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RETRACTED: Yizhi Qingxin Formula Extract Ameliorates Cognitive Decline in Aged Rats *via* the Brain-Derived Neurotrophic Factor/Tropomyosin Receptor Kinase B Pathway

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Cognitive impairment and decline in old age are primarily driven by the accumulation of age-related neuropathologies, and old age is thus the primary risk factor for neurodegenerative diseases such as AD. Here, we investigated the effects of Yizhi Qingxin formula (YQF) extract on cognitive impairment in aged rats and determine the role of the brain-derived neurotrophic factor (BDNF)/*tropomyosin receptor kinase B* (TrkB) pathway underlying the neuroprotective effects of the YQF extract. Fifty male Wistar rats were randomly divided into five groups: Control group, Model group, Donepezil group, and YQF extract groups (treatment with YQF extract at two different doses). After treatment with YQF extract for 8 weeks, learning and cognitive abilities were assessed using the Morris water maze. Morphological changes in the hippocampus were observed using hematoxylin-eosin. Activated microglia and astrocytes were assessed using immunohistochemistry. Expressions of proteins and genes were examined using western blotting and real-time PCR. The results revealed that oral treatment with YQF extract dramatically improved spatial learning and memory ability and ameliorated histopathological and morphological characteristics in aged rats. YQF extract significantly increased acetylcholine and interleukin (IL)-10 levels but markedly decreased amyloid-β peptide, tumor necrosis factor alpha (TNFα), IL-2, and IL-6 levels. In addition, it inhibited the excessive activation of microglia and astrocytes, downregulated the expressions of TNFα and IL-2, and upregulated nerve growth factor, BDNF, and TrkB expressions. Furthermore, hippocampal extracellular signal-related kinase (Erk) and protein kinase B (Akt), the upstream signaling of BDNF/TrkB, were also activated by treatment with YQF extract. Our findings indicate that YQF extract activates the BDNF/TrkB pathway through the upregulation of Erk and Akt signaling, and the activated signaling pathway might contribute to the protective effects of YQF extract on cognitive impairment in aged rats.

Keywords: Yizhi Qingxin formula, Alzheimer's disease, cognitive impairment, aged rats, brain-derived neurotrophic factor, tropomyosin receptor kinase B

INTRODUCTION

With the population of older adults is increasing greatly worldwide, "Aging & Disease" has become a hot topic in our society (Shetty et al., 2019), and aging is the predominant risk factor for various age-related diseases (Franceschi et al., 2018), including cancer, cardiovascular disorder, diabetes mellitus, Parkinson's disease, Huntington's disease, and Alzheimer's disease (AD). AD is a severe neurodegenerative disease and the most prevalent form of dementia, with specific neuropathological features, including amyloid protein deposits, neurofibrillary tangles, synaptic dysfunction (Marsh and Alifragis, 2018), and neuronal loss (Ossenkoppele et al., 2015). The increasing incidence of AD imposes a heavy economic burden on family and society (Lane et al., 2018). However, current United States Food and Drug Administration (FDA)-approved drugs for the treatment of AD, such as memantine, rivastigmine, and donepezil, are known to be effective only in mild and moderate AD patients by improving clinical symptoms without slowing disease progression (Godyń et al., 2016; Epperly et al., 2017). Thus, studies are needed to develop/discover novel, potentially effective agents for the treatment of AD.

Neurotrophic factors belong to the growth factor family, including brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-3 (nt-3), and neurotrophin 4/5 (nt-4/5), of which BDNF and NGF are two important neurotrophic factors in the body (Wei et al., 2015). BDNF mainly exists in the cerebral cortex, hippocampus, and cerebellum, and plays a vital role in neuronal survival, differentiation, synaptic plasticity, and neurogenesis after binding to its cognate TrkB receptor (Bekinschtein et al., 2014; Bathina and Das, 2015; Avgan et al., 2017). Importantly, accumulating evidence indicates that dysfunction of the BDNF/TrkB system is involved in a variety of brain diseases, including AD and depressive disorder (Zhang et al., 2016; Yin et al., 2018). Therefore, the activation of the BDNF/TrkB pathway can be strategically positioned to improve memory deficits (Luo et al., 2019; Tang et al., 2019).

Traditional Chinese medicine is a promising source with immense chemical diversity and multiple-target characteristics, and it thus is suitable for the modulation of multiple signaling pathways/cascades in AD (Liu et al., 2014; Mana et al., 2019). Yizhi Qingxin formula (YQF, previously called Fuzheng Quxie Decoction), a traditional herbal formula composed of *Panax ginseng* C. A. Mey. (radix et rhizoma), *Coptis chinensis* Franch. (rhizoma), and Conioselinum anthriscoides 'Chuanxiong' syn. *Ligusticum chuanxiong* (rhizoma), has been used clinically for the treatment of AD or cognitive impairment. As predicted by network pharmacological approaches, the targets of YQF extract include acetylcholine esterase (AchE), tumor necrosis factor alpha (TNFα), caspase 3, and BDNF, which are involved in neural transmission, inflammation, cellular apoptosis, and nerve regeneration (Wang et al., 2018). However, the exact mechanism underlying the effects of YQF extract on cognitive function still needs further clarification. The present study investigated the effects of YQF extract on cognitive function and elucidated the role of the activated BDNF/tropomyosin receptor kinase B (TrkB) pathway in neuroprotective effects of YQF extract in aged rats.

MATERIALS AND METHODS

Animals

Fifty male Wistar rats (650–750 g; 18 months of age) were purchased from Beijing Weitong Lihua Experimental Animal Technology Co. Ltd. All animals were housed in groups under standard conditions (12 h light–dark cycle; light on 7 am–7 pm; temperature of 22 ± 1°C) with free access to water and food. All animal experiments were performed in accordance with the standards established by the Institutional Animal Care and Use Committee of Institute of Basic Medical Sciences of Xiyuan Hospital and were approved by the Ethics Committee of Xiyuan Hospital, China Academy of Chinese Medical Sciences (NO. 2018XLC009-1).

Drug and Chemicals

YQF is composed of *Panax ginseng* C. A. Mey. (radix et rhizoma), *Coptis chinensis* Franch. (rhizoma), and Conioselinum anthriscoides 'Chuanxiong' syn. *Ligusticum chuanxiong* (rhizoma). They were deposited at the Herbarium of National Resource Center for Chinese Materia Medica (CMMI), China Academy of Chinese Medical Sciences. The codes for *Panax ginseng*, *Coptis chinensis*, and *Ligusticum chuanxiong* specimens were 0220582LY0015, 420528LY0075, and 331127LY0805, respectively.

In this study, YQF extract is a mixture of three kinds of powder, including *P. ginseng* extract powder, *C. chinensis* extract powder, and *C. anthriscoides* extract powder, with a weight ratio of 9:5:6. *P. ginseng* extract powder (Batch number: K177215) and *C. chinensis* extract powder (Batch number: K173559) were purchased from Xi'an Kai lai Biological Engineering Co., Ltd. (Xi'an, China). C. anthriscoides extract powder (Batch number: 20170612) was prepared at the Department of Pharmaceutical Preparation of Xiyuan Hospital, China Academy of Chinese Medical Sciences. The major bioactive compounds were identified using HPLC (High Performance Liquid Chromatography), where the characterization can be found in the **Supplementary Materials**. The certificates of analysis of *P. ginseng* extract powder (Ginsenoside Rg1, 8.6%; ginsenoside Rb1, 3.2%; ginsenoside Re, 19.2%; ginsenoside Rd, 6.1%), *C. chinensis* extract powder (Total alkaloid of Coptis, 12.5%), and *C. anthriscoides* extract powder (Ligustrazine, 0.07 mg/g; ferulic acid, 1.08 mg/g; ligustilide, 37.2 mg/g) were also shown in the **Supplementary Materials**, respectively. Donepezil hydrochloride tablets (5 mg, batch number: H20050978) produced by Eisai (China) pharmaceutical., Ltd. (Suzhou, China). When used, the YQF extract and donepezil hydrochloride tablets were dissolved separately in distilled water at intended concentrations. YQF was dissolved in distilled water, and its final appearance was brown suspension after dissolving in water.

Rabbit polyclonal antibodies against BDNF, TrkB, nerve growth factor (NGF), interleukin-2 (IL-2), and TNFα were

purchased from Abcam (Cambridge, UK), and those against extracellular signal-regulated protein kinases 1 and 2 (ERK1/2), phospho-ERK1/2 (Thr202/Tyr204), protein kinase B (Akt), and phosphor-Akt (Ser473) were obtained from Cell Signaling Technology (CST, Beverly, MA, USA).

Animal Grouping and Drug Administration

Aged rats with cognitive impairment were screened using Morris water maze (MWM). According to the results of MWM, the mean escape latency range of 95% normal value of young rats was taken as the lower limit, while 99% normal value range was taken as the upper limit, then the rats were divided into the cognitive impairment group and cognitive normal group (Wang et al., 2015). The cognitive impairment group was randomly divided into four separate groups: (1) Model group (n = 10), orally administrated distilled water; (2) Donepezil group (n = 10), orally administrated donepezil (0.5 mg/kg); (3) YQF extract low-dose group (n = 10), orally administrated YQF extract (0.3 mg/kg); and (4) YQF extract high-dose group (n = 10), orally administrated YQF extract (0.6 mg/kg). The cognitive normal group was used as a control group (n = 10), and they were orally administrated the same volumes of distilled water. All groups were treated once a day for 8 consecutive weeks.

Morris Water Maze

Following 8 weeks of intragastric water or drug administration, the cognitive function of rats was assessed using MWM with a video analysis system (Beijing ZS Dichuang Technology Development Co., Ltd., Beijing, China). The MWM apparatus consisted of a circular pool (120 cm in diameter and 50 cm in height) surrounded by a white curtain, platform (10 cm in diameter and 50 cm in height), video camera, and computer. The pool was divided into four quadrants and filled with opaque water that was maintained at 23 ± 2°C. The escape platform was placed in one quadrant, with 1 cm below the water surface. One day before the experiment, rats were habituated to the pool environment for 120 s without the platform. The MWM experiment consisted of two parts: navigation trials and a spatial probe trial. The trials were conducted in one morning and one afternoon blocks from 8:00 am to 12:00 pm and 1:00 pm to 5:00 pm, respectively.

In the navigation trials, rats were placed into the water facing the wall of the pool at the 1/2 radian position in one of the four quadrants. Rats were allowed 90 s to find the platform, where they remained for at least 5 s. If rats failed to find the platform within 90 s, they were manually guided to the platform and remained for 10 s by the experimenter. During the 5-day training period, rats underwent four trials per day, with an intertrial interval of 15 min. Time to find the platform (escape latency, EL) and search strategies were recorded using video tracking software. The maximum value for EL was regarded as 90 s.

The spatial probe trial was performed on the 6th day. During the trial, the platform was removed, and rats were allowed to swim freely for 60 s inside the pool. The number of the original platform location crossing was recorded. The ratio of time or distance in the original platform quadrant to that in the whole pool was also calculated.

Hematoxylin-Eosin (HE)

After behavioral evaluation, rats were sacrificed for histopathological examination. The brain tissue was fixed in 10% neutral-buffered formalin, embedded in paraffin, and then cut into 10 μm sections. Deparaffinization was performed by heating the sections for 25 min at 60°C. After gradual hydration through graded alcohols, the sections were rinsed in distilled water and were stained using HE. All pathological changes in neurons of the hippocampal CA1 region was observed under a light microscope (Olympus, Japan).

Enzyme-Linked Immunosorbent Assay (ELISA)

Blood was collected from the abdominal aorta of rats. After blood samples were centrifuged at 1,050 g for 2 ×10 min, serum samples were collected and then stored at −80°C until use. ELISA kits (Beijing Sino-uk institute of Biological Technology, Beijing, China) were used to detect the levels of amyloid-β peptide (β-AP), Ach, TNFα, IL-2, IL-6, and IL-10 according to the manufacturer's instructions.

Immunohistochemistry

Brain sections were deparaffinized and incubated in a 3% H_2O_2-deionized water solution for 5–10 min. After washing three times in phosphate buffered saline (PBS), the sections were incubated in primary antibodies (Ionized calcium binding adaptor molecule 1 [Iba1] and glial fibrillary acidic protein [GFAP]) at 4°C overnight. After washing three times in PBS, they were incubated in secondary antibodies for 20 min, stained using 3,3′-diaminobenzidine, dehydrated in graded alcohol, cleared in xylene, and mounted with neutral gum. Average immunohistochemical staining was assessed from multiple samples of each group. Integrated optical density analysis was performed using the Image-Pro Plus image analysis system (Media Cybernetics, Bethesda, MD, USA).

Western Blot Analysis

Proteins (30 μg of protein per lane) were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and electrotransferred to polyvinylidene difluoride membranes (Millipore). The membranes were blocked with 5% bovine serum albumin and then incubated overnight at 4°C with specific antibodies against the following proteins: TNFα, IL-2, BDNF, TrkB, NGF, Erk1/2, p-Erk1/2, Akt, and p-Akt. After washing three times in Tris phosphate-buffered saline, the membranes were incubated with HRP-conjugated secondary antibodies, followed by electrochemiluminescent detection (Millipore). Subsequently, blot densitometry was performed, and the optical density of the bands was analyzed using a Gene Genius Bio Imaging System.

Quantitative RT-PCR Analysis

Total RNA was extracted from brain tissues using a total RNA extraction kit (Biomed) according to the manufacturer's instruction. The quantity and quality of RNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific).

A first-strand cDNA synthesis kit (Thermo Scientific) was used to synthesize cDNA according to the manufacturer's instruction. RT-PCR was performed using an ABI7500 real-time PCR system (Applied Biosystems) with SYBR Premix Ex Taq mix (Invitrogen) according to the manufacturer's instructions. The thermal reaction cycles were as follows: 95°C for 30 s and then 40 repetitions of 95°C for 5 s and 60°C for 40 s. GAPDH was used as a housekeeping gene to normalize threshold cycle (Ct) values. The following primers were used: TNFα (forward: TGAACTTC GGGGTGATCGGT; reverse: GGCTACGGGCTTGTCAC TCG), IL-2 (forward: GCACTGACGCTTGTCCTCCTTG; reverse: ATGTTTCAATTCTGTGGCCTGCTT), BDNF (forward: GGAGTGGAAAGGGTGAAACAAAG; reverse: TCC AAAAACAGGGGATTCAGTG), TrkB (forward: TGGACCAC GCCAACTGACATCG; reverse: GGCAATCACCACCACGGC ATAGA), NGF (forward: ACAGATAGCAATGTCCCAGAGG; reverse: ATCCAGAGTGTCCGAAGAGGTG), and GAPDH (forward: TTCCTACCCCCAATGTATCCG; reverse: CCACC CTGTTGCTGTAGCCATA). The relative amount of mRNA was calculated using the relative quantification (ΔΔCT) method. The ratio of the amplified target gene and the amplified internal control GADPH was calculated.

Statistical Analyses

Data were analyzed using SPSS 19.0 software. The values are expressed as mean ± SEM. In the MWM test, EL was analyzed by two-way repeated measures analysis of variance (ANOVA), followed by Fisher's least significant difference *post hoc* test. All other data were analyzed by ANOVA, followed by Bonferroni correction, to identify differences in mean values between groups. The results were considered significant at $p < 0.05$.

RESULTS

YQF Extract Ameliorates Cognitive Deficits in Aged Rats

The MWM test is a well-established paradigm for evaluating learning and cognitive ability. As shown in **Figure 1**, during the 5 day training (**Figure 1A**), the EL on day 1 had no statistically significant differences among groups ($p > 0.05$), indicating that rats have similar initial cognitive performances. Moreover, significantly decreased escape latencies were observed in the fifth day in the donepezil, YQF-L, and YQF-H groups compared with the model group ($p < 0.01$), suggesting that YQF extract-treated rats show better acquisition of the task. In addition, significant differences in the typical swimming tracking path were observed among these groups (**Figure 1B**), and the findings further support the observed improvements in cognition performances. In the probe test (**Figure 1C**), rats with YQF extract administration had significantly shorter searching distances in the target quadrant than those in the model group ($p < 0.01$); however, no significant differences were observed in searching time and platform-crossing number were observed among these groups ($p > 0.05$).

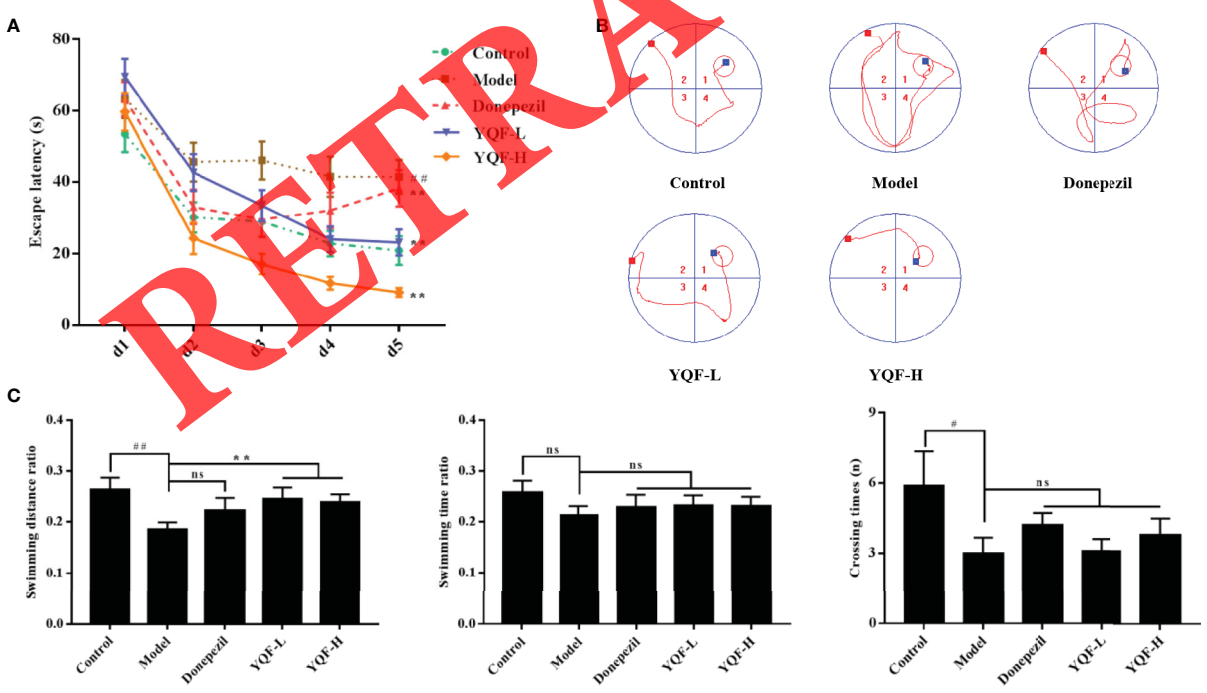

FIGURE 1 | YQF extract ameliorated cognitive deficits of aged rat in the MWM test. **(A)** Mean EL during the training phase from day 1 to day 5. **(B)** Representative swimming paths of aged rat on 5th day of MWM test. **(C)** Percentage of time spent, swimming distance in the target quadrant, and number of crossings of the platform on the 6th day. The data are expressed as mean ± SEM, n = 10. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with control group; $^{**}p < 0.01$, compared with model group. ns, no significant difference.

Effects of YQF Extract on Morphological Changes in the Hippocampus

HE staining showed that neurons were tightly aligned, and cytoplasm and nuclei were clear in the hippocampal CA1 region in control rats; however, neurons were scattered and loose with no clear boundaries, neuron quantity and neuron layers were decreased, and chromatic agglutination and karyopyknosis were detected in the model group. Higher number of normal neurons, more compact cell arrangement, and richer chromatin were found in the drug-treated groups than in the model group, as shown in **Figure 2**.

Effects of YQF Extract on Expressions of Iba-1 and GFAP

Microglia can be labeled by Iba-1, and astrocytes can be labeled by GFAP. When cells were positively expressed, brown-yellow granules can be deposited in the cytoplasm. Iba-1 and GFAP expressions were examined using immunohistochemical analysis. As shown in **Figure 3**, the expression levels of Iba-1 and GFAP were significantly increased in the model group ($p < 0.01$) compared with the control group. Notably, the increased Iba-1 and GFAP expressions were significantly attenuated after treatment with drugs ($p < 0.01$ or $p < 0.05$).

Effects of YQF Extract on β-AP and Ach Levels

As shown in **Figure 4**, the β-AP level was significantly elevated in the model group compared with the control group ($p < 0.05$). Notably, YQF extract dose-dependently attenuated the increased β-AP levels ($p < 0.01$), while no significant difference was observed between vehicle and donepezil treatments ($p > 0.05$). Moreover, the Ach level was significantly decreased in the model group compared with the control group ($p < 0.01$), and significantly increased Ach levels were found in the drug treatment groups compared with the model group ($p < 0.01$ or $p < 0.05$).

Effects of YQF Extract on TNFα, IL-2, IL-6, and IL-10 Levels

ELISA kits were used to assess TNFα, IL-2, IL-6, and IL-10 levels. As shown in **Figure 5**, the levels of TNFα, IL-2, and IL-6 significantly increased in the model group compared with the control group ($p < 0.01$), and the increased levels of TNFα, IL-2, and IL-6 were significantly attenuated after treatment with YQF extract ($p < 0.01$ or $p < 0.05$). Significant decreases in TNFα levels ($p > 0.05$) were found, but no significant changes in IL-2, IL-6, and IL-10 levels were observed after donepezil treatment. Moreover, the IL-10 level had an upward trend in the model group compared with the control group ($p > 0.05$). However, significantly increased IL-10 levels were found in the drug treatment groups compared with the model group ($p < 0.01$ or $p < 0.05$).

Western blotting was used to further assess the protein expressions of TNFα and IL-2. As shown in **Figures 6A, B**, protein expression levels of TNFα and IL-2 in the hippocampus were significantly increased in the model group compared with the control group ($p < 0.05$). However, both TNFα and IL-2 expression levels were decreased in YQF extract-treated groups compared with the model group ($p < 0.01$ or $p < 0.05$). Notably,

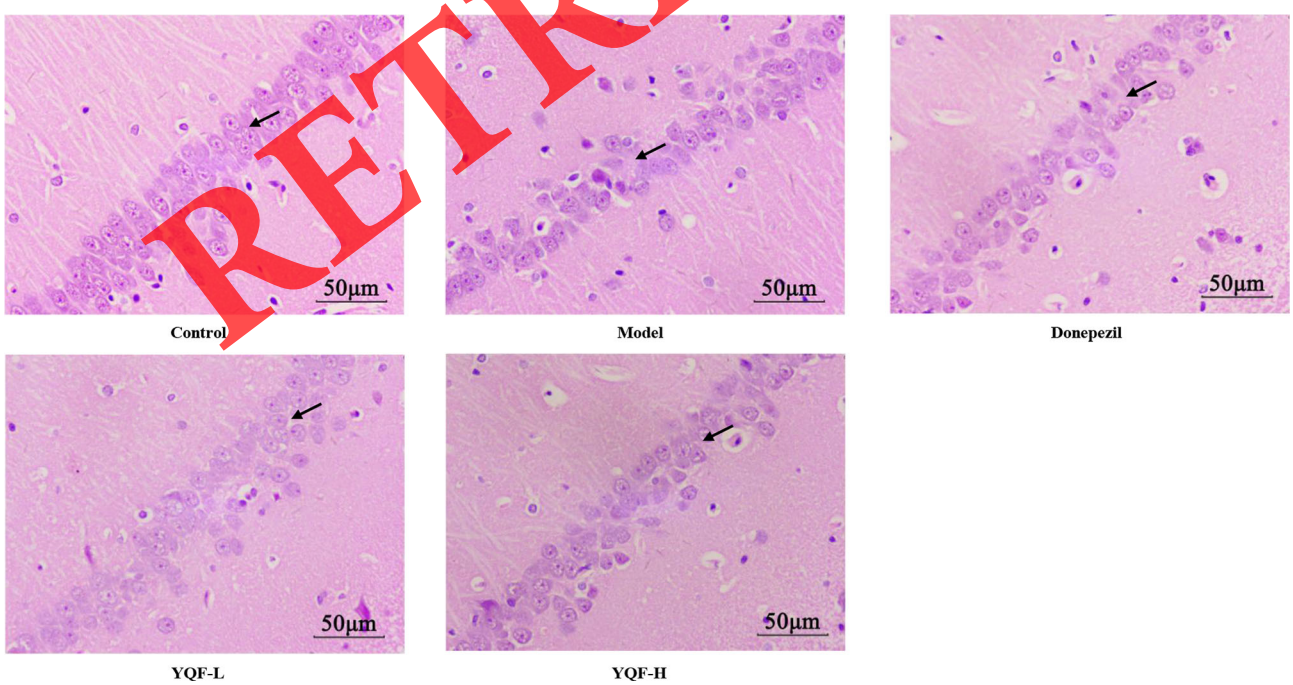

FIGURE 2 | HE staining of hippocampal CA1 (n = 5).

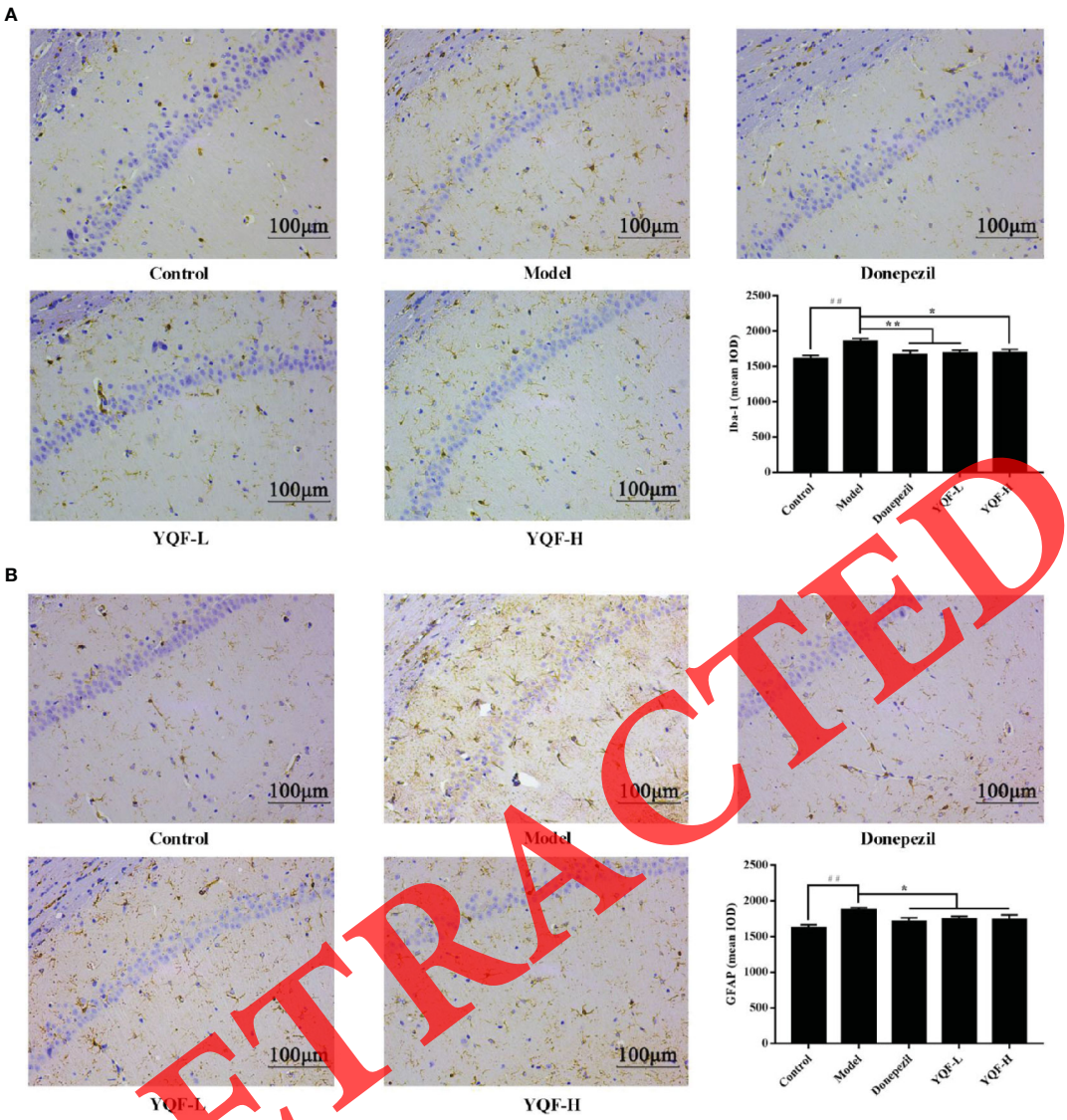

FIGURE 3 | Iba-1 and GFAP expression were detected by immunehistochemistry. **(A)** Iba-1 expression in aged rats. **(B)** GFAP expression in aged rats. The data are expressed as mean ± SEM, n = 5. $^{\#\#}p < 0.01$, compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with Model group.

no significant changes in protein expressions of TNFα and IL-2 were found after donepezil treatment.

In addition, we also investigated the potential effects of YQF extract on TNFα and IL-2 mRNA by RT-PCR (**Figures 6C, D**). TNFα mRNA levels were significantly decreased in the YQF extract-treated groups compared with the model group ($p < 0.01$ or $p < 0.05$); however, no significant changes in IL-2 mRNA levels were found after YQF extract treatment ($p > 0.05$).

Effects of YQF Extract on Expressions of BDNF, TrkB, and NGF

Using western blotting, we evaluated relative protein expressions of BDNF, TrkB, and NGF, which play major roles in cognitive function, in the hippocampus. As shown in **Figures 7A–C**, the expressions of BDNF, TrkB, and NGF in the hippocampus were significantly lower in the model group than in the control group ($p < 0.01$ or $p < 0.05$). Significantly increased expressions of these three proteins were found in aged rats in the YQF extract-treated groups compared with the model group ($p < 0.01$ or $p < 0.05$). In addition, significantly increased BDNF levels were found ($p > 0.05$), but no significant changes in TrkB and NGF levels were observed after donepezil treatment. Further, we investigated the effects of YQF extract on BDNF, TrkB, and NGF mRNAs by RT-PCR (**Figures 7D–F**). The levels of BDNF, TrkB, and NGF mRNAs showed a significant reduction in the model group compared with the control group ($p < 0.01$ or $p < 0.05$); however, treatment with YQF extract or donepezil significantly attenuated the reduction ($p < 0.01$ or $p < 0.05$).

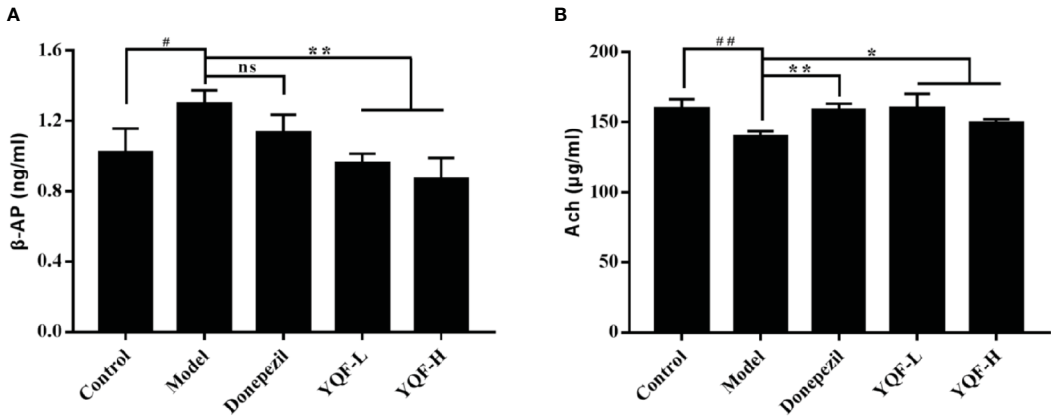

FIGURE 4 | Effects of YQF extract on β-AP and Ach levels. **(A)** β-AP level in aged rats. **(B)** Ach level in aged rats. The data are expressed as mean ± SEM, n = 10. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with model group. ns, no significant difference.

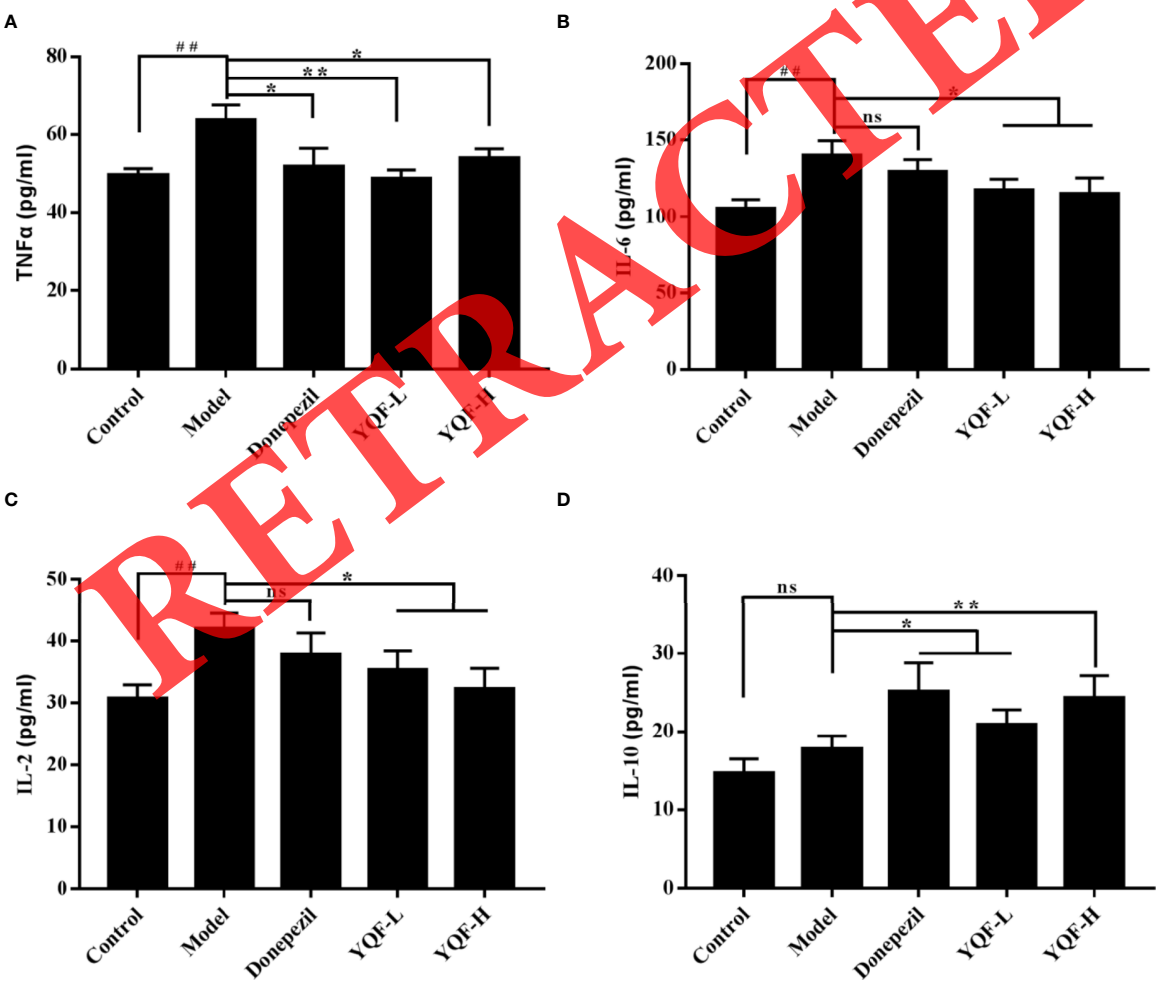

FIGURE 5 | Effects of YQF extract on TNFα, IL-2, IL-6, and IL-10 levels in serum. **(A)** TNFα level in aged rats. **(B)** IL-2 level in aged rats. **(C)** IL-6 level in aged rats. **(D)** IL-10 level in aged rats. The data are expressed as mean ± SEM, n = 10. $^{\#\#}p < 0.01$, compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with Model group. ns, no significant difference.

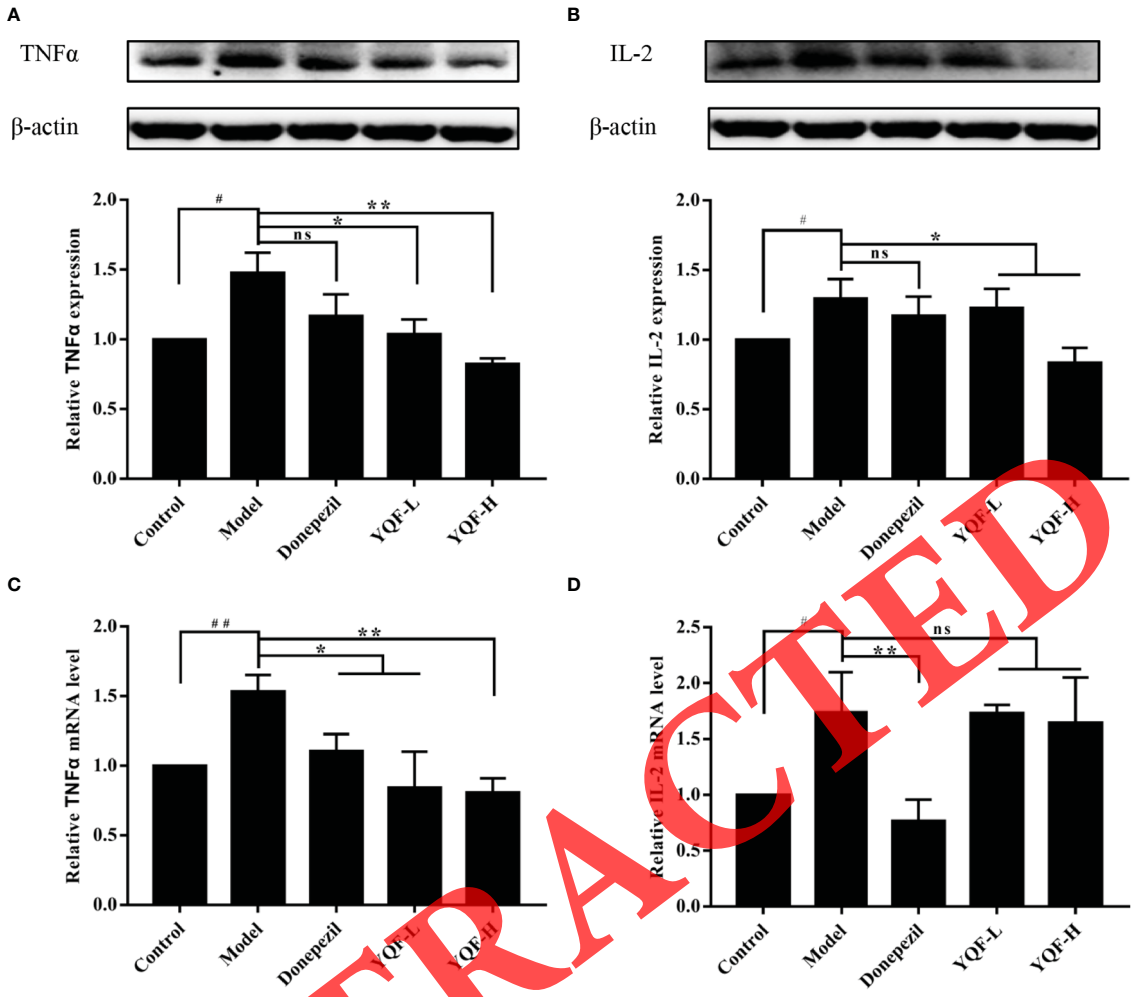

FIGURE 6 | Effect of YQF extract on the expression of TNFα and IL-2. **(A)** YQF extract suppressed TNFα expression. **(B)** YQF extract decreased IL-2 expression. **(C)** Quantitative analysis of TNFα mRNA by RT-PCR. **(D)** Quantitative analysis of IL-2 mRNA by RT-PCR. The results were obtained from three independent experiments. The data are expressed as mean ± SEM, n = 4. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with Model group. ns, no significant difference.

Effects of YQF Extract on Phosphorylation of Erk and Akt in the Hippocampus

Erk and Akt are upstream proteins of the BDNF/TrkB signaling pathway. We sought to determine whether YQF extract affects Erk and Akt phosphorylation by western blotting. As shown in **Figures 8A, B**, Erk and Akt phosphorylation decreased significantly in the model group compared with the control group ($p < 0.01$). Notably, YQF extract dose-dependently increased Erk and Akt phosphorylation levels. However, no significant changes in Erk and Akt phosphorylation levels were detected after donepezil treatment ($p > 0.05$).

DISCUSSION

Aging is an inevitable process, which promotes a range of degenerative pathologies characterized by a progressive loss of physiological function (Murtha et al., 2019). In the central nervous system, cognitive impairment and decline in old age are primarily driven by the accumulation of age-related neuropathologies, and old age is thus the primary risk factor for neurodegenerative diseases such as AD (Boyle et al., 2017). Previous studies have shown that YQF extract significantly ameliorates cognitive impairment and protects cerebrovascular function in SAMP8 and APP/PS1 mice (Yang et al., 2017; Wang et al., 2018; Yang et al., 2019). Moreover, the main active ingredients of YQF extract were detected by liquid chromatography tandem mass spectrometry (LC-MS/MS), and alkaloids like berberine, palmatine, worenine, and protopine, and ginsenosides like Rg1, Re, Rb1, Rd, and Rc were found in plasma, while berberine, palmatine, epiberberine, coptisine, and ginsenoside Rb1 were found in brain tissue (Yang et al., 2019). These findings are in agreement with those of studies, which have revealed that the major components of YQF extract (i.e.,

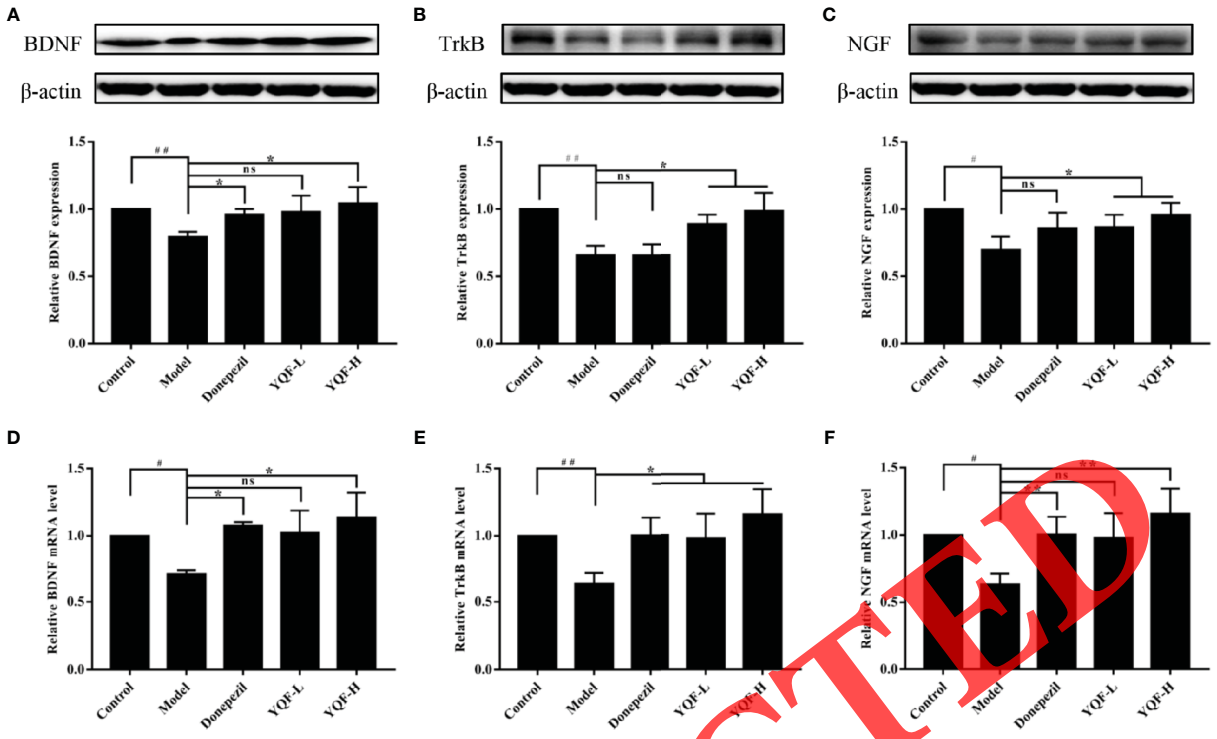

FIGURE 7 | Effect of YQF extract on the expression of BDNF, TrkB and NGF. **(A)** YQF extract increased BDNF expression. **(B)** YQF extract increased TrkB expression. **(C)** YQF extract increased NGF expression. **(D)** Quantitative analysis of BDNF mRNA by RT-PCR. **(E)** Quantitative analysis of TrkB mRNA by RT-PCR. **(F)** Quantitative analysis of NGF mRNA by RT-PCR. The results were obtained from three independent experiments. The data are expressed as mean ± SEM, n = 4. $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, compared with control group; $^{*}p < 0.05$, $^{**}p < 0.01$, compared with Model group. ns, no significant difference.

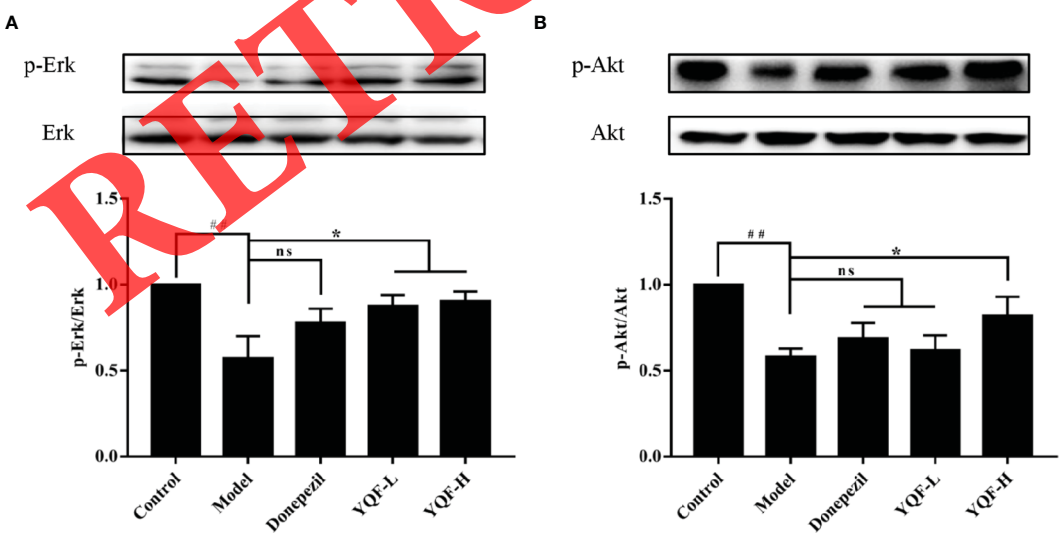

FIGURE 8 | Effect of YQF extract on phosphorylation of Erk and Akt in hippocampus. **(A)** YQF extract increased Erk phosphorylation. **(B)** YQF extract increased Akt phosphorylation. The data are expressed as mean ± SEM, n = 4. $^{\#\#}p < 0.01$, compared with control group; $^{*}p < 0.05$, compared with Model group. ns: no significant difference.

ginsenoside Rg1, Rb1, berberine, and ligustilide) exert antidementia effects in various animal models of the following mechanisms: induction alpha-processing of APP and Klotho and potential Aβ clearance, increasing the expressions of neurotrophic factors and signaling (Kuang et al., 2017; Cao et al., 2018; Yang et al., 2018). Donepezil, an acetylcholinesterase inhibitor (AChEI), has been widely used to treat Alzheimer's disease (AD) in China (Zhang and Gordon, 2018), so we used donepezil as the positive control in the present study. Our findings demonstrated that treatment with YQF extract also significantly increased the total number of neurons in the hippocampal CA1 region, enhanced the Ach level, and decreased the β-AP level. Moreover, YQF extract also inhibited the excessive activation of microglia and astrocytes and decreased the expression levels of inflammatory factors, including TNFα and IL-2. Furthermore, we assessed the molecular mechanism underlying neuroprotective properties of YQF extract, and the findings indicate that increased expressions of BDNF and TrkB are linked to Erk and Akt phosphorylation. Collectively, these findings suggest that YQF extract has neuroprotective effects against cognitive impairments during natural aging through activating the BDNF/TrkB pathway and restoring several memory-associated proteins in aged rats.

MWM is widely used to evaluate hippocampus-dependent spatial memory in rodents (Cazakoff et al., 2010). Therefore, we used MWM to evaluate cognition and memory of aged rats. In the present study, aged rats exhibited significantly prolonged escape latencies during the training sessions. During the 5-day training sessions, shorter escape latencies were observed in rats in the YQF extract administration group than in the model group. In the probe test, YQF extract-treated rats also had significantly shorter searching distance in the target quadrant. Collectively, these behavioral results suggest that YQF extract improves learning and spatial memory in aged rats.

