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RESEARCH ARTICLE OPEN ACCESS

Xixin Decoction May Treat Vascular Dementia by Modulating the NPTX2/C1q/C3 Complement Pathway

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Received: 4 September 2024 | **Revised:** 26 November 2024 | **Accepted:** 30 December 2024

Funding: This study was supported by grants 82405523 from the National Natural Science Foundation of China, grants 2022CFB962 and 2024AFB922 from the Natural Science Foundation of Hubei Province, and grants ZY2023Q025 from the Hubei Provincial Administration of Traditional Chinese Medicine.

Keywords: C1q/C3 | NPTX2 | vascular dementia | Xixin decoction

ABSTRACT

Vascular dementia (VaD) is a type of dementia that results from brain injury caused by cerebrovascular disease or vascular risk factors. Accumulating evidence from clinical studies has found that Xixin decoction can effectively improve the cognitive function of patients with VaD and improve their daily living ability. However, the pathogenesis of VaD is not fully understood, and the therapeutic mechanism of Xixin decoction is also unclear. The Morris water maze, new object recognition, transmission electron microscopy observation, Golgi staining, Nissl staining, Western blotting, and quantitative real-time polymerase chain reaction were employed to explore the therapeutic mechanism of Xixin decoction. The results showed decreased learning and cognitive abilities, hippocampal neuron damage, decreased NPTX2 protein expression, and increased expression of inflammatory factors such as C1q and C3 in the model group compared with the control group. Furthermore, compared with the model group, the above symptoms were improved after administration of Xixin decoction, and the activity of the NPTX2/C1q/C3 complement pathway was altered. In conclusion, these results suggested that Xixin decoction might treat VaD by modulating the NPTX2/C1q/C3 complement pathway.

1 | Introduction

Vascular dementia (VaD), a cognitive disorder resulting from brain damage due to cerebrovascular disease or vascular risk factors [1, 2], poses a significant challenge, particularly in developing countries. In Asia, VaD accounts for up to 30% of such cases in individuals over 65 years old and is second only to Alzheimer's disease in terms of prevalence [3–6]. The aging population has led to a steady increase in the number of patients with VaD in China [7], imposing a substantial social and economic burden. Consequently, the study of VaD prevention and treatment is not only medically important but also holds significant social relevance.

Although the exact pathogenesis of VaD remains elusive, the prevailing hypotheses suggest a strong link to neuroinflammation and oxidative stress, among which cerebral neuroinflammation is one of the important pathological signs of the disease [8, 9]. Cerebral neuroinflammation is characterized by elevated levels of proinflammatory factors, synaptic damage, and the hyperactivation of glial cells [10]. Given that the pathogenesis of VaD is unknown there are no specific drugs available. Accordingly,

studies on the use of drugs for treatment are crucial [11]. Some clinical studies have indicated that the serum levels of inflammatory factors (like interleukin [IL]-6, tumor necrosis factor [TNF]-α, IL-1β, or C-reactive protein) are significantly elevated in patients with VaD, with a marked decrease in these inflammatory indices following anti-VaD treatment [12]. Moreover, experimental studies have found increased levels of inflammatory factors in the VaD model rats, alongside evident neuronal injury [13, 14].

In traditional Chinese medicine, VaD symptoms can be categorized as dementia, forgetfulness, and unwisdom, with the core pathogenesis attributed to phlegm obstructing orifices, blood stasis obstruction, medullary reduction and brain depletion. The ancient text Dialectical Record by Chen Shiduo describes the role of phlegm in dementia, suggesting that its accumulation can cloud the mind [15]. Xixin decoction, augmented with Chuanxiong and peach kernels to invigorate blood circulation and eliminate blood stasis, aligns with the treatment principles for VaD. Some studies have demonstrated that modified Xixin decoction in combination with Oxiracetam, enhances the cognitive status of patients with VaD caused by infract. Furthermore, the combination of Xixin decoction with acupuncture has been found to improve the cognitive function, life ability, and psychological state of patients with vascular mild cognitive impairment.

Numerous studies have shown that aberrant synaptic function and pruning are key pathogenic mechanisms in neurodegenerative diseases [16, 17]. NPTX2, an important biomarker for diagnosing neurodegenerative diseases, is localized at synapses and is important in promoting excitatory synapse formation and neuronal dendritic growth [18, 19]. The initiator of the classical complement pathway, C1q, binds to immune complexes and activates C3, thereby inducing microglia to phagocytose complement-regulated synapses [20], and aberrant activation in a variety of CNS disorders leads to synaptic loss and induced cognitive decline [21, 22]. Several studies found that in the mature brain, NPTX2 is involved in the progression of neurodegenerative diseases mediated by the complement pathway by regulating the activity of the complement pathway to modulate synaptic modifications mediated by microglia [23–25]. Meanwhile, the binding of NPTX2 to C1q can downregulate C3, mitigate microglia-mediated neuroinflammation, and reduce the levels of neuroinflammation-related proteins such as GPNMB, IL-6, and TNF-α, thereby lessening synaptic damage [23].

