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Poliumoside inhibits apoptosis, oxidative stress and neuro-inflammation to prevent intracerebroventricular Streptozotocin-induced cognitive dysfunction in Sprague-Dawley Rats: in *in-vivo*, *in-vitro* and *in-silico* study

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ABSTRACT

Background: Alzheimer's disease (AD) is a severe neurological illness causes cognitive decline and mortality if not treated early. However, the current therapeutic modalities are inefficient to manage the cognitive dysfunction of AD. Therefore, in the present manuscript,

we have enumerated the pharmacological benefit of Poliumoside in the Streptozotocin-induced cognitive dysfunction in Sprague-Dawley (SD) rats.

Materials and methods: Initially, the cognitive dysfunction in rats was induced by the intracerebroventricular administration of Streptozotocin, then rats received PMD (5 mg and 10 mg/kg body weight) was given. Various behavioural analysis, such as Morris water maze (MWM), and object recognition tests (ORT), and locomotor analysis was conducted in PMD treated group. Various biochemical analysis was conducted to analyze the effect of PMD on hippocampus oxidative-nitrosative stress and pro-inflammatory cytokines. MTT assay and annexin V/PI staining was performed to analyse the effect of PMD on the cell viability and neuronal toxicity of PC12 cells, respectively. Molecular docking analysis was also conducted with crystal structure of human AChE.

Results: PMD treatment improved cognitive capacity in rats in MWM and ORT. Compared to STZ rats, PMD-treated rats had significantly higher locomotor activity and lower AChE activity. PMD also restores dopamine, 5-HT, and NE levels and reduces metabolic their deactivation as evidenced by increased levels of DOPAC, HVA, 5-HIAA. Nitrite, MDA, SOD, CAT, and GSH levels were restored near normal in PMD-treated rats, reducing hippocampus oxidative-nitrosative stress. Pro-inflammatory cytokines were similarly lowered in PMD-treated rats. In *in-vitro* studies, PMD did not affect PC12 cell survival at the maximal dose of 10 μM. In addition, PMD concentration-dependently prevents H_2O_2-induced neuronal death in PC12 cells. The in-silico docking analysis showed that the PMD fit snugly into the active site of human AChE by engaging with the anionic domain and the catalytic triad of Trp86, Tyr337, Phe338, and Gly121 residues.

Conclusions: In conclusion, our study demonstrated that PMD have significant impact on AD by inhibiting ACheE and restoring neurotransmitter levels, which enhances Ach levels in rats and improves cognitive impairment in STZ rats.

Keywords: behavioural activity, locomotor activity, neurotransmitter, apoptosis, docking

INTRODUCTION

Alzheimer's disease (AD) is a neurological condition that worsens over time and mainly affects the elderly [31]. It causes significant cognitive deterioration and eventually results in death. The global prevalence of Alzheimer's is projected to increase substantially, driven by aging populations, with estimates suggesting that by 2050, nearly 152 million individuals will be living with the disease [46]. The pathological underpinnings of AD are complex and multifactorial. Hallmark features include the accumulation of amyloid-beta (Aβ) plaques and neurofibrillary tangles composed of hyperphosphorylated tau protein. These pathological entities disrupt neuronal function and integrity, leading to synaptic loss and neuronal death [8]. The precise mechanisms through which Aβ and tau contribute to neurodegeneration remain subjects of intense research, but it is clear that their interaction plays a critical role in the disease's progression. Additionally, chronic neuroinflammation, oxidative stress, mitochondrial dysfunction, and dysregulated calcium homeostasis are implicated in the pathogenesis of AD [11].

Most cases of Alzheimer’s disease are classified as sporadic, with no clear inheritance pattern, and typically manifest after the age of 65. Sporadic Alzheimer’s disease (SAD) contrasts with the rarer familial forms, which are often linked to mutations in genes such as *APP*, *PSEN1*, and *PSEN2*, and usually present earlier in life [8, 46].

Despite advances in our understanding of AD pathology, effective treatments remain elusive. Current pharmacological interventions, including cholinesterase inhibitors and NMDA receptor antagonists, offer only symptomatic relief without altering the course of the disease [14]. The lack of disease-modifying therapies highlights a critical gap in Alzheimer’s research and treatment. Given the increasing prevalence and substantial impact of AD, there is a pressing need for novel therapeutic strategies that can halt or reverse disease progression.

Flavonoids are the class of plant based medicinal compounds that are well known for their medicinal properties [41]. They have a significant impact on a variety of human maladies, such as inflammation and cancer, and they have the potential to alleviate cognitive dysfunction associated with AD [7, 9]. These compounds exhibit potent antioxidant, anti-inflammatory, and neuroprotective properties, making them promising candidates for addressing the complex pathology of Alzheimer’s [18]. Research indicates that flavonoids can improve memory, learning, and overall cognitive function by scavenging free radicals and inhibiting inflammatory pathways. For instance, studies on animals and humans have

demonstrated that regular consumption of flavonoid-rich foods can enhance cognitive performance and slow the progression of cognitive decline [34].

Poliumoside (PMD), a natural phenylpropanoid glycoside that is present in a variety of medicinal plants, demonstrates substantial pharmacological effects [2, 20]. It is recognized for its potent antioxidant and anti-inflammatory properties, which aid in the reduction of oxidative stress and inflammation [16, 25, 43, 45]. However, till now, none of the research has been conducted to define the pharmacological benefit of PMD against cognitive dysfunction. Thus, the present study was conducted to elucidate the effect of PMD on the Streptozotocin-induced cognitive dysfunction in Sprague-Dawley Rats.

Figure 1. Chemical structure of Poliumoside.

MATERIALS AND METHODS

Chemicals

The chemicals utilized in the investigation were acquired from Sigma Aldrich, USA.

In vivo activity

Animal

Male Wistar rats weighing between 220 and 260 grams were acquired from the Institutional animal home and maintained in a highly sanitary environment. The rats were provided with unlimited access to food and water and were housed in a 12-hour alternating cycle of day and

night. The study has been approved the Institutional Ethical Committee of Northern Jiangsu, People's Hospital, China *via* letter no. 2024KY-036.

Intracerebroventricular (ICV) injection of STZ

A surgical incision was made along the midline of the scalp to reveal the bregma after depilating the animal's head. A 5 μL dose of artificial cerebrospinal fluid (ACSF) was given to the sham control group, while the other rat groups were given 5μL of STZ (3 mg/kg) bilaterally using a micro syringe at a rate of 1 μL/min. It was made sure that the syringe was left undisturbed for at least two minutes after the infusion was ended in order to avoid backflow. Antiseptic was applied after the rats were sutured. The animals were given palliative care after surgery. The administration of STZ was repeated on day 3 using the identical protocols [1].

Experimental design

The rats were randomly assigned into five groups (n = 6) as follows:

Group 1: Control (ICV artificial cerebrospinal fluid (ACSF), 5 μL per ventricle on day 1 and day 3).

Group 2: STZ (3 mg/kg, 5 μL), ICV per ventricle on day 1 and day 3.

The rest three groups received PMD in the various dosage as given below. The treatment was provided to the rats one hour after the oral administration of STZ at the specified dose, and continued for a duration of 21 days.

Group 3: STZ (3 mg/kg, 5 μL), ICV per ventricle on day 1 and day 3 + PMD (5 mg/kg body weight) administration for 21 consecutive days.

Group 4: STZ (3 mg/kg, 5 μL), ICV per ventricle on day 1 and day 3 + PMD (10 mg/kg body weight) administration for 21 consecutive days.

Group 5: STZ (3 mg/kg, 5 μL), ICV per ventricle on day 1 and day 3 + Donepezil at 10 mg/kg *p.o.* administration for 21 consecutive days.

A study utilizing the Morris Water Maze (MWM) was conducted on the 22nd day to investigate behavioural patterns. The animals were euthanized using a high dosage of

anesthaesia (thiopental sodium, 80 mg/kg, *i.p.*). Their brains were then extracted and the hippocampus and frontal cortex were separated. These brain regions were kept in a freezer at –80°C until further biochemical examination. The brain samples were pulverized in ice-cold phosphate buffer (pH 7.4) with a high-speed blender for biochemical analysis.

Behavioural evaluation

Morris Water Maze Test. The rats' spatial memory was assessed using the Morris Water Maze (MWM) test, using a previously described protocol. The device consists of a circular water tank with a depth of 50 cm. Skimmed milk powder was added to make the floor imperceptible. The duration spent in the designated quadrant indicated the level of memory consolidation following the initial learning phase [40].

Object Recognition Test. The ORT was conducted according to the previously documented protocol with a wooden open box device. The discrimination index, which measures the capacity of rats to differentiate between a novel and familiar item, was calculated using the formula D = (N – F) / (N + F), where F represents the familiar item and N represents the novel item [1].

Measurement of locomotor activity. The animals were tested and monitored in a 30 × 30 cm^2 square arena for 10 minutes each. A computerized actophotometer and infrared light-sensitive photocells were installed in the arena. The count of animal crossings the laser beam was documented.

Acetylcholinesterase (AChE) activity. The activity of acetylcholinesterase (AChE) was assessed using 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), also known as the Ellman reagent, following the method reported by Ellman et al. with slight adjustments [10].

Quantification of antioxidant biomarkers. The assessment of antioxidant biomarkers, such as nitrite, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione (GSH) was conducted using commercially available ELISA kits (Cayman Kits) in accordance with the instructions provided by the manufacturer.

Enzyme-linked immunosorbent analysis (ELISA). The quantification of tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, and IL-6 was carried out using commercially available ELISA kits (Nanjing Jiancheng Bioengineering Institute, China) following the instructions provided by the manufacturer.

In vitro activity

Cell culture and treatment

The PC12 cells were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA) and were cultured as per the given procedure. The cells were pretreated with various concentrations of PMD (5, 10, and 20 μg/mL) for 24 h and then exposed to H_2O_2 (500 μM) for 4 h.

Cell viability and Fluorescein isothiocyanate (FITC)-annexin/propidium iodide (PI) staining

Cell viability was measured using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as per the previously reported procedure [38]. The apoptotic and necrotic cells were evaluated and quantified by the FITC Annexin V Apoptosis Detection Kit I (BD Bioscience, San Jose, CA, USA) in accordance with the manufacturer's instructions. In brief, after treatment, the cells were washed with ice-cold PBS, collected by centrifugation, and then resuspended in binding buffer. Five microliters of Annexin V-FITC and 5 μL of propidium iodide (PI) were added and incubated for 15 min at room temperature in the dark. Cells were analyzed using flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA, USA). The PMD was used in the concentration of (2.5, 5, and 10 μM).

Molecular docking

The webserver CBDock2 was utilized for the docking of PMD into the active site of 3D crystal structure of recombinant human AChE in complex with donepezil using the default

settings [22, 23]. The interactions were visualized in BIOVIA Discovery Studio Visualizer [27].

Statistical analysis

The data were expressed as the mean ± standard deviation (SD). The statistical analysis was performed using ANOVA followed by a Bonferroni post hoc multiple comparison test using GraphPad Prism 5.0 (San Diego, CA, USA). A P value of less than 0.05 was determined to be statistically significant.

RESULTS

Effect of PMD on the spatial memory (MWM) task

During the MWM test, rats received STZ showed weakened learning skills in both the acquisition and retention trials (Fig. 2). The animals underwent a 5-day training period in the Morris Water Maze (MWM), which began on day 17 after the STZ-administration. However, no significant difference was observed in the means latencies of all group and the day 1 (day 17 after STZ infusion). As expected, the STZ rats had an inability to perform the task on days 18 and 19, as seen by increased latency. Furthermore, in subsequent trials, they did not exhibit substantial learning as compared to the control and donepezil-treated group. Nevertheless, the effects of 5 and 10 mg/kg of PMD were comparable to donepezil and considerably reduced the STZ-induced acquisition deficit.

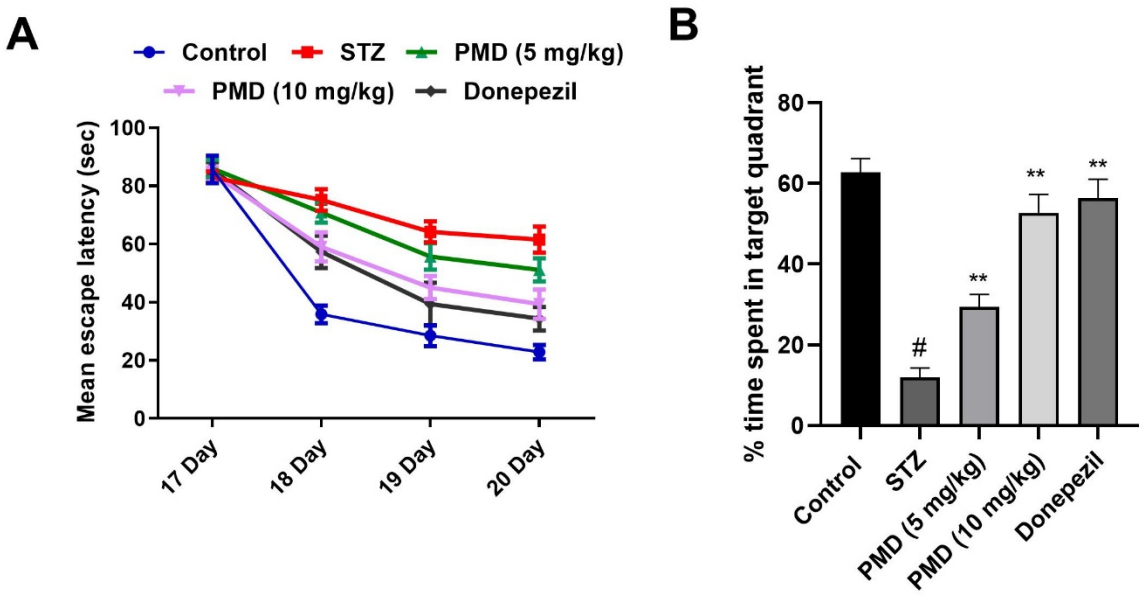

Figure 2. Effect of PMD on the spatial memory deficit using the MWM test. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect of PMD on the non-spatial memory deficit

In object recognition test to identify the effect of PMD on the non-spatial memory deficit, all animals were exposed to similar objects after the 14th day of first STZ administration (Fig. 3). On the 15th day, the animals were then exposed to familiar and new objects. It was found that rats administered with STZ were unable to differentiate between novel and familiar object. However, a rats treated with PMD showed significant ability to segregate between novel and familiar object. These effects of PMD were found almost similar to donepezil as standard.

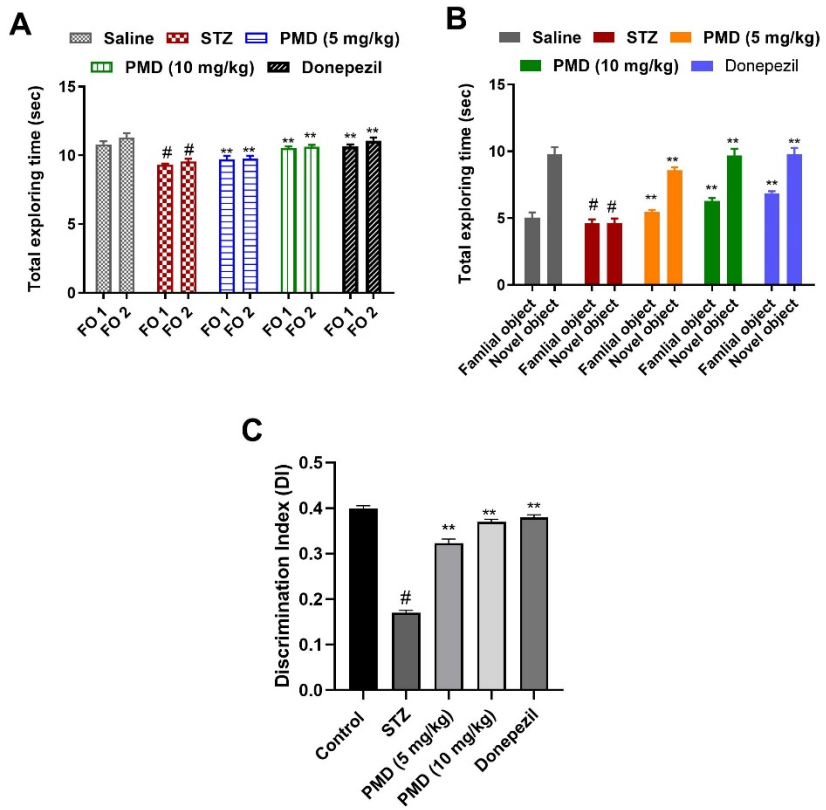

Figure 3. Effect of PMD on the non-spatial memory deficit using the identification of various familial and novel objects. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect of PMD on the locomotor activity

Figure 4 indicates that the rats administered with STZ saw a significant reduction in their locomotor capacity compared to the control group. After the administration of PMD, a significant increase in locomotor activity was seen, which was shown to be equivalent to the normal donepezil. The group that received a dosage of 10 mg/kg of PMD had the most significant improvement in locomotor activity.

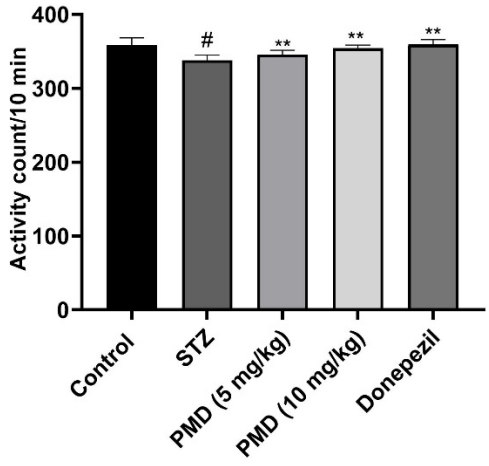

Figure 4. Effect of PMD on the locomotor activity rats. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect of PMD on the STZ induced AChE activity in rats

Hippocampal tissues were extracted from rat brains to investigate the impact of PMD on the amount of AChE activity. According to Figure 5, the level of AChE was considerably higher in the rats treated with STZ compared to the control group. Nevertheless, the administration of PMD resulted in a significant decrease in the level of AChE. These effects were determined to be similar to the effects of donepezil, which is considered the benchmark.

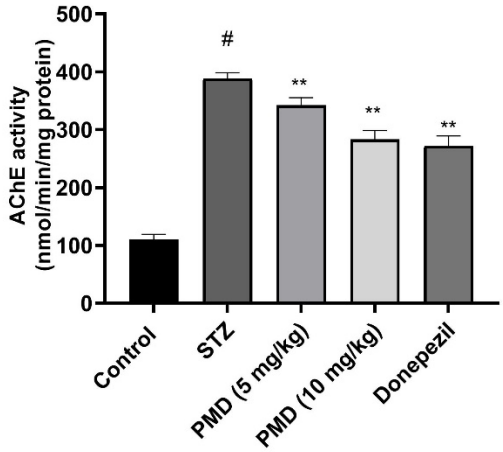

Figure 5. Effect of PMD on the AChE activity. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect of PMD on the neurotransmitter's level

The effect of PMD was investigated on the level of various neurotransmitters such as, dopamine, NE, and 5-HT. As shown in fig. 6, the levels of these neurotransmitters were found significantly reduced in STZ treated rats compared to control. However, the administration of PMD causes significant restoration of these neurotransmitters near to normal.

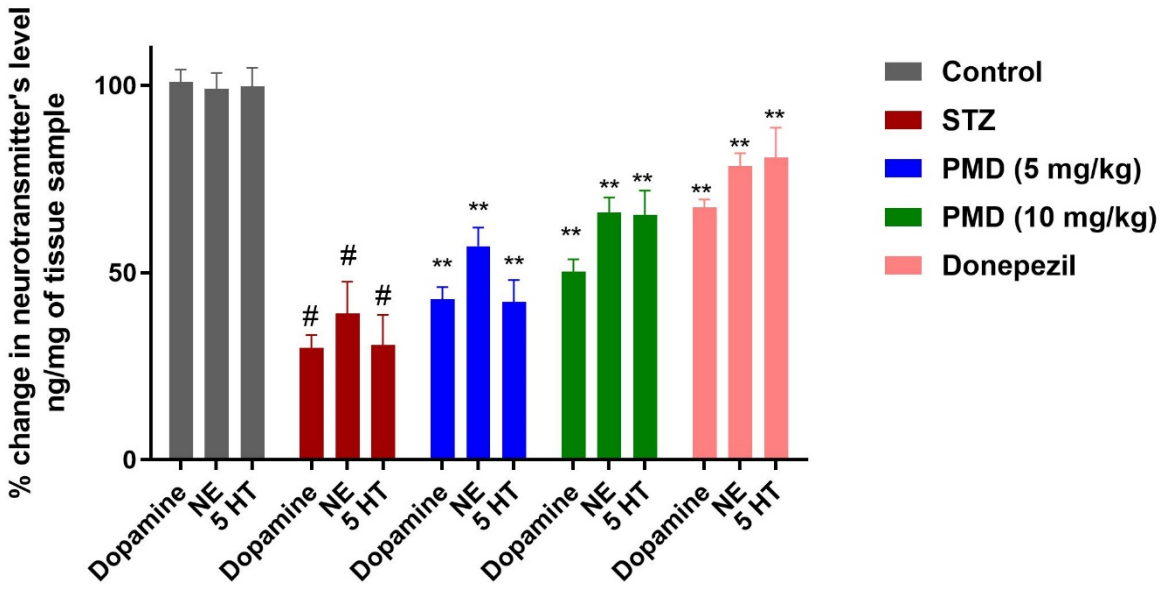

Figure 6. Effect of PMD on the neurotransmitter level of STZ rats. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect of PMD on the neurotransmitter's metabolite levels

The effect of PMD was investigated on the levels of neurotransmitters metabolite (HVA, DOPAC and 5-HIAA) and results are presented in Figure 7. The level of HVA, DOPAC and

5-HIAA was found significantly elevated in the STZ treated rats as compared to control group. However, administration of PMD causes significant reduction these metabolites as compared to STZ rats. The inhibitory pattern was found similar to that of donepezil as standard.

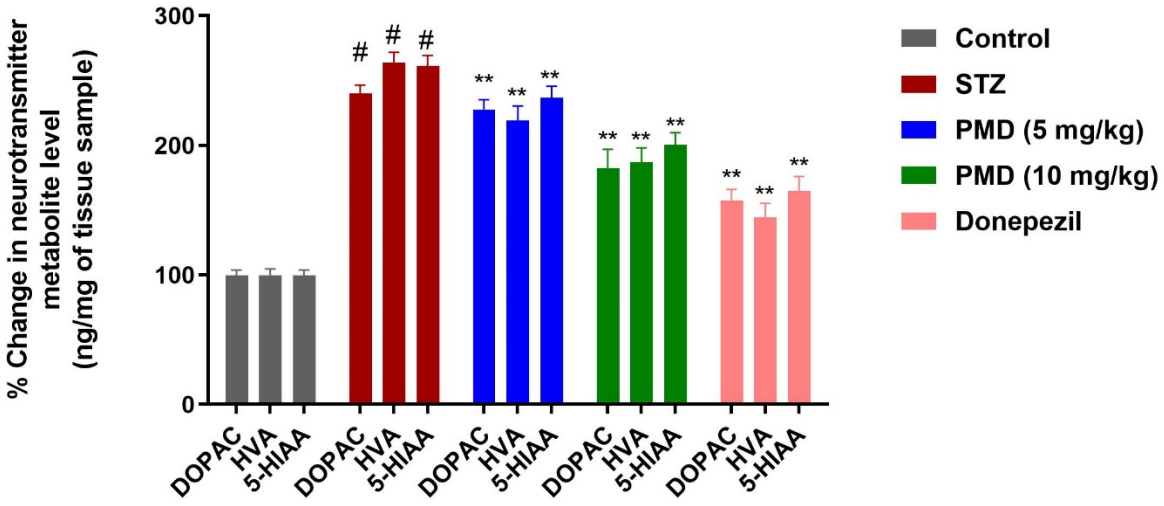

Figure 7. Effect of PMD on the % Change in neurotransmitter metabolite level. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect on the hippocampal oxidative-nitrosative stress

The impact of PMD was further examined on different biomarkers to ascertain its influence on oxidative-nitrosative stress. According to the data presented in Figure 8, there was a considerable increase in the levels of nitrite and MDA, accompanied by a decrease in the levels of SOD, CAT, and GSH. However, the levels of these biomarkers were observed to be greatly restored to a state close to normal, and were shown to be comparable to the standard donepezil.

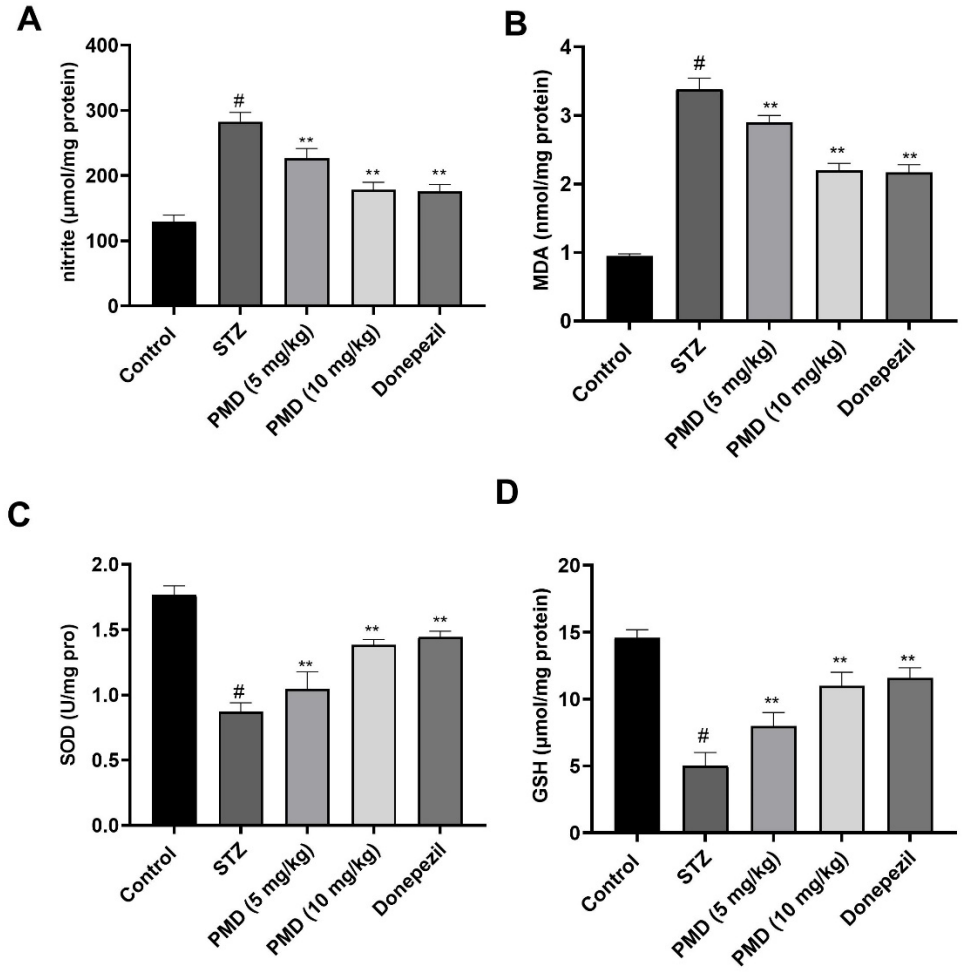

Figure 8. Effect of PMD on the hippocampal oxidative-nitrosative stress. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect of PMD on the hippocampal pro-inflammatory cytokine's levels

The brain hippocampal homogenate was used to ascertain the effect of PMD on the levels of various pro-inflammatory cytokines. As shown in Fig. 9, the level of TNF-α, IL-1β, and IL-6 was found significantly increased in STZ administered rats as compared to control. However, after the administration of PMD, the levels of these cytokines were reduced significantly with maximum reduction activity was achieved in the case of 10 mg/kg treated group. The effect of PMD on the cytokines was found almost similar to donepezil as standard.

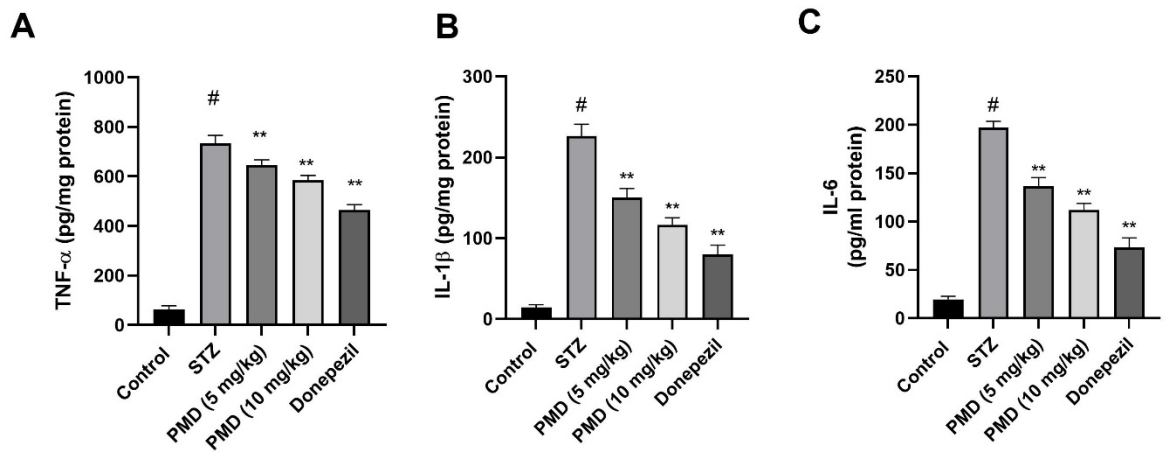

Figure 9. Effect of PMD on the hippocampal pro-inflammatory cytokine's levels. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Effect of PMD on the cell viability and apoptosis

The impact of PMD was also examined on cell viability and apoptosis, and the findings are displayed in Figure 10. It has been found that the viability of PC12 cells were not found appreciably altered at the maximum tested concentration of 10 μM for 24 h. Moreover, the rate of apoptosis of PC12 cells was found significantly induced in the H2O2 treated group as compared to control, however, cells treated with PMD showed significant reduction the rate of apoptosis in the concentration-dependent manner.

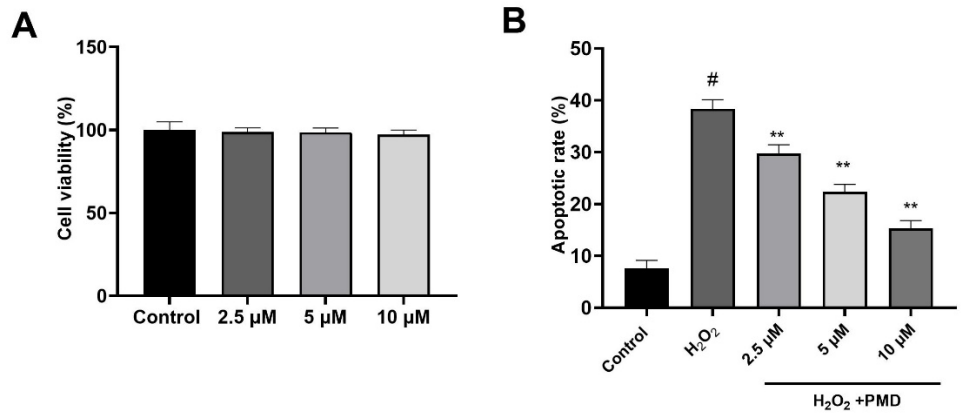

Figure 10. Effect of PMD on the (A) cell viability of PC12 cells and (B) rate of apoptosis of PC12 cells after H_2O_2 neurotoxicity. The results represent the mean ± SEM from triplicate experiments. $^{\#}P < 0.001$ as compared with control. $^{**}P < 0.001$ as compared with STZ.

Docking analysis of PMD with human AChE

The docking process was conducted to assess the binding strength between PMD and the 3D-crystal structure of human AChE. This analysis was performed using the CBDock2 webserver, and it was determined that PMD successfully docked into the active site with a Vina Score of –9.9. This webserver incorporates the curvature-based cavity detection method and the AutoDock Vina-based molecular docking method. Figure 11 displays the docked orientation of PMD, whereas Figure 12 provides a high-resolution 3D representation of same orientation within the entire protein perspective. The illustration in Figure 13 in 2D clearly demonstrates the interaction of PMD. It can be noted that PMD forms hydrogen bonds with Glu292, Glu285, Ser293, Gln291, Phe295, and Tyr341. In addition, it generated many non-covalent contacts, including pi-pi stacking, pi-pi T shaped, and alkyl interactions with His287, Leu289, and Asp286. The docking data unambiguously demonstrated that PMD effectively interacted with the anionic domain and the catalytic triad composed of Trp86, Tyr337, Phe338, and Gly121 residues. These findings align with prior research indicating that PMD can interact with the same residues that regulate the activity of human AChE.

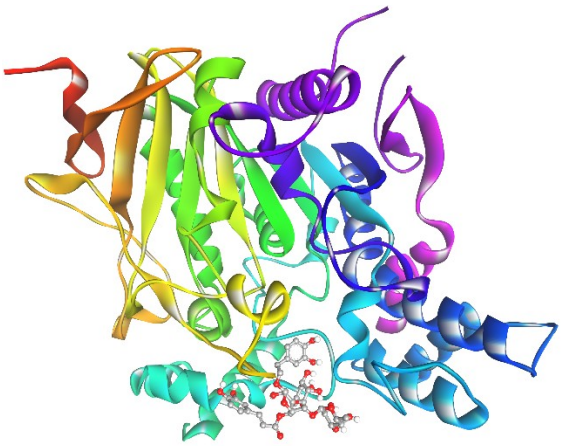

Figure 11. Docked orientation of PMD into the active site of human AChE in whole protein view.

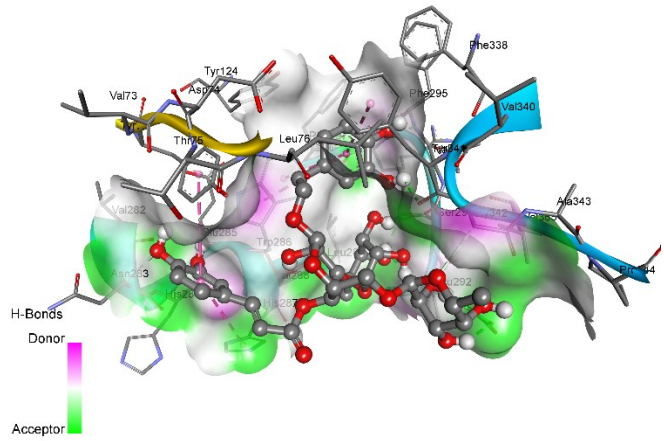

Figure 12. High resolution docked pose of PMD in the active site of human AChE.

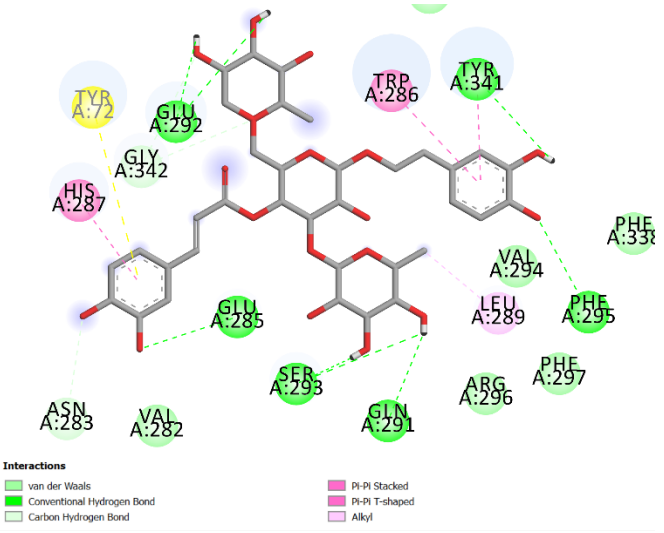

Figure 13. 2D interaction of PMD in the active site of human AChE.

DISCUSSION

The fast aging of China's population is adding to the country's already heavy load from AD [44]. There is a growing strain on healthcare systems and family finances due to the rising prevalence of AD and related cognitive impairment. Lifestyle changes, such as less physical exercise and altered dietary habits, as well as urbanization and an increase in life expectancy, are major contributors to this issue. Also, heredity plays a big role; certain groups are just more predisposed to AD than others. Furthermore, environmental factors including air pollution and limited access to mental health services worsen the disease's impact. Research

on effective treatments and preventative measures, together with efforts to increase public awareness, improve care facilities, and speed diagnosis, are all part of the solution to these problems[15]. Reducing the personal and societal impact of AD requires immediate and effective action. Research indicates that cognitive impairment, characterized by slow but steady deterioration in memory, cognition, and reasoning, is a hallmark of AD. Amnesia, confusion, and diminished capacity to perform everyday tasks are signs of AD, which mainly affects the brain regions accountable for cognitive functioning. Neuronal connections is disrupted and brain cells die as a result of the connection between amyloid plaques and tau tangles. Cognitive impairment worsens with the progression of AD, drastically impacting the patient's standard of living. To slow the cognitive decline associated with this neurodegenerative disease, early diagnosis and treatment are crucial.

Through its multi-target action, the current study demonstrated that PMD ameliorates cognitive impairment in STZ-rats, hence protecting them against AD. At first, we have used the MWM test to look at how PMD affected the spatial memory of rats. This test is essential for studying rats' spatial memory since it evaluates their ability to find a hidden platform in a water labyrinth [33]. The capacity to learn new things, remember what you've learned, and create mental maps are all tested here. This provides important insight into the neural mechanisms that underlie spatial memory, and its replicability and longevity are key to its significance [4]. Since the structures and processes in the hippocampus that are involved for spatial memory are conserved across species, the findings of MWM research performed on rats can be applied to humans with great accuracy. Understanding human cognitive disorders like AD and coming up with new ways to treat them are both made easier by this association [32]. By considerably mitigating the impact of STZ-induced acquisition deficit, PMD considerably enhances the spatial memory of the rats in the current investigation. The ORT test was able to detect problems in non-spatial memory. It is critically important to evaluate cognitive impairment using both familiar and new objects [3, 21]. Crucial for daily tasks, this method evaluates a person's memorization and recognition abilities [17]. PMD significantly improves object identification in rats compared to STZ rats in the present investigation. Additionally, the PMD greatly enhances the locomotor activity of rats.

In Alzheimer's disease, the levels of acetylcholine decrease as a result of the breakdown of acetylcholine by acetylcholinesterase (AChE), which leads to cognitive impairments. Acetylcholinesterase inhibitors (AChEIs) play a vital role in the treatment of AD by inhibiting the degradation of acetylcholine. This action enhances the transmission of

cholinergic signals, leading to improved cognitive function [26, 29]. Donepezil, rivastigmine, and galantamine are inhibitors that reduce symptoms such as memory loss and disorientation in individuals with AD, thereby improving their quality of life [12]. In the present study, PMD showed significant AChE inhibitory which was found comparable to donepezil. Thus, it was suggested that PMD improves the locomotor activity and cognitive dysfunction in STZ rats by preventing the degradation of Ach in rats by inhibiting AChE. Mounting data indicates that neurotransmitter levels are profoundly impacted, leading to cognitive and behavioural problems. Decreased dopamine levels frequently result in apathy and impaired motivation. The drop in norepinephrine (NE) levels is caused by the degeneration of the locus coeruleus, which impacts attention, arousal, and mood control. Depression, anxiety, and sleep difficulties are also caused by a reduction in serotonin (5-HT) levels. The abnormalities of neurotransmitters worsen cognitive impairment and neuropsychiatric symptoms in individuals with AD [30, 35, 37]. Thus, agents restoring these levels have beneficial effect against AD. In this investigation, we have demonstrated that PMD effectively restored the levels of these neurotransmitter near to normal and prevented the breakdown of these neurotransmitters, as indicated by the increased levels of their metabolites (DOPAC, HVA, and 5-HIAA) in rats.

Oxidative-nitrosative stress and pro-inflammatory cytokines are crucial factors in the development of cognitive dysfunction in AD. Oxidative-nitrosative stress occurs when there is an unequal synthesis of reactive oxygen and nitrogen species compared to the body's antioxidant defenses. This imbalance leads to damage and death of neurons. This stress worsens the production of amyloid-beta plaques and the hyperphosphorylation of tau, which are important characteristics of AD pathogenesis [6]. Cytokines with pro-inflammatory properties, such as TNF-α, IL-1β, and IL-6, are produced as a result of the buildup of amyloid-beta and oxidative stress. This leads to the development of a persistent inflammatory state inside the brain. The inflammation exacerbates synaptic dysfunction and neuronal damage, hastening the decrease in cognitive function [5]. Therefore, it is crucial to focus on addressing oxidative-nitrosative stress and inflammation in order to develop successful therapeutic approaches that can delay or prevent cognitive impairment in individuals with AD. The current study demonstrates that PMD effectively decreases oxidative-nitrosative stress by reducing the levels of nitrite and MDA. Additionally, it enhances the enzymatic antioxidant defense system, as indicated by the increased levels of SOD and GSH. It also reduces the generation various pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) near to

normal in STZ rats. Neuronal apoptosis, also known as programmed cell death, leads to cognitive impairment by decreasing the quantity of functioning neurons in the brain. This loss hampers the functioning of brain networks that are crucial for acquiring knowledge, retaining information, and carrying out various mental tasks. Conditions such as AD expedite apoptosis, resulting in substantial cognitive deterioration [13, 24]. Preserving neurons against programmed cell death, known as apoptosis, is a crucial therapeutic approach to reduce cognitive deficits and maintain brain function in older populations [19, 42]. Therefore, in the present study, we have determined the effect of PMD on the H_2O_2 induced apoptosis of PC12, where PMD showed concentration dependent reduction of rate of apoptosis of PC12 cells.

In past few decades, we have witnessed the successful implication of docking analysis in the drug discovery process. It plays a vital role in drug discovery by accurately predicting the interaction between tiny compounds and target proteins. Docking is a process that simulates how a drug fits into the active site of a protein [28]. It is used to find new drug candidates and improve their ability to bind to the protein with high affinity and specificity. This computational technology expedites the process of discovery, decreases expenses, and offers understanding into the molecular underpinnings of medication action [39]. Thus, concerning to this, we have enumerated the binding characteristic of PMD in the active site of AChE using the CBDock2 webserver [23, 36]. It is a is an improved version of the protein-ligand blind docking tool that inherits the curvature-based cavity detection procedure and the AutoDock Vina-based molecular docking procedure in CB-Dock server. Results suggest that PMD was deeply buried into the active site of AChE via creating close-interatomic H-bonds and non-interacting (pi-pi bonds) bonds with residues at the anionic site of AChE.

CONCLUSIONS

In summary, our findings for the first time demonstrated the protective role of PMD on AD by suppressing the action of AcheE and restoring the levels of other neurotransmitters. This leads to an increase in Ach levels in rats, resulting in an improvement in cognitive impairment in rats with STZ-induced AD. The PMD also causes decrease in oxidative-stress, inflammation and neuronal apoptosis which further boosted the effect PMD against the cognitive deficit of AD.

ARTICLE INFORMATION AND DECLARATIONS

Data availability statement

The data will be available from the corresponding author on reasonable request.

Ethics statement

The study has been approved by the Animal Ethical Committee of Liuzhou Traditional Chinese Medicine Hospital (LTCM/AE/2024/02, China.

Author contributions

Yanan Zuo, and **Bineng Chen:** Contributed equally to the work. Both are involved in experiments, data analysis, writing first draft of manuscript. **Xiaokun Li:** data analysis and writing first draft of the manuscript **Guocheng Liu:** conceptualization, supervision, data analysis, final editing and reviewing.

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Conflict of interest

None declared.

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