample	Total alkaloid content (% ± RSD)	Total protein content (% ± RSD)	Total flavonoid content (% ± RSD)	Total acid soluble-lignin content (% ± RSD)
KG-E7	21.6 ± 1.9	ND	5.2 ± 0.1	5.2 ± 0.3
RSD: Relative standard deviation				

Zebrafish Husbandry

Adult male and female zebrafish of aged four to six months old were purchased at Faculty of Biotechnology & Biomolecular Sciences, University Putra Malaysia (UPM) with an average body weight of 0.9 ± 0.1 g. The zebrafish were acclimated for a minimum of 2 weeks in a 10 L glass fish tank. Both the housing and experimental tanks were filled with facility water that had been filtered. Air bubbles were added to provide aeration, and the water temperature was maintained at 28 ± 2˚C. The fish were fed twice daily at 9:30 and 16:30 and were maintained on a light-dark cycle of 14 hours of light and 10 hours of darkness.

Experimental Design

All zebrafish were divided into five groups: normal (without MPTP and kratom treatment, n = 6), negative (with MPTP-induced but without kratom treatment, n = 6), low dose (LD) (with MPTP-induced and kratom treatment [25 mg/L], n = 6), medium dose (MD) (with MPTP-induced and kratom treatment [50 mg/L], n = 6), and high dose (HD) (with MPTP-induced and kratom treatment [100 mg/L], n = 6) (Figure 1). The fish were fed one hour prior to the commencement of the treatment during the experimental days. The MPTP solution was administered in accordance with the participants' designated group. Swimming behaviour was observed at 0-, 24- and 96-hours post-injection until symptoms resembling PD were observed. Zebrafish that had been treated with MPTP were exposed to three distinct concentrations of kratom, based on their assigned group, after 96 hours. The experiment was conducted over a 40-day period, during which all zebrafish were euthanised promptly following 28 days of exposure to the kratom treatment. The experiments were conducted with the approval of the Research Management Centre (RMC), Animal Care and Use Committee, Management and Science University (MSU) (MSURMC02/FR01/02/L3/020).

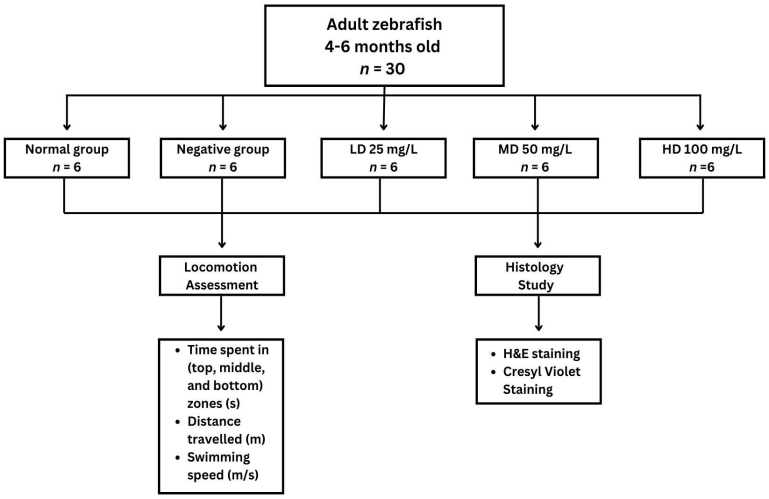

FIGURE 1: Grouping of experimental animals

Ethical Considerations and Sample Size

To obtain the required statistical power, a total of thirty (30) zebrafish were used in the study, with six (6) animals assigned to the normal, negative, and three treatment groups, respectively. The sample size, calculated using a structured formula below was determined to be six animals per group (Naduvilath et al. 2020):

Adjusted sample size =
Sample size / (1 - [% attrition / 100])

MPTP Induced Parkinsonism

A 5 mg quantity of powdered MPTP was diluted in 1 mL of normal saline solution to create a stock solution with a concentration of 5 mg/L. This stock solution was sufficient for inducing MPTP in 24 zebrafish. Prior to delivering MPTP, the zebrafish were rendered unconscious using ice-cold water. The zebrafish were given 40 µL of a prepared MPTP solution through intraperitoneal injection using a 31G needle linked to an insulin syringe. A single administration of 40 µL of MPTP solution comprised 200 µg of MPTP, which can produce Parkinson's symptoms in zebrafish without causing death (Bashirzade et al. 2022). The injection was administered at the abdominal cavity with an angle of 45 degrees. The zebrafish were promptly transferred to a recovery tank and closely observed for any adverse effects or injuries during the recovery period.

Kratom Treatment

To administer the treatment, we produced kratom extract with concentrations of LD (25 mg/L), MD (50 mg/L), and HD (100 mg/L) in 1 litre tanks. The highest dose that can be tested according to OECD Guidelines No. 203 is 100 mg/L. The doses selected for this study (25, 50 and 100 mg/L) aligned with those used in previous zebrafish toxicology studies on natural products, such as Luo and his collaborators in 2024 who investigated *Danggui Shaoyao san* at similar concentrations, supporting their relevance to our model (Luo et al. 2024). Groups of 18 animals were transferred to the

tanks, namely zebrafish that were induced with MPTP. The water treatment was altered on a triweekly basis to uphold the desired treatment concentration. The groups receiving treatments were exposed for a duration of four weeks to examine the progression of PD and evaluate the therapeutic effects of the treatment (Gad 2006).

Locomotion Assessment

Novel Tank Diving Test (NTDT) test was performed to assess site preference behaviour in zebrafish, as described by (Aparna & Patri 2021). The test was conducted to examine the locomotor activity and swimming behaviour of zebrafish. A 3-liter glass tank was utilised, with a white background, employing polystyrene to augment the contrast with the animal throughout testing. A camera was positioned in front of the tank to record the frontal perspective. Each zebrafish was subjected to an individual test. Before the test, the zebrafish were acclimated in the assay tank for five minutes to familiarise themselves with the surroundings. The zebrafish were allowed to swim freely in the tank for a duration of five minutes, during which their swimming conduct was continuously observed and recorded. The videos were stored in the mp4 file type. The zebrafish could only be observed through a monitor screen during the recording session to ensure that the recorder remained hidden from view. The observed behaviours were evaluated using SMART v3.0 - Panlab Harvard Apparatus. The unique tank was digitally partitioned into three horizontal zones: the top, middle and bottom. The study assessed locomotor activity by measuring the amount of time spent in the top, middle and bottom zones (in seconds), the total distance walked (in metres) and the speed within each zone (in metres per second). An assessment was conducted on the swimming pattern in trajectory maps across various groups. Each parameter was examined among the normal group, negative group and therapy groups.

Histology Study

Following a 28-day period of administering kratom, zebrafish were sacrificed by submerging in ice water bath and then were preserved in a 10% formalin solution for a duration of seven days. Brain samples were collected and subsequently treated using the Leica Tissue Processor ASP300S (Wetzlar, Germany), an automated method for preparing tissue samples. The tissues were immersed in paraffin and then cut into cross sections that were 10 m. The forebrain sections were subjected to staining with haematoxylin and eosin (H&E) using an automated stainer (Leica Autostainer XL, Wetzlar, Germany) and subsequently viewed under a microscope (Leica, Wetzlar, Germany) (Onger et al. 2017). The remaining brain tissue sections were stained with cresyl violet manually to facilitate histological assessment and quantification of neuronal damage (Vastegani et al. 2023).

Data Analysis

Locomotion assessment data was updated in the Graph Pad Prism Version 10.0. These data were expressed mean ± structural equation modelling (SEM) and analysed with One-Way Analysis of Variance (ANOVA). In view of multiple group comparisons, Tukey's test was used to compare the mean of each group with the mean of every other group. A p-value of <0.05 was considered statistically significant.

RESULTS

Locomotion Assessment

Figure 2 illustrated the locomotor activity and swimming behaviour of zebrafish under different conditions, including normal control, negative control and treatment groups with different various doses of kratom (25 mg/L, 50 mg/L and 100 mg/L). The negative group showed clear motor impairments, such as a strong preference for the bottom zone of the tank and reduced overall movement, as evidenced by a significant mirror the motor symptoms of PD, demonstrating the neurotoxic effects of MPTP.

In contrast, zebrafish in treatment group displayed marked improvements in locomotor behaviour. Higher doses, particularly 100 mg/L, led to increased exploration of the upper sections of the tank, suggesting reduced anxiety and enhanced well-being. Additionally, kratom significantly boosted overall swimming performance, with noticeable increases in both the distance covered and speed of movement effects observed at higher concentrations. These results highlighted kratom's potential to reverse MPTP-induced motor impairments, restoring to normal functions swimming behaviour and activity levels in zebrafish.

Histological Study

The histological examination, using H&E and cresyl violet staining, revealed key observations on the effects of different treatments on neuronal integrity. In the normal group (Figure 3A), neurons were evenly distributed with distinct nuclei, indicating a healthy neuronal population. In contrast, negative group (Figure 3B) displayed a significant reduction in neuron count and abnormal neuron morphology, reflecting severe neuronal injury and degeneration caused by MPTP's neurotoxic effects, which mimic PD.

Notably, the treatment groups (Figure 3C to 3Z) showed significant improvement in neuronal health. There was an increase in neuron quantity and a more uniform neuronal structure, suggesting enhanced neural regeneration. These findings highlighted kratom's protective and restorative effects on neurons, mitigating the damage caused by MPTP-induced degeneration. Kratom appeared to promote neuronal growth and offer neuroprotection in degenerative conditions.

DISCUSSION

The alkaloid content, particularly mitragynine as displayed in Table 1, presents a higher percentage in the ethanolic extracts derived from kratom. This suggests that this specific compound could play a significant role in slowing neuronal damage, which we believe could be attributed to its anti-inflammatory properties. These properties are potentially expressed through the inhibition of pro-inflammatory cytokines such as IL-1. This hypothesis is supported by research conducted by Tuntiyasawasdikul et al. (2024) and Kafo et al. (2023), both of which demonstrated that ethanolic kratom extracts exert significant anti-inflammatory effects by inhibiting pro-inflammatory mediators. These findings further support the potential of kratom extract as a natural anti-inflammatory agent, suggesting its possible application in the treatment of conditions characterised by inflammation.

Based on the trajectory locomotion pattern in Figure 2A, the normal zebrafish initially spent more time in the bottom zone of the tank but gradually began exploring the upper zones as they acclimated to the new environment (Dos Santos et al. 2020). However, the time spent in the top zone of the negative group

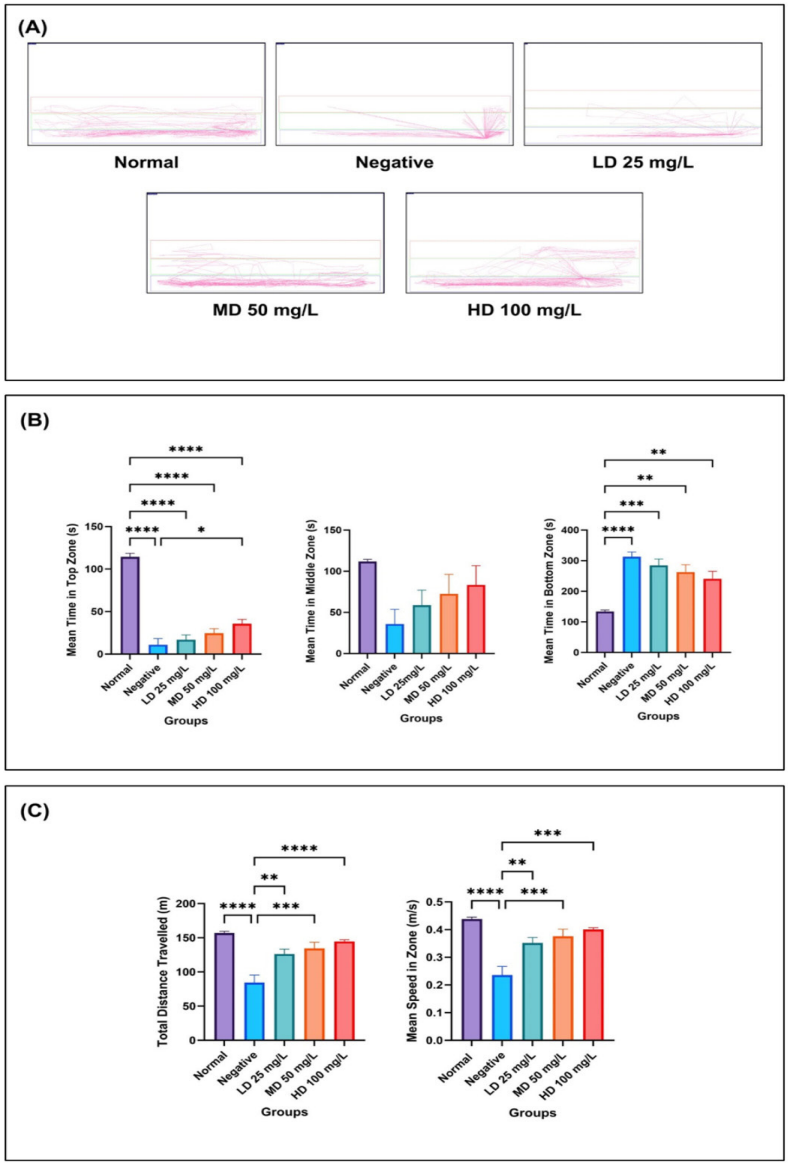

FIGURE 2: Locomotion assessment of the zebrafish models. (A) Representative of locomotion tracking pattern of the normal, negative (MPTP-treated), kratom treatment groups (25, 50, and 100 mg/L); (B) Representative of the time spent in top, middle, and bottom zones by zebrafish in the tank in different groups; (C) Representative of the total distance travelled and speed in zone by zebrafish in the tank in different groups. Values were means ± S.E.M (n = 10). p<0.05 was considered statistically significant. For Tukey's post hoc analyses: (B) Top zone (Normal vs. Negative ****p < 0.0001, Normal vs. LD 25 mg/L ****P < 0.0001, Normal vs. MD 50 mg/L ****P < 0.0001, Normal vs. HD 100 mg/L ****P < 0.0001 and Negative vs. HD 100 mg/L *P 0.0395) and Bottom zone (Normal vs. Negative ****P < 0.0001, Normal vs. LD 25 mg/L ***P 0.0002, Normal vs. MD 50 mg/L **P 0.0012 and Normal vs. HD 100 mg/L **P 0.0073). (C) Total distance (Normal vs. Negative ****P < 0.0001, Negative vs. LD 25 mg/L **P 0.0048, Negative vs. MD 50 mg/L ***P 0.0008 and Negative vs. HD 100 mg/L ****P < 0.0001) and speed in zone (Normal vs. Negative ****P < 0.0001, Negative vs. LD 25 mg/L **P 0.0053, Negative vs. MD 50 mg/L ***P 0.0008 and Negative vs. HD 100 mg/L ***P 0.0001)

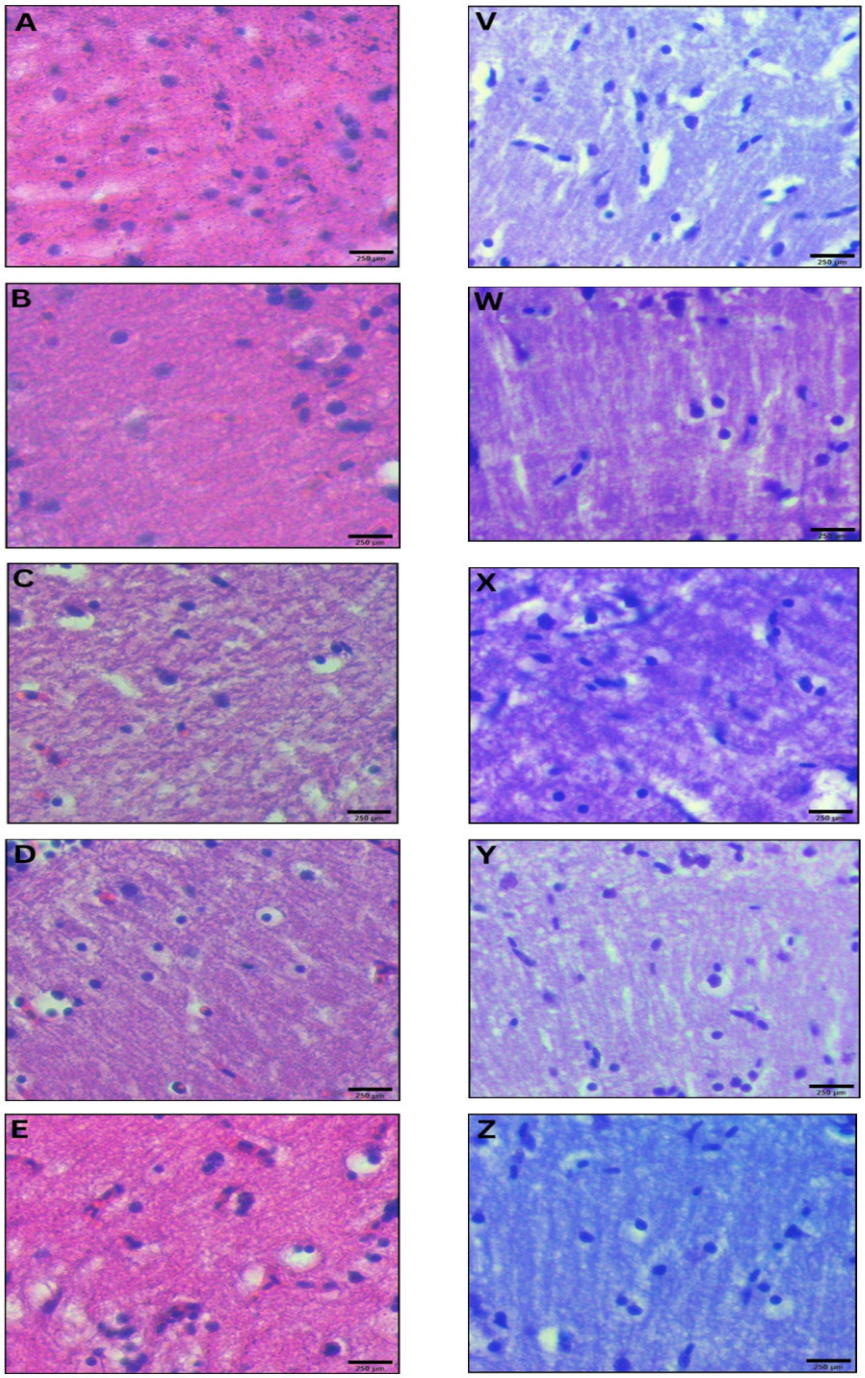

FIGURE 3: Microscopic appearance of ventral diencephalon stained with H & E (left) and cresyl violet (right) in different treated groups. A and V: normal group; B and W: negative (MPTP-treated) group; C and X: LD (25 mg/L) group; D and Y: MD (50 mg/L) group and E and Z: HD (100 mg/L) group

(Figure 2B) was significantly ($P < 0.05$) lower than the HD kratom-treated group, which demonstrated a strong preference for the bottom zone. One of the primary reasons for this preference is the motor deficits exhibited by zebrafish with PD model, showing reduced swimming speed and decreased movement (Razali et al. 2021). According to the findings reported by Bashirzade and his collaborators in 2022, MPTP-treated zebrafish displayed considerable cognitive impairments, a characteristic indicative of the advanced stages of PD. The cognitive deficits observed in these zebrafish models included impairments in learning, memory, and executive function, which are typical symptoms associated with late-stage PD. These behavioural and cognitive dysfunctions mirror the challenges experienced by individuals suffering from advanced Parkinson's, thereby validating the utility of the zebrafish model in exploring cognitive decline and its underlying mechanisms in neurodegenerative diseases (Bashirzade et al. 2022). These motor impairments lead to increased resting time at the bottom of the tank, a hallmark of PD observed in both the fish and human patients (Wang et al. 2017). Another factor contributing to the preference for the bottom zone is anxiety-like behaviour, which is shown by increased time at the tank bottom and in the light compartment during tests, mirroring the anxiety and depression seen in human patients (Najib et al. 2020).

Dopamine depletion, is another key factor in the altered swimming behaviour of zebrafish with PD, contributing to motor deficits and altered swimming patterns due to its role as a crucial neurotransmitter regulating movement (Vaz et al. 2018). Finally, olfactory dysfunction, an early non-motor symptom of PD, contributes the preference for the bottom of the tank, as zebrafish with PD models show reduced olfactory preference for amino acids, which is crucial for their survival and motivation, leading to decreased exploration and more bottom-dwelling behaviour (Nadig et al. 2022). Hence, the kratom treatment can alter this swimming behaviour by reducing motor deficits, anxiety-like behaviour, dopamine depletion and olfactory dysfunction.

Based on the graphs in Figure 2C, the distance travelled, and swimming speed of treatment groups were significantly ($P < 0.05$) higher than the negative group. This indicates that the kratom treatment promotes a restoration of motor function and reduced neurodegeneration. Decreased distance and speed by zebrafish from the negative group can be attributed to the neurotoxic effects of MPTP on dopaminergic neurons in zebrafish, which induces PD-like symptoms. MPTP, a neurotoxin that specifically targets and destroys dopaminergic neurons in the substantia nigra, leading to motor impairments and changes in locomotor activity, has been shown in studies involving zebrafish to induce PD-like symptoms, including slower swimming velocity, shorter distance travelled and longer freezing maintenance compared to normal zebrafish (Omar et al. 2023b).

The swimming velocity of MPTP-injected zebrafish is significantly reduced compared to normal zebrafish, as evidenced by a study finding a 30% decrease in swimming velocity 24 hours post-injection, which remained low 96 hours, indicating the motor impairments characteristic of PD caused by the loss of dopaminergic neurons in the substantia nigra (Razali et al. 2022). Furthermore, the minished nigral dopaminergic neurons in the MPTP-induced zebrafish model cause movement abnormalities, that mimic PD-like dyskinesia and bradykinesia, resulting in the slower swimming speed observed in the MPTP-injected group compared to normal (Ke et al. 2021).

Histopathological analysis of zebrafish brain tissues has yielded significant insights into the processes of neurodegeneration, particularly in the context of PD models. Zebrafish treated with the neurotoxin MPTP exhibited numerous instances of neuronal degeneration (Figure 3B). The findings from this study are consistent with the research conducted by Kalyn and Ekker (2021), where they emphasised that exposure to MPTP, a neurotoxin commonly used to model PD, leads to a pronounced loss of dopaminergic neurons in zebrafish. This significant reduction in dopaminergic neurons highlights the utility of the zebrafish model in replicating key aspects of neurodegeneration observed in PD. As such, the zebrafish model proves to be a valuable system for investigating the mechanisms underlying neurodegeneration, particularly in relation to dopaminergic neuronal loss and its broader implications for neurodegenerative diseases like Parkinson's (Kalyn & Ekker 2021). This degeneration was characterised by the presence of darkly stained cells on the surface of neurons, referred to as Lewy spots (Figure 4A), and within axons (Figure 4B), referred to as Lewy neurites. These findings are critical as they are indicative of oxidative stress, contrasting markedly with observations in the normal group.