Therefore, this study designed relevant experiments to explore the role and mechanism of Xixin decoction in the treatment of VaD by regulating the NPTX2/C1q/C3 pathway. The results of this study might lay a foundation for the development of VaD-targeted therapy, ultimately improve the quality of life for patients with VaD, and reduce the societal and economic burden of this growing health concern.

2 | Materials and Methods

2.1 | Medicament Preparation

The Xixin decoction consists of the following Chinese herbal ingredients that are listed in Table 1.

TABLE 1 | The consists of Xixin decoction.

Chinese herbal ingredients	Dosage (g)	Batch number
Ginseng Radix Et Rhizoma (Ren Shen)	30	2424070107
Poria cocos sclerotium with pine root (Fu shen)	30	2424100106
Fried Ziziphi Spinosae Semen (Chao Suan Zao Ren, crushed)	30	2424100112
Pinellia Rhizoma Praeparatum (Fa Ban Xia)	15	2424080112
Citri Reticulatae Pericarpium (Chen Pi)	9	2424070139
Jianqu, a Fermented Traditional Chinese Medicine (Jian Qu)	9	2424080111
Glycyrrhiza Radix Et Rhizoma (Gan Cao)	3	2424070111
Acori Tatarinowii Rhizoma (Shi Chang Pu)	3	2424080104
Prepared Aconiti Lateralis Radix Praeparata (Hei Shun Pian, pre-boiled)	3	2424070118
Chuanxiong Rhizoma (Chuan Xiong)	12	2424070120
Persicae Semen (Tao Ren)	10	2424060138

All of the Chinese medicines were produced by Hubei Chenmei Traditional Chinese Medicine Co., Ltd, passed the factory inspection, purchased from the Chinese medicine pharmacy of Huanggang Hospital of Traditional Chinese Medicine, and identified as authentic by Jian Yu of the Pharmacy Department.

The total weight of raw herbs in Table 1 was 154 g, which was decocted and administered once a day for adults, according to the "Code of Practice for the Management of Chinese Medicine Decoction Rooms in Medical Institutions" published by the National Administration of Traditional Chinese Medicine on March 16, 2009. Black Shun Tablet was added to 200 mL of ddH_2O and decocted for 30 min. Subsequently, the remaining traditional Chinese medicine was incorporated and decocted for 30 min, after which the liquid was filtered. The dregs of the medicine were added to 200 mL of ddH_2O again and decocted for 30 min, and the liquid was filtered. The two medicinal liquids were mixed, concentrated, and lyophilized into lyophilized powder via low-temperature decompression, which was sealed and stored in a dry dish, and dissolved in ddH_2O when used. Upon gavage, ddH_2O was added to make a medicinal solution with a concentration of 1 g/ml of raw drug quantity. According to the method of converting the body surface area of humans and rats, the amount of gavage for rats in the group of heart-washing soup was 11.88 g/(kg·d), and finally, the gavage was carried out according to the body weight of each rat. Donepezil hydrochloride tablets

were dissolved in ultrapure water when dispensed as a positive drug.

2.2 | Animals and Treatment

Seventy-five male Sprague-Dawley rats (weighted 315 ± 25 g) were purchased from the Beijing Weitong Lihua Experimental Animal Technology Co, LTD. All rats were kept in the SPF environment with an independent ventilation cage and housed with food and water available randomly in a temperature- and humidity-controlled room (23.5 ± 1.5°C, 45%–55% humidity), maintained with a 12-h light/dark cycle.

The rats were randomly allocated into five groups, the blank control, the VaD model control, the sham, the Xixin decoction, and the donepezil groups. Each comprises 15 individuals. The blank control group was not treated; the sham group left only isolated unilateral common carotid artery without ligation; and the remaining groups left unilateral common carotid artery ligation. On the 8th postoperative day, the Morris water maze experiment was performed to evaluate the model. Subsequent successful models were gavaged (the Xixin decoction group = 11.88 g/(kg·d) and the donepezil group = 0.45 g/(kg·d)), and the blank control group, the VaD model control group, and the sham group received an equal amount of double distilled water daily. Gavage was performed for 4 consecutive weeks. All experiments were performed in compliance with the requirements of the Experimental Animal Ethics Committee of the Hubei University of Chinese Medicine (IEC2024-163).

2.3 | Morris Water Maze

The Morris water maze method detects the behavioral changes in each group of mice. The test procedure includes two parts, namely, the positioning navigation test and the space exploration test, which detects the motor ability and spatial memory ability of mice.

After intragastric administration, a positioning cruise test was started for 5 days with 4 training sessions per day. On the last day, the incubation time of the rat platform was recorded, including the total swimming distance (cm) before reaching the platform or within 90 s, and the average swimming speed (cm/s) was calculated. The movement trajectory of each rat in the pool was recorded to generate a kinematics map for the positioning navigation test.

After the space exploration test, the underwater platform in the pool was removed, and the number of times (times) of the original platform and the movement route in the pool were recorded to generate the movement trajectory map of the space exploration test.

2.4 | New Object Recognition

After the rats were familiar with the characters and the environment, they were placed into the object environment. We then recorded the exploration time (s) on each object, as well as the number of times (times), time (s), and distance (cm) of each object within 5 min. After 1 h, one of the objects was replaced, and the above record was repeated.