The hippocampus is an important component of the limbic system, which is implicated in fundamental learning, memory, emotion, and behaviors (Cha et al., 2016; Travaglia et al., 2018). Cognitive deficits in aged rats are often associated with some structural and functional changes, such as incomplete hippocampal morphology and an apparent decrease in the total numbers of pyramidal neurons (Yu et al., 2017). Thus, HE was used to observe the morphological changes of the hippocampus in each group. A significant reduction in the number of neurons was found in the CA1 region of the hippocampus, and the findings are consistent with those in previous studies (Bettio et al., 2017). After treatment with different doses of YQF extract, the hippocampal structure, and quantity of neurons were improved significantly, suggesting that YQF extract prevents age-related neuronal degeneration.

Neuroinflammation, one of the main causes of AD, is orchestrated by the activation of microglial cells and astrocytes and the subsequent overproduction of pro-inflammatory cytokines (Sudduth et al., 2013; Bagyinszky et al., 2017; Ahmad et al., 2019). As demonstrated in our previous study, YQF extract significantly inhibited the activation of microglia and astrocytes in the brain in aged rats, further confirming its anti-neuroinflammatory properties. Moreover, we observed a reduced release of TNFα, IL-2, and IL-6, all of which are the inflammation-related cytokines, as well as β-AP level. In addition, both donepezil and YQF extract significantly increased the concentration of acetylcholine, and donepezil is more effective. Hippocampal neurons are suggested to be involved in neuroinflammation by increasing the expression of inflammatory proteins (Luo et al., 2019). To further explore the mechanism of the anti-inflammatory action of YQF extract, we investigated the effect of YQF extract on TNFα and IL-2 expressions. We found that YQF extract could reduce the expressions of TNFα and IL-2, indicating that YQF extract can suppress inflammatory protein expressions in aged rats. Therefore, YQF extract has a significant effect on cognitive impairment by attenuating inflammation.

In order to explore the underlying mechanism, we evaluated the BDNF/TrkB signaling. Our results showed that the expressions of BDNF, TrkB, and NGF in the hippocampus were greatly reduced in aged rats, which is consistent with previous reports (Siuda et al., 2017; Li et al., 2018). After 8 weeks of early

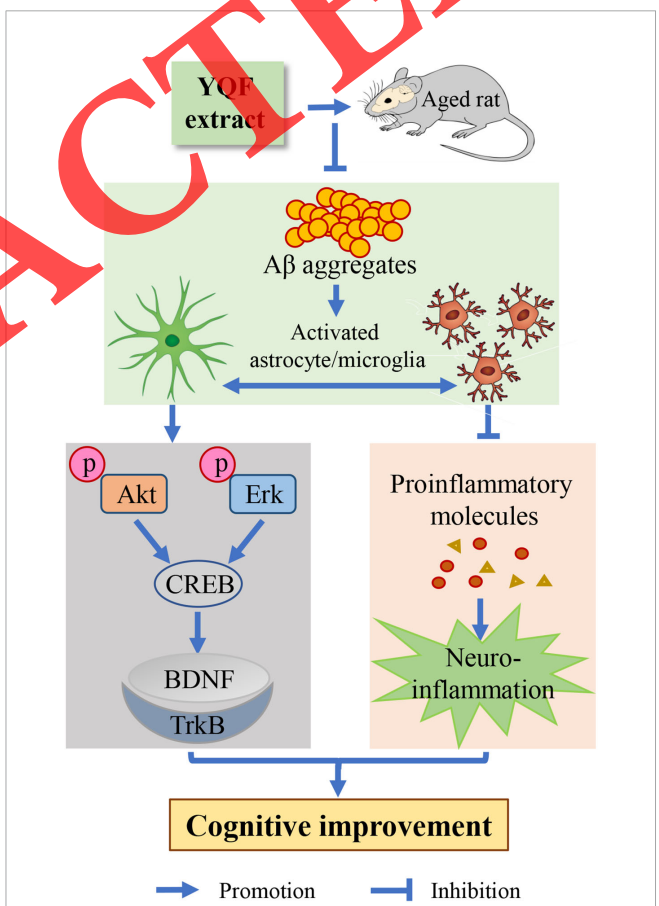

FIGURE 9 | Possible role of microglia/astrocyte in the onset/progression of the AD pathology through proinflammatory molecules mediated neuro-inflammation (Ahmad et al., 2019). A proposed pathway for YQF extract-induced activation of BDNF/TrkB to explains the cognitive improvement effects in aged rats.

YQF extract intervention, the expressions of BDNF, TrkB, and NGF were increased, indicating that the YQF extract improves cognitive function through the regulation of nerve regeneration. Increased BDNF mRNA expression in the hippocampus has been demonstrated to be implicated in the acquisition of spatial reference and working memory (Mizuno et al., 2000). The present study showed that YQF extract significantly increased mRNA and protein expression levels of BDNF, TrkB, and NGF. Thus, activation of the hippocampal BDNF/TrkB signaling might be responsible for cognitive and behaviors changes in YQF extract-treated rats. The Erk signaling pathway is involved in various cell responses, such as proliferation, migration, differentiation, and death (Li et al., 2014; Krawczyk et al., 2019). Indeed, Erk activity has been suggested to be important for synaptic plasticity and memory formation (Carlezonjr et al., 2005; Bekinschtein et al., 2007). Previous literature has reported that soy isoflavones have neuroprotective effects may be partially due to suppression of oxidative stress and activation of the Erk/CREB/BDNF signaling pathway, suggesting that BDNF may be a downstream target of Erk (Lu et al., 2018). PI3K/Akt has been demonstrated to regulate a wide array of biological function in the hippocampus, such as anti-inflammation, anti-apoptosis, and neuroprotection, which may affect NMDA receptors and downstream signaling through TrKβ and BDNF to improve cognitive deficits (Mizuno et al., 2003; Li et al., 2015; Srivastava et al., 2018). In addition, the activity of Akt has been linked with consolidation of memory and for the stabilization of activity dependent forms of synaptic plasticity (Bekinschtein et al., 2007). Therefore, we investigated the phosphorylation levels of hippocampal Erk and Akt in rats and found that YQF extract increased Erk and Akt phosphorylation levels. These findings suggest that YQF extract-induced upregulation of BDNF, TrkB, and NGF expressions is linked to increased Erk and Akt phosphorylation. Moreover, YQF extract protects cognitive functions probably through multiple targets.

In conclusion, our study is the first to show that YQF extract has protective effects on cognitive decline *via* potentiating the activation of BDNF/TrkB signaling, along with upregulation of upstream kinases such as Erk and Akt, in aged rats (**Figure 9**). Therefore, YQF extract may be a potential drug candidate for the treatment of cognition impairment in neurodegenerative disease, and the present findings warrant further pre-clinical testing in other models.

DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

ETHICS STATEMENT

The animal study was reviewed and approved by Ethics Committee of Xiyuan Hospital, China Academy of Chinese Medical Sciences.

AUTHOR CONTRIBUTIONS

LM performed experiments, data analysis and wrote the manuscript. YC, FW, and ZL assisted in the experiments and figures. ZW and YY assisted in the data analysis. HP supervised the experiments. HL conceived the study and designed the experiments. All the authors reviewed and approved the final version of manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2020.00510/full#supplementary-material

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.