The identification of Lewy spots and Lewy neurites in the brain tissues of MPTP-treated zebrafish suggests early pathogenic changes that precede the formation of Lewy bodies. These observations are significant because Lewy neurites are considered an early marker of PD pathology, appearing before the formation of Lewy bodies (Seidel et al. 2015). This early appearance is crucial for understanding the progression of neurodegenerative diseases and provides a window into the initial stages of neural damage. A hallmark of this pathological buildup is the accumulation of misfolded alpha-synuclein protein in neural processes, especially within axons. This misfolding is a critical aspect of PD pathology, leading to the degeneration of neural structures (Bridi & Hirth 2018). The presence of these markers in zebrafish models treated with MPTP underscores the validity of this model for studying PD and other neurodegenerative diseases.

Interestingly, when comparing the MPTP-treated groups to those treated with kratom, a notable reduction in histopathological markers of neurodegeneration was observed.

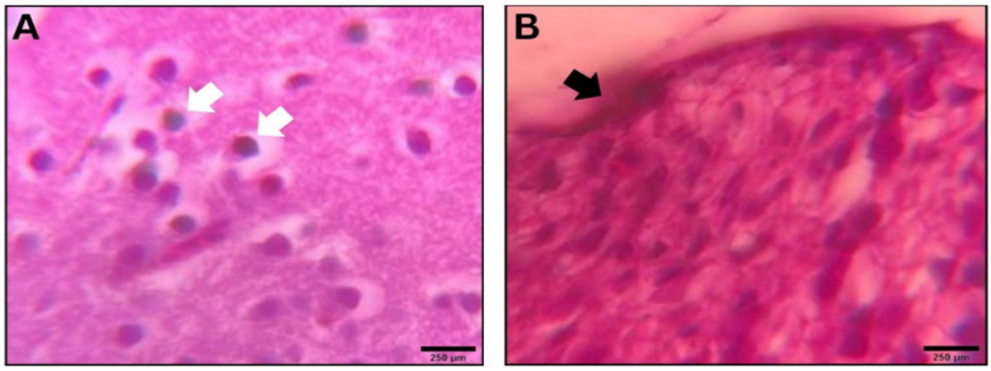

FIGURE 4: Microscopic appearance of neurons in the brain tissues of zebrafish. A: presence of Lewy dots (white arrows) in MPTP-treated zebrafish group. B: presence of Lewy axons (black arrow).

Specifically, the kratom-treated groups showed improved neuronal integrity and a reduced presence of Lewy spots and Lewy neurites. These findings suggest that kratom may have neuroprotective properties that counteract the neurodegenerative processes induced by MPTP. This reduction in histopathological markers indicates that kratom could potentially slow down the progression of neuronal damage and improve overall neural health. The implications of these findings are significant, suggesting that kratom might offer a therapeutic benefit in mitigating the early stages of PD pathology by preserving neuronal integrity and reducing oxidative stress markers.

Figure 5 illustrated neurons filled with numerous vacuoles containing dense granules, a hallmark of granulovacuolar degeneration (GVD) which was found in the negative group. GVD is a distinctive pathological feature frequently observed in the neurons of individuals suffering from various neurodegenerative diseases, including PD (Moda et al. 2023). A study highlights that the presence of neurons with a significant number of such vacuoles is indicative of

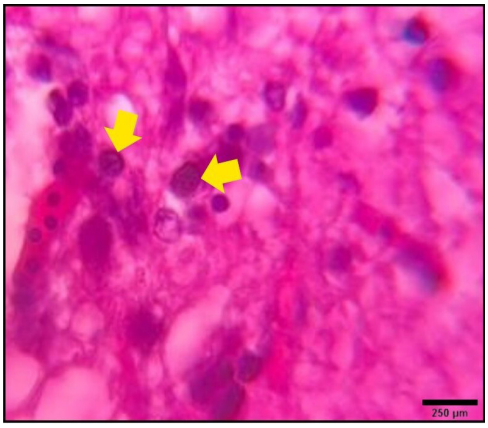

FIGURE 5: Microscopic appearance of neurons in the brain tissues of zebrafish. Yellow arrows indicated the presence of vacuoles and dense granules found in neurons.

GVD, which is integral to understanding the cellular degradation processes in PD (Köhler 2016). The vacuoles observed in GVD are believed to originate from the endosomal-lysosomal pathway, a crucial cellular process involved in the degradation and recycling of cellular components. Typically, these vacuoles contain a dense core granule (Peric & Annaert 2015). The formation of these vacuoles is thought to be a cellular response to various stressors, particularly oxidative stress, which accumulates in neurons over time (Höhn et al. 2017).

Oxidative stress plays a crucial role in the development of PD. It refers to the imbalance between the production of ROS and the cell's ability to detoxify these harmful byproducts or repair the resulting damage. This stress disrupts various cellular processes, leading to neuronal degradation and death, which are key features of PD pathology (Jiang et al. 2016). Oxidative stress escalation can result in mitochondrial malfunction, protein misfolding and compromised autophagy-lysosomal pathways (Bhatia & Sharma 2021). These cellular disruptions ultimately lead to the creation of GVD vacuoles as a compensatory mechanism to isolate damaged organelles and misfolded proteins (Xiong et al. 2013). The existence of GVD vacuoles in PD neurons implies that the cells are trying to deal with the accumulation of stresses and preserve homeostasis (Kumar & Maity 2021). Nevertheless, if the stress continues or the cellular repair processes become unable to cope, the neurons may ultimately undergo apoptosis or necrosis, resulting in the distinct neurodegeneration seen in PD (Puspita et al. 2017).

Mitochondrial dysfunction, a critical consequence of oxidative stress, severely impairs cellular energy production. Mitochondria are the powerhouses of the cell, generating ATP through oxidative

phosphorylation. When oxidative stress damages the mitochondrial membranes and enzymes involved in ATP production, the energy supply of the neuron is compromised. This energy deficit can impede various cellular processes, including protein synthesis and ion homeostasis, further contributing to neuronal dysfunction and death (Muddapu et al. 2020). Protein misfolding is another major issue arising from oxidative stress. Proper protein folding is crucial for maintaining cellular function, and misfolded proteins can aggregate to form toxic species that disrupt cellular integrity. In PD, the accumulation of misfolded alpha-synuclein protein is a hallmark. These aggregates can interfere with cellular functions and promote the formation of Lewy bodies, another pathological feature of PD (Mahul-Mellier et al. 2020).

The autophagy-lysosomal pathway is essential for degrading and recycling cellular components. Oxidative stress can impair this pathway, leading to the accumulation of damaged organelles and proteins within the cell. The formation of GVD vacuoles represents an attempt by neurons to sequester these damaged components and prevent their detrimental effects on cellular function (Huang et al. 2019). However, the persistent stress and impaired cellular repair mechanisms can overwhelm these protective responses. When the accumulation of damage exceeds the cell's capacity for repair and containment, it triggers apoptotic or necrotic pathways. Apoptosis, a programmed cell death mechanism, involves a cascade of molecular events leading to cell death without provoking inflammation. In contrast, necrosis is a form of cell death characterised by cell lysis and inflammation. Both processes contribute to the progressive loss of neurons observed in PD (Callizot et al. 2019).

CONCLUSION

In summary, the study discusses the potential therapeutic effects of kratom on PD symptoms, particularly focusing on its ability to counteract neurodegeneration induced by MPTP in zebrafish models. It highlights that kratom treatment improves locomotor behaviour and neuronal integrity, as evidenced by behavioural assays and histopathological analysis. The findings suggest that kratom possesses anti-inflammatory properties and may have neuroprotective effects, indicating its potential as a treatment for neurodegenerative disorders like PD. Further research on observing alpha-synuclein and pro-inflammatory cytokines through immunohistochemistry, as well as determining the levels of tyrosine hydroxylase, homocysteine, dopamine and serotonin through ELISA tests, is recommended in PD studies using a zebrafish model because these factors are crucial biomarkers involved in PD pathology.

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