2.5 | Transmission Electron Microscopy Observation

After rats were anesthetized, heads were severed, brains were removed and hippocampal tissue was isolated. The cerebral cortex was carefully dissected to fully expose the hippocampus. This procedure necessitates functioning in a sterile environment on ice to gently remove the intact hippocampus and then cut it into approximately 1 mm^3 pieces using a sharp scalpel. For small tissue blocks, 2–3 tissue blocks were cut from each hippocampus, immediately put into the sample bottle equipped with electron lens fixative (Servicebio, Wuhan, Hubei, China), fixed for 2 h at room temperature, and transferred to 4°C for storage.

The synaptic structures of rat hippocampal neurons, nucleus, mitochondria, ribosome, and rough endoplasmic reticulum were observed under transmission electron microscopy (HITACHI, Tokyo, Japan).

2.6 | Golgi Staining

Briefly, Golgi staining uses the silophilic of neurons to deeply dye neurons in rat brain tissue as black with a gray or colorless background, using image processing to highlight dendritic spines, which can then be quantified. The hippocampal tissue was fixed with Golgi staining fixative (Servicebio, Wuhan, Hubei, China).

2.7 | Nissl Staining

The hippocampal tissue was fixed with formaldehyde. The formaldehyde-fixed specimens were embedded in paraffin and cut into slices. The sections were deparaffinized with xylene and rehydrated in a graded series of alcohol. Samples were stained in toluidine blue solution for 40 min at 50–60°C. After clearing in distilled water, the slides were gradually dehydrated for 3 min in successive baths of ethanol, with one pass each in 70%, 80%, and 95% and two passes in 100%. All slides were then given two passes for 5 min in 100% dimethylbenzene, and coverslips were applied with neutral balsam. Finally, the basic neural structure of rat hippocampal tissue was observed [26].

2.8 | Western Blotting

The rat hippocampus tissue was lysed in RIPA lysis buffer and total protein was extracted. Then the protein was separated by 10 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. Next, the membranes were blocked with 5 % skim milk and incubated with primary antibody overnight at 4°C. The primary antibody was NPTX2 (1:1000; 10889-1-AP; Proteintech), C1q (1:1000; 11602-1-AP; Proteintech, Chicago IL, USA), C3 (1:1000; 21337-1-AP;

TABLE 2 | Primer sequences.

Primers	Primer sequences(5'-3')	Primer length (bp)
NPTX2	Forward:AGAGTCCTGAGCCACTTCCT	167
	Reverse:CCGGTAGGATGACCACACAG	
C1q	Forward:TGGCAACGTGGTTGTCTTTG	84
	Reverse:ACCGCACAGATGAAGTGACC	
C3	Forward:TTGTCCCCTTGAAGATCGGC	111
	Reverse:TCATTCCTTCTGGCACGACC	
PSD95	Forward:CTGCCCCATCATAACTCCCC	164
	Reverse:TCCACTCATGCAAACCAGC'	
TNF-α	Forward:GGAGGGAGAACAGCAACTCC	93
	Reverse:GCCAGTGTATGAGAGGGACG	
β-Actin	Forward:CCGCGAGTACAACCTTCTTG	207
	Reverse:TGACCCATACCCACCATCAC	

Proteintech, Chicago IL, USA), PSD95 (1:5000; CY5407; Abways, Shanghai, China), TNF-α (1:1000; 60291-1-lg; Proteintech, Chicago IL, USA) and β-actin (1:10000; 66009-1-lg; Proteintech, Chicago IL, USA). After being washed five times with PBST, the membranes were incubated with secondary antibody HRP-conjugated Affinipure Goat Anti-Mouse IgG (H+L) (1:10000; SA00001-1; Proteintech, Chicago IL, USA) or HRP-conjugated Affinipure Goat Anti-Rabbit IgG (H+L) (1:10000; SA00001-2; Proteintech, Chicago IL, USA). Finally, the blots were analyzed with ECL luminescent liquid and chemiluminescent systems.

2.9 | Quantitative Real-Time Polymerase Chain Reaction

Total RNAs were extracted with TRNzol Universal RNA Reagent (TIANGEN, Beijing, China). A micro spectrophotometer (Kaiao Technology, Beijing, China) was used to detect RNA quality and quantity. The cDNA was prepared by the FastKing One-Step Genomic DNA Elimination cDNA First Strand Synthesis Premix Reagent (TIANGEN, Beijing, China). The quantitative real-time polymerase chain reaction (RT-qPCR) was performed using FastReal Rapid Fluorescence Quantitative PCR Premix Reagent (TIANGEN, Beijing, China) on the PCR Amplifier (TC-96/G/H(b)B; Bioer Technology, Hangzhou, Zhejiang, China). In brief, 20 μl total reaction volume contained 10 μl SYBR Green Supermix (2×), 0.5 μl each forward and reverse primer, and 50 ng cDNA. The RT-PCR conditions were performed with an incubation at 95°C for 2 min, followed by 40 cycles with 5 s denaturation at 95°C and 30 s extension at a temperature depending on different primers. The β-actin was used as an internal standard for standardization. The relevant expressions of the target genes were detected by Fluorescence quantitative polymerase chain reaction detection system(Bioer Technology, FQD-96A, Hangzhou, Zhejiang, China) and were calculated using the $2^{-\Delta\Delta Ct}$ method. All primer sequences are listed in Table 2.

