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Neuroprotective efficacy of folecitin from *Hypericum oblongifolium* against LPS-induced oxidative stress, neurodegeneration, and memory dysfunction

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Abstract

Neurological disorders, such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease, are commonly associated with persistent neuro-inflammation, and there is an urgent need to discover new therapeutic agents that may target the various pathways involved in neurodegeneration. In this study, we investigated the therapeutic potential of folecitin, a flavonoid isolated from *Hypericum oblongifolium*, against lipopolysaccharide (LPS)-induced oxidative stress associated with neurodegeneration, amyloidogenic Aβ production pathway, and memory dysfunction in mice. LPS was administered i.p. at 250 µg/kg/day for 3 weeks, followed by the administration of folecitin at a dose of 30 mg/kg/day for the last two weeks. A Western blot technique was used to assess the expression of different proteins involved in oxidative stress, neurodegeneration, and neuronal synapse. Results indicated that folecitin significantly reduced LPS-induced apoptotic neurodegeneration, including the expression of BAX, Caspase-3, and PARP-1 proteins, inhibited BACE1, and the amyloidogenic Aβ production pathway. Folecitin improved both pre- and post-neuronal synapse, as well as memory dysfunction. Furthermore, folecitin significantly activated endogenous antioxidant proteins such as Nrf-2 and HO-1 via stimulating the phosphorylation of Akt proteins. These findings suggest that folecitin may be a suitable lead to design new drugs for neurotoxin-triggered neurodegenerative disorders.

Key words: Folecitin; LPS; Memory impairment; Neurodegenerative disease; Neuroprotection

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1. Introduction

Neurological disorders (NDs), such as amyotrophic lateral sclerosis (ALS), Parkinson's disease (PD), and Alzheimer's disease (AD), are the main progressive neurodegenerative diseases worldwide. It has been reported that 46.8 million people suffered from NDs in 2015, incurring an estimated treatment cost of US$ 818 billion. By 2040, NDs are expected to be the second most common cause of death (Raz et al., 2010). NDs are characterized by the depletion (or inadequate synthesis) of neurotransmitters, oxidative stress, inflammation, and the aggregation of misfolded proteins in the central nervous system (CNS), such as *β*-amyloid and Tau proteins in AD (Bhat et al., 2015). The treatment of AD uses various therapeutic agents of natural and synthetic origin (Shaikh et al., 2014; Salehi et al., 2020), some of which such as the acetylcholinesterase inhibitors present adverse side-effects (Colovic et al., 2013). Other treatments such as anti-Tau protein and *β*-amyloid antibodies, and β-secretase 1 (BACE1) inhibitors are very expensive (Felder et al., 2000; Adewusi et al., 2011).

Plants have a long history of contributing to the discovery of new drugs. There are more than 300,000 species of higher plants worldwide, of which more than 85% have not been explored for the presence of bioactive principles (Barata et al., 2016). Plants produce a diverse range of chemicals (e.g. alkaloids, polyphenols, flavonoids, glycosides, terpenoids, and saponins) that exhibit various biological properties (Koirala et al., 2016; Enioutina et al., 2017; Koirala et al., 2020; Panthi et al., 2020; Modi et al., 2021). In Asian countries, more than 120 traditional plants-based medicines are used for the management of CNS disorders (Rodrigo et al., 2011). This includes extracts (and phytoconstituents) from various plants such as *Panax ginseng* (ginsenosides) (Bao et al., 2005), *Curcuma longa* (curcumin) (Cole et al., 2007), *Hypericum perforatum* (hyperoside) (Oliveira et al., 2016), *Centella asiatica* (catechin) (Lokanathan et al., 2016), *Bacopa monneri* (bacosides) (Abdul Manap et al.,

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2019), *Withania somnifera* (withanolