Effects of Huanglian Jiedu Decoction on Cognitive Function in Mice with Alzheimer's Disease Induced by *Porphyromonas gingivalis* Infection

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Abstract [**Objectives**] To investigate the ameliorative effects of Huanglian Jiedu Decoction (HLJDD) on cognitive function impairment in an Alzheimer's disease (AD) mouse model induced by *Porphyromonas gingivalis* infection. [**Methods**] Thirty-six male C57BL/6 mice were randomly assigned to six groups: control group, model group, low-dose HLJDD group, medium-dose HLJDD group, high-dose HLJDD group, and positive drug group (treated with moxifloxacin). With the exception of the control group, all groups underwent an 8-week *P. gingivalis* chronic infection model induced via oral administration. Subsequently, each treatment group received corresponding doses of HLJDD (2.5, 5, and 10 mg/g) or moxifloxacin for 8 weeks intervention. The novel object recognition test was employed to evaluate the non-spatial memory abilities of mice, and the novel object exploration preference index was calculated to assess cognitive function. [**Results**] Compared to the control group, the novel object exploration preference index of mice in the model group was significantly reduced ($P<0.01$), indicating that *P. gingivalis* infection effectively induced cognitive impairment. Relative to the model group, mice treated with medium and high doses of HLJDD exhibited a significant, dose-dependent increase in the novel object exploration preference index, whereas the low-dose group showed no significant improvement. Additionally, the positive drug moxifloxacin demonstrated a significant neuroprotective effect on cognition. [**Conclusions**] HLJDD effectively improves cognitive function impairment in AD model mice induced by *P. gingivalis* infection, offering novel experimental evidence supporting the heat-clearing and detoxification approach as well as the therapeutic potential of traditional Chinese medicine (TCM) compounds in the intervention of AD.

Key words Huanglian Jiedu Decoction (HLJDD), *Porphyromonas gingivalis*, Alzheimer's disease (AD), Novel object recognition, Neuroinflammation

1 Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder marked by progressive cognitive impairment and memory decline. Neuroinflammation is considered a central mechanism underlying the pathogenesis and progression of AD[1]. Amyloid-beta (Aβ) and abnormal Tau proteins activate neural cells, inducing the sustained release of inflammatory factors. These inflammatory mediators subsequently facilitate the production and deposition of Aβ, exacerbate pathological phosphorylation of Tau protein, and contribute to synaptic dysfunction and neuronal death[2-3]. Studies have demonstrated that infection with *Porphyromonas gingivalis* can induce neuroinflammation and accelerate the progression of AD[4]. *P. gingivalis* and its virulence factors may enter the bloodstream via compromised periodontal mucosa, traverse the blood-brain barrier, and invade the central nervous system[5]. Within the brain, *P. gingivalis* activates microglia, initiating a sustained neuroinflammatory response, promoting the production of Aβ, and impairing its clearance, ultimately resulting in neuronal damage[6].

According to the theory of traditional Chinese medicine (TCM), the pathogenesis of AD is associated with the "internal accumulation of heat toxins", with *P. gingivalis* considered a "toxic pathogenic factor" invasion[7]. In clinical TCM practice, Huanglian Jiedu Decoction (HLJDD) is frequently employed as a therapeutic agent for AD[8]. HLJDD, which originates from *Waitai Miyao* (*Essential Secrets from Outside the Metropolis*), comprises four bitter and cold herbs: *Coptis chinensis*, *Scutellaria baicalensis*, *Phellodendron amurense*, and *Gardenia jasminoides*. These components collectively exert effects of heat clearance, fire purging, and detoxification[9]. Contemporary pharmacological studies have demonstrated that HLJDD and its principal active components exhibit multiple pharmacological activities, including broad-spectrum antibacterial, anti-inflammatory, antioxidant, and neuroprotective effects[10-11].

This study aimed to establish an AD model in mice through chronic oral infection with *P. gingivalis*, employing the novel object recognition test as the primary behavioral index to assess non-spatial memory. The objective was to elucidate the therapeutic effects of HLJDD on cognitive impairments induced by *P. gingivalis* infection and to investigate its underlying mechanisms, thereby providing a novel experimental foundation for the etiological prevention and treatment of AD by integrating traditional Chinese and

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Western medical approaches.

2 Materials and methods

2.1 Animals and grouping Thirty-six healthy male C57BL/6 mice, aged 8 weeks and weighing (20 ± 2) g, were obtained from Henan SCBS Biotechnology Co., Ltd. (license No.: SYXK G 2020-0005). The animals were housed under standard specific pathogen-free (SPF) conditions, with a 12 h light/dark cycle and *ad libitum* access to food and water. Following a one-week acclimation period, the mice were randomly assigned to six groups ($n = 6$) using a random number table method: control group, model group, low-dose HLJDD group, medium-dose HLJDD group, high-dose HLJDD group, and positive drug group.

2.2 Preparation of HLJDD A total of 54.0 g of *C. chinensis*, 36.0 g of *S. baicalensis*, 36.0 g of *P. amurense*, and 54.0 g of *G. jasminoides* were accurately weighed according to the prescribed ratio, yielding a total weight of 180.0 g. The medicinal materials were soaked in 10 times their volume of distilled water for 30 min, then heated to boiling and decocted for 1.5 h. The resulting liquid was filtered, and the filtrate was collected. The residue was subsequently subjected to two additional extractions by adding 8 and 6 times the volume of distilled water, respectively, each followed by boiling for 1 h and filtration. The three filtrates were combined and concentrated under reduced pressure to achieve a crude drug concentration of 2 g/mL. The final extract was sealed and stored at 4 ℃ for future use. Moxifloxacin was obtained from Aladdin Company, with the production batch No.: B2512338.

2.3 Model establishment and drug administration The *P. gingivalis* strain was cultured under anaerobic conditions at 37 ℃ and subsequently resuspended in sterile PBS to a concentration of 1×10^9 CFU/mL. With the exception of the control group, mice in the experimental groups received 100 μL of the bacterial suspension orally each day for 8 weeks to establish a chronic infection model. The control group was administered an equivalent volume of PBS concurrently. After 8 weeks, the low-dose, medium-dose, and high-dose groups received oral administrations of HLJDD at dosages of 2.5, 5, and 10 mg/g, respectively, based on the crude drug dose per mouse body weight (mg/g). These dosages were determined according to the adult clinical prescription and the animal-to-human dose conversion formula[12-13]. The positive drug group was administered moxifloxacin via intraperitoneal injection at a dosage of 10 μg/g. The control and model groups received equivalent volumes of normal saline. All groups were treated once daily for 8 consecutive weeks.

2.4 Novel object recognition test The novel object recognition test was performed 24 h following the final administration. The experimental apparatus consisted of an open cube box measuring 40 cm × 40 cm × 40 cm. The entire procedure was conducted in a quiet and dusky environment. After testing each mouse, the box and objects were meticulously cleaned with 75% ethanol to remove any residual odors and prevent interference[2]. The experiment was conducted in three stages: (i) adaptation period (day 1), during which mice were placed in an empty box and allowed to explore freely for 5 min to acclimate to the experimental environment; (ii) training period (day 2), wherein two identical red cylindrical objects (3 cm in diameter) were symmetrically positioned 10 cm from the box walls; the mice faced the box wall, and their exploratory behavior toward the two objects was recorded over a 5-min session; (iii) test period (day 3), in which one red cylinder was replaced with a green cube (side length 3 cm); the procedure from the training period was repeated to record the exploration time and frequency directed toward the novel and familiar objects within 5 min. Exploratory behavior was defined as instances in which a mouse's nose was directed toward an object at a close distance (≤2 cm) or when the mouse engaged in contact behaviors such as sniffing and licking. Physical contact with only the body, without nose orientation, was classified as non-exploratory behavior. The following evaluation parameters were calculated: Recognition index = Time spent exploring novel objects/Total time spent exploring both novel and familiar objects; Object preference = Number of explorations of novel objects/Total number of explorations of both novel and familiar objects.

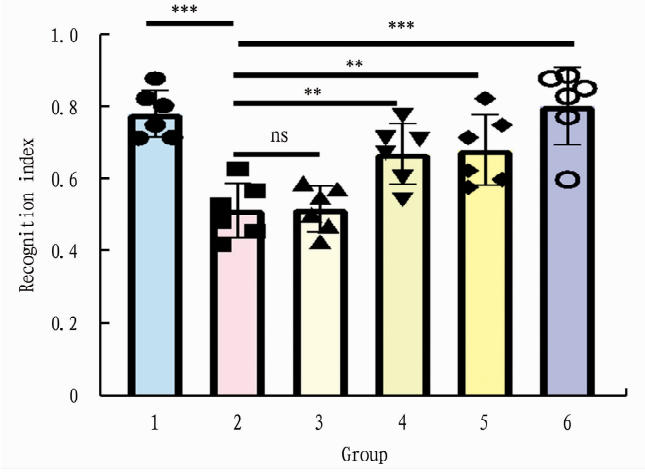
2.5 Statistical analysis All experimental data were presented as mean ± standard error (mean ± SEM). Quantitative and statistical analyses were performed using GraphPad Prism 8.0 software, and results were illustrated graphically. One-way analysis of variance (ANOVA) was employed for comparisons among multiple groups. When variances were homogeneous, unpaired *t*-tests were conducted for statistical analysis. A *P*-value less than 0.05 was considered indicative of statistical significance.

3 Results and analysis

The model validation results demonstrated that, following 8 weeks of oral administration of *P. gingivalis*, mice in all experimental groups except the control group exhibited pronounced redness and swelling of the oral mucosa. Additionally, the abundance of *P. gingivalis* colonies in the oral tissues of these groups was significantly greater than that observed in the control group, thereby confirming the successful establishment of a chronic *P. gingivalis* infection model and an associated periodontal inflammatory state. Furthermore, the novel object recognition test revealed that, compared to the control group, mice in the model group showed a significant reduction in both the recognition index and object preference ($P < 0.01$). Analysis of the exploration paths indicated a marked decrease in the exploration trajectory of novel objects, suggesting that *P. gingivalis* infection effectively induced non-spatial memory impairment in the mice.

As illustrated in Fig. 1, the analysis of the recognition index revealed that mice in the control group demonstrated a significant

preference for exploring novel objects. In contrast, the recognition index of the model group was significantly reduced, indicating impaired memory recognition ability. Compared to the model group, the low-dose HLJDD group did not exhibit a statistically significant difference in the recognition index ($P>0.05$). However, the medium-dose and high-dose HLJDD groups showed a significant, dose-dependent increase in the recognition index ($P<0.01$). Additionally, the positive drug group treated with moxifloxacin displayed a significant cognitive protective effect ($P<0.01$).



NOTE 1. Control group; 2. Model group; 3. Low dose HLJDD group; 4. Medium dose HLJDD group; 5. High dose HLJDD group; 6. Positive drug group; $n=6$; Compared to the model group, ns indicates $P>0.05$, $^{**}P<0.01$, $^{**}P<0.001$. The same below.

Fig. 1 Recognition index results in the novel object recognition test

As illustrated in Fig. 2, the results of object preference aligned with the trend observed in the recognition index. Specifically, the model group exhibited a significant decrease in object preference compared to the control group ($P<0.01$). Conversely, the medium-dose and high-dose HLJDD groups, as well as the moxifloxacin group, demonstrated significantly higher object preference than the model group ($P<0.01$). No significant improvement was observed in the low-dose group. The mouse trajectory mapping (Fig. 3) further confirmed that the exploration trajectories of mice in the model group were predominantly concentrated around familiar objects, with significantly reduced exploration time and path length for novel objects. In contrast, the pathways of mice in the medium-dose and high-dose HLJDD groups, as well as the moxifloxacin group, were significantly biased towards novel objects, exhibiting more diverse exploration trajectories. These findings suggest that cognitive functions were effectively restored. The results presented above consistently demonstrated that medium and high doses of HLJDD significantly enhanced non-spatial memory abilities in AD model mice induced by *P. gingivalis* infection, exhibiting a dose-dependent effect. In contrast, the intervention effect of low doses was not statistically significant.

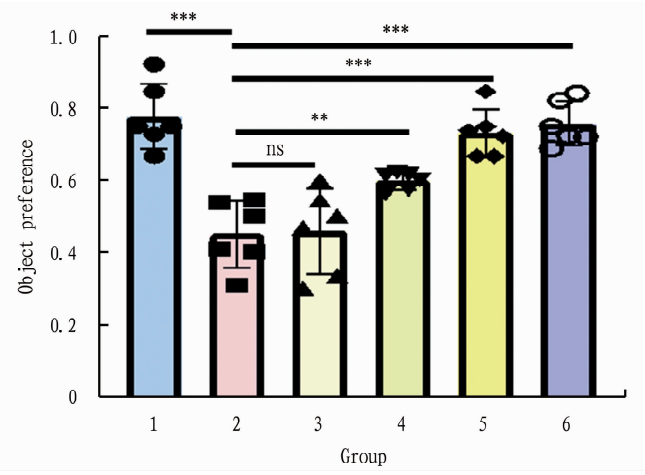


Fig. 2 Object preference results in the novel object recognition test

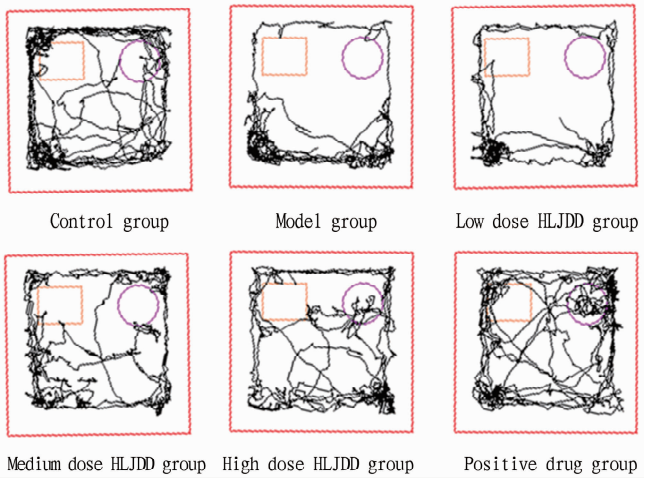


Fig. 3 Mouse trajectory mapping in the novel object recognition test

4 Discussion

This study demonstrated that HLJDD significantly ameliorates cognitive dysfunction in AD model mice induced by *P. gingivalis* infection. These findings provide experimental evidence supporting the use of heat-clearing and detoxifying TCM formulations for the prevention and treatment of AD, and further substantiate the pathological link among "periodontal infection, neuroinflammation, and AD".

This study successfully established a mouse model of AD induced by exogenous *P. gingivalis* infection. Behavioral evaluation demonstrated that *P. gingivalis* infection resulted in significant impairment of non-spatial memory in mice, corroborating findings from previous research[4]. The underlying mechanism is likely associated with the invasion of the central nervous system by *P. gingivalis* and its virulence factors, such as gingipains, which activate the pro-inflammatory phenotypic transformation of microglia and initiate persistent neuroinflammation[14-15]. This inflammatory response disrupts the equilibrium between Aβ production and clearance, promotes abnormal phosphorylation of Tau protein, and establishes a deleterious cycle of "inflammation and Aβ/Tau pathology", ultimately leading

to synaptic dysfunction and cognitive deficits[16].

The improvement effect of HLJDD on cognitive impairment in this model is hypothesized to result from a multi-target synergistic mechanism involving "antibacterial, anti-inflammatory, and neuroprotective actions"[17-18]. Primarily, the broad-spectrum antibacterial properties of HLJDD can reduce the load of *P. gingivalis* in the oral cavity and systemically, thereby decreasing the translocation of its virulence factors to the central nervous system and preventing the initiation of inflammation at its source[10]. Secondly, the primary active components of HLJDD, including berberine and baicalin, modulate the M1/M2 phenotypic polarization of microglia by inhibiting critical signaling pathways such as NF-κB and the NLRP3 inflammasome. This modulation results in the downregulation of pro-inflammatory factor expression and the upregulation of anti-inflammatory factor levels[19-20]. These findings align with recent studies that components of HLJDD can specifically target microglia and ameliorate AD pathology through the regulation of autophagy and inflammatory pathways[4]. Furthermore, the antioxidant properties of HLJDD can mitigate inflammation-induced oxidative stress damage, protect hippocampal neurons and synaptic plasticity, thereby enhancing cognitive function[9]. The significant therapeutic effects observed in the medium-dose and high-dose HLJDD groups in this study further validate the scientific nature of the TCM approach of clearing heat and detoxification in the treatment of AD. These findings also indicate that appropriate dosage control is essential in clinical applications.

This study presents several limitations. First, it exclusively employs novel object recognition tests to assess non-spatial memory. Future research should incorporate a comprehensive evaluation of spatial memory and learning abilities in mice by utilizing additional methods such as the Morris water maze and passive avoidance tests. Second, the study provides only behavioral evidence, and the specific regulatory effects of HLJDD on Aβ deposition, Tau phosphorylation, microglial phenotypes, and the inflammatory factor profile within the brain require validation through subsequent molecular biology and morphological investigations. Third, the synergistic mechanisms underlying the individual components of HLJDD remain insufficiently explored, necessitating further research focused on formula decomposition.

In conclusion, this study demonstrated that HLJDD can dose-dependently ameliorate cognitive dysfunction in an AD mouse model induced by *P. gingivalis* infection. The underlying mechanism may involve the inhibition of neuroinflammation and modulation of microglial activation. These findings offer a novel strategy for the prevention and treatment of AD based on heat-clearing and detoxification principles. Future research will focus on elucidating the molecular mechanisms and optimizing the formulation to facilitate the clinical translation of HLJDD.

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