2.10 | Statistical Method

All data were described as mean ± standard deviation (SD). Experimental data were analyzed by one-way ANOVA, and comparisons between groups were made by least significant difference method for two-group comparisons. A statistical difference was considered as $p < 0.05$.

3 | Results

3.1 | Effect of Xixin Decoction on Cognition in the VaD Model Rats

The rat positioning navigation and space exploration abilities were observed through the Morris water maze experiment (Figure 1A). The results showed no significant difference in the mean swimming speed of the groups compared with the control group (Figure 1B). However, in contrast to the control group, we found no statistical variance in the total swimming distance of the sham group, whereas the total swimming distance of the other groups was significantly prolonged. Compared with the model group, the total swimming distance of each group was shortened (Figure 1C). Compared with the control group, no alteration was observed in the platform incubation period of the sham group, but the incubation period of the other groups was significantly elongated. In contrast to the model group, the incubation period on the platform was shortened in all groups (Figure 1D). Compared with the control group, no difference was noted in the frequency of crossing the platform in the sham group, but the frequency of crossing the platform in the other groups decreased significantly. Compared with the model group, the number of times crossing the platform increased in all groups (Figure 1E).

Moreover, the new object recognition experiment observed the number, time, and distance of the rat activity around the old and new objects. Compared with the control group, the new object recognition coefficient of the sham group remained unchanged, whereas that of the other groups decreased significantly. In contrast to the model group, the new object recognition coefficient of each group increased markedly (Figure 1F).

3.2 | Effects of Xixin Decoction on the Ultrastructure of Hippocampal Nerve Cells in the VaD Model Rats

Transmission electron microscopy revealed that rat hippocampal neurons in the control and sham groups had intact cell structures and nuclei, with aggregated in the nucleus, and abundant and structurally intact mitochondria. Meanwhile, the rat hippocampal neurons in the model group exhibited cell death, including nucleus collapse, border cracking or disappearance, and chromatin shrinkage or disappearance. Moreover, the mitochondria decreased significantly, and most of them were swollen or even ruptured. The hippocampal neurons in the donepezil and Xixin decoction groups also had different degrees of damage, but the nuclei of the cells were generally normal in morphology and structurally intact, and some of the mitochondria were swollen (Figure 2).

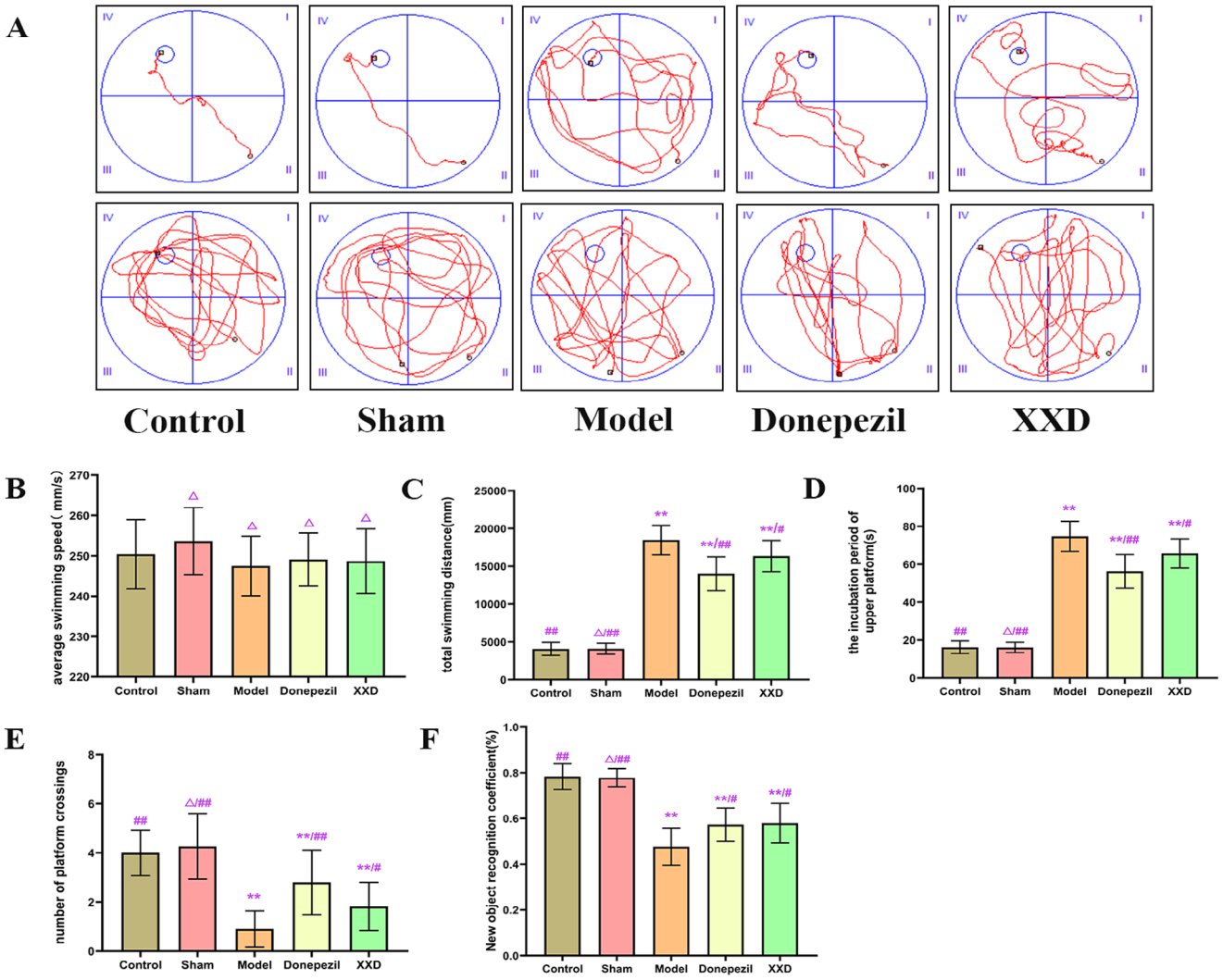

FIGURE 1 | Experimental results of Morris water maze and new object recognition in each group of rats.
(A) Trajectory diagrams of the Morris water maze, the upper figure depicted the trajectory of the localization navigation test and the lower figure presented the trajectory of the spatial exploration test; (B) average swimming speed (mm/s); (C) total swimming distance (mm); (D) the incubation period of upper platform (s); (E) number of platform crossings; (F) new object recognition coefficient (%). Comparison of each rat group with the control group, $*p < 0.05$, $**p < 0.01$, $\Delta p > 0.05$; comparison of each rat group with the model group, $\#p < 0.05$, $\#\#p < 0.01$.

3.3 | Xixin Decoction Improves Neuron Number and Dendritic Spine Density in the VaD Model Rats

Changes in the number of neurons in the CA3 area were observed by Nissl staining. Compared with the control group, the number of neurons in the CA3 area of rats in the sham group did not change significantly, and the number of neurons in the CA3 area of rats in the rest of the groups decreased. The most obvious decrease was noted in the model group. Furthermore, the number of neurons in the CA3 area of rats in the Xixin decoction and donepezil groups increased compared with the model group, suggesting that Xixin decoction improved the number of neurons in the VaD rats (Figure 3A and B).

Changes in the dendritic spine density of neurons in the CA3 area were observed by Golgi staining. Compared with the density of neuronal dendritic spines in the CA3 area of rats in the control group, we found no significant change in the sham group, whereas the rest of the rats in all groups showed a significant decrease. Compared with the model group, the density of neuronal dendritic spines in the CA3 area of rats in the Xixin decoction and donepezil groups significantly increased (Figure 3C,D).

3.4 | Xixin Decoction Might Ameliorate Neuronal Cell Damage in the VaD Rats by Affecting the NPTX2/C1q/C3 Complement Pathway

Compared with the control group, NPTX2, and PSD95 protein expression decreased, and C1q, C3, and TNF-α protein expression increased in the hippocampal tissues of rats in the model group; while NPTX2 and PSD95 protein expression increased and C1q, C3, and TNF-α protein expression decreased in rats of the Xixin decoction and donepezil groups compared to the model group, suggesting that the Xixin decoction improved

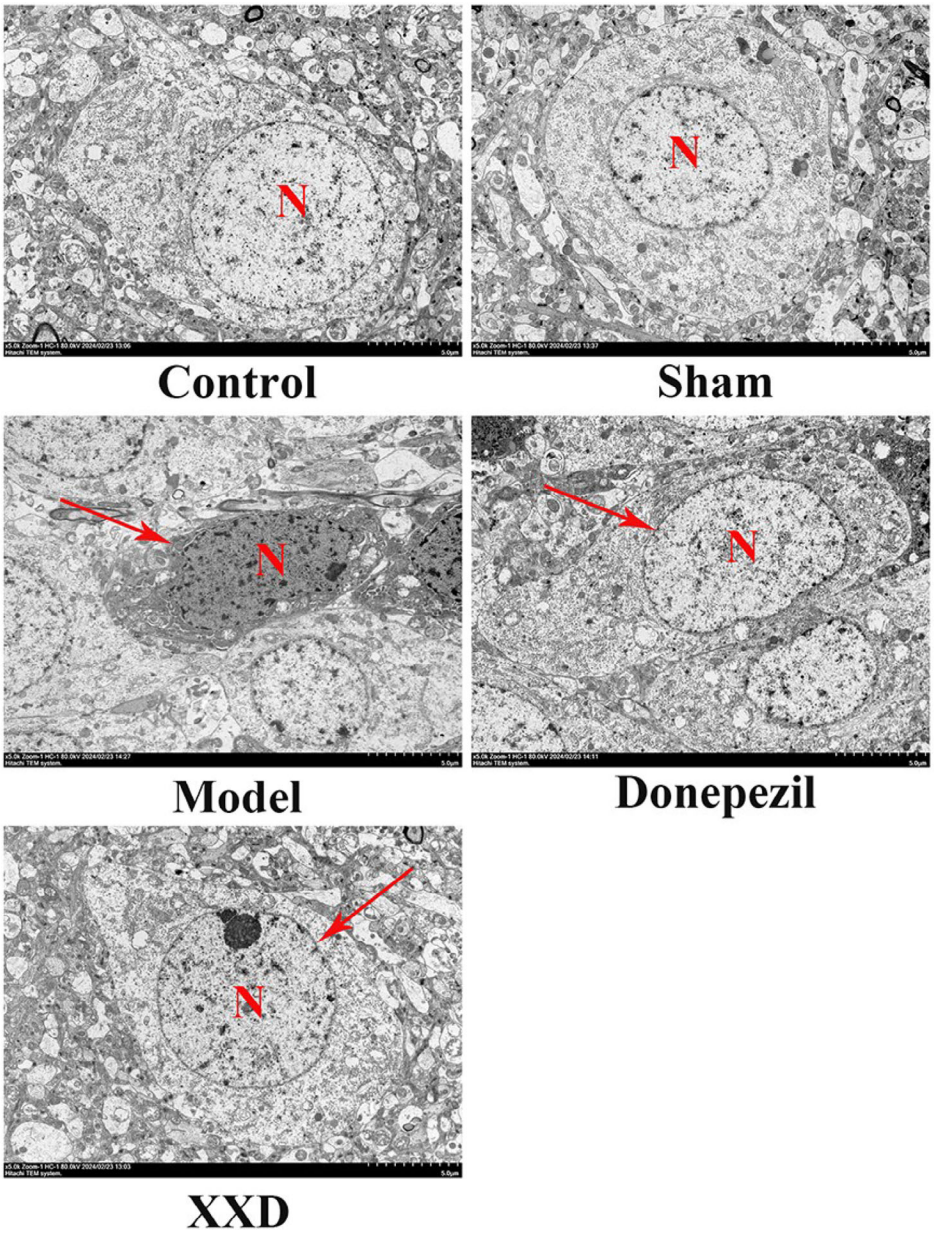

FIGURE 2 | Transmission electron microscopy results of neurons in the hippocampal region of rats in each group. (×5000, Bar = 5 μm)
N is for cell nucleus, the red arrow points to nuclear shrinkage or cell death.

the neuronal cell injury symptoms in the VaD model rats was associated with the activation of NPTX2/C1q/C3 complement pathway (Figure 4A). The PCR results were consistent with the above results (Figure 4B-F).

4 | Discussion

The aggravation of population aging has led to a rapid increase in the prevalence of VaD in the world, especially in China, where aging is becoming increasingly serious. Therefore, the prevention and treatment of VaD have attracted extensive attention, but its pathogenesis is not completely clear, among which cranial nerve inflammation is one of its important pathological signs. Recent studies have shown that Xixin decoction might improve spatial learning and memory in rats, as well as alleviate inflammatory responses in the brain might improve spatial learning and memory in rats, as well as alleviate inflammatory responses in the brain [27]. Therefore, in this study, ligation of the unilateral common carotid artery in rats was adopted to mimic humans suffering from VaD. This rat model attempted to elucidate the molecular mechanism of Xixin decoction in the treatment of VaD. In this study, we explored the mechanism of Xixin decoction in which NPTX2 regulates the C1q/C3 complement pathway, and we revealed that Xixin decoction might treat VaD by regulating the NPTX2/C1q/C3 complement pathway. The results of motor function and cognitive function testing showed that the intervention of donepezil and Xixin decoction might improve the motor and cognitive conditions of the model rats. In addition, our results showed that donepezil and Xixin decoction might increase the number of neurons and the density of neuron dendritic spines in the hippocampus of model rats. In addition, our results showed

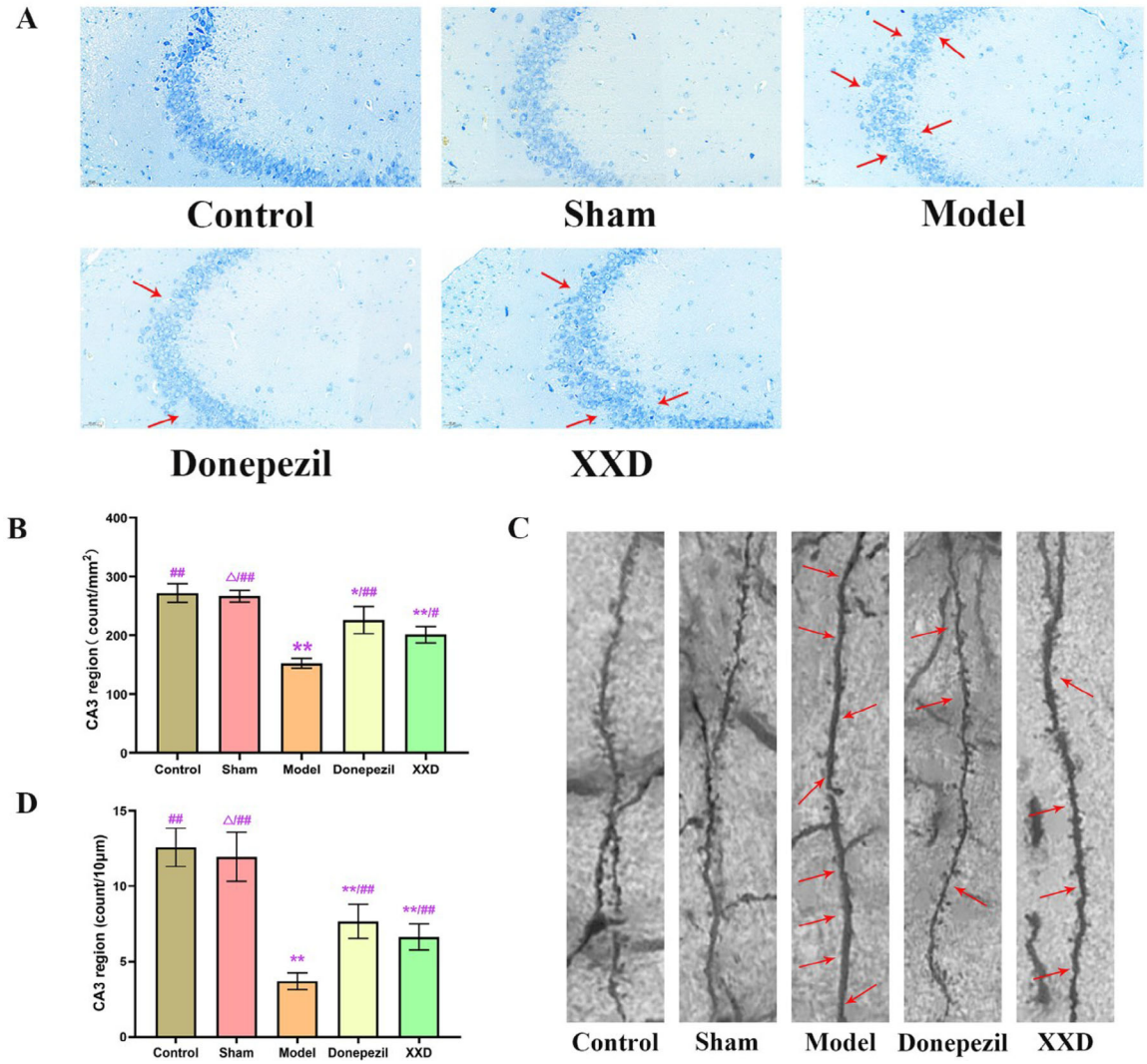

FIGURE 3 | The results of Nissl staining and Golgi staining for rats in each group.
Nissl staining of rat hippocampal CA3 area in each group (×200, Bar = 50 μm). The area indicated by the red arrow was where the neuronal loss occurs. (B) results of Nissl staining in the CA3 area of rats in each group (count/mm^2). (C) Golgi staining of the hippocampal area of rats in each group (×1000). The area indicated by the red arrow was where dendritic spine loss occurs. (D) results of Golgi staining in the CA3 area of rats in each group (count/10 μm). Comparison of each rat group with the control group, *$p < 0.05$, **$p < 0.01$, $^{\Delta}P > 0.05$; comparison of each rat group with the model group, #$p < 0.05$, ##$p < 0.01$.

that donepezil and Xixin decoction might increase the number of neurons and the density of neuron dendritic spines in the hippocampus of model rats. Our results demonstrated that the protein expression levels of NPTX2 and PSD95 increased, but the protein expression levels of C1q, C3, and TNF-α decreased in the Xixin decoction rats. These results suggested that Xixin decoction's improvement of neuronal cell injury symptoms in the VaD model rats was related to the activation of the NPTX2/C1q/C3 complement pathway.

NPTX2, as a neuronal activity regulator, plays an important role in neurological diseases [28]. In this study, the expression of NPTX2 decreased in the VaD model rats, and Xixin decoction was able to upregulate its expression, indicating that Xixin decoction might affect the pathological process of VaD through NPTX2. Meanwhile, the complement system is an important component of the immune system, and the role of C1q and C3 in neurodegenerative diseases has been extensively studied [29, 30]. Previous studies have shown that NPTX2 specifically binds to C1q, regulates complement C1q signaling-dependent synaptic loss during disease progression, inhibits complement activity, and ameliorates microglia-mediated synaptic loss [23]. Previous studies have shown that Xixin decoction effectively improves the cognitive function and daily living ability of patients with VaD [31]. Our study found that Xixin decoction might reduce the expression of C1q and C3, which might be related to its anti-inflammatory and neuroprotective effects, indicating that the improvement of Xixin decoction in patients with VaD might be associated with the decrease in the expression of C1q and C3. In addition, the relationship between the complement system and neuroinflammation is particularly important in the pathogenesis of VaD. This study speculated that Xixin decoction might reduce the neuroinflammatory response by modulating the complement system, thereby protecting nerve cells from damage. This finding

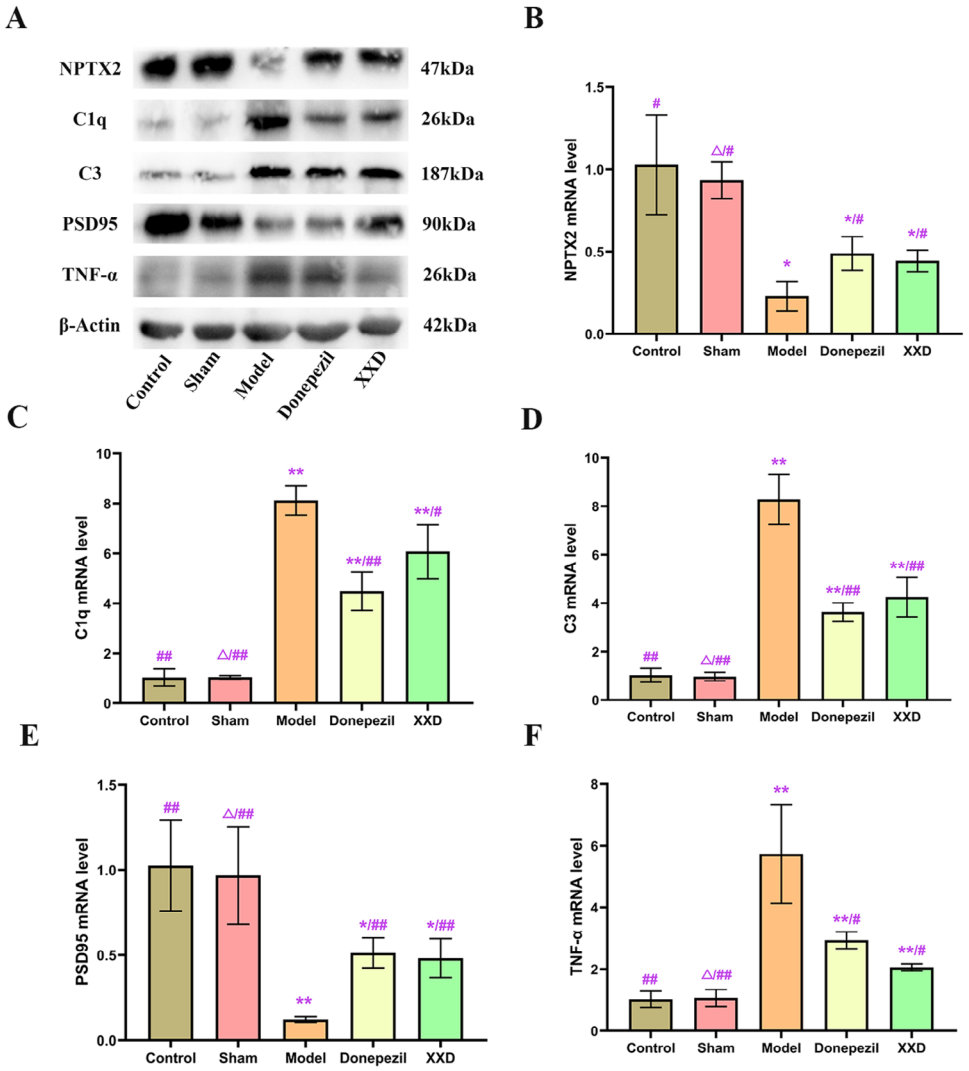

FIGURE 4 | Changes in the activity of the NPTX2/C1q/C3 complement pathway. (A) Western blotting to detect changes in the NPTX2/C1q/C3 complement pathway protein levels. (B–F) RT-qPCR detected mRNA expression levels of NPTX2, C1q, C3, PSD95, and TNF-a, respectively. Comparison of each rat group with the control group, $*p < 0.05$, $**p < 0.01$, $\Delta p > 0.05$; comparison of each rat group with the model group, $\#p < 0.05$, $\#\#p < 0.01$.

was consistent with previous findings that the activation of the complement system is closely correlated with the pathological process of VaD [32].

This study elucidated the theoretical basis and scientific connotation of VaD treatment, preliminarily revealed the mechanism of action of Xixin decoction prescription in preventing and controlling VaD, and provided a research basis for further enriching anti-VaD treatment and effective prescription of traditional Chinese medicine. However, this study had some limitations, such as the lack of relevant clinical studies to elucidate the regulation of NPTX2 and C1q/C3 complement system by Xixin decoction. Overall, the involvement of Xixin decoction as a therapeutic agent for VaD in regulating key proteins and pathogenic pathways involved in the pathogenesis of the disease suggests that it may offer a new therapeutic avenue.

In summary, Xixin decoction might exert its therapeutic effect on VaD by modulating NPTX2 and the C1q/C3 complement system. Future studies could further explore its molecular mechanisms and evaluate its potential for application in clinical trials.

5 | Conclusion

In conclusion, our study suggested that Xixin decoction might downregulate the C1q/C3 complement pathway by regulating NPTX2, further inhibiting microglial hyperactivation, reducing neuroinflammation and synaptic damage, and improving cognitive function in the VaD model rats.

Author Contributions

Shuo Yang: Conceptualization, data curation, project administration, visualization, formal analysis, and funding acquisition. **Xin-qi Gao**: Writing—original draft. ai-hua tan: project administration, and funding acquisition. **Pan Ge**: Methodology, software, and writing—review & editing.

Acknowledgments

This study was supported by grants 82405523 from the National Natural Science Foundation of China, grants 2022CFB962 and 2024AFB922 from the Natural Science Foundation of Hubei Province, and grants

ZY2023Q025 from the Hubei Provincial Administration of Traditional Chinese Medicine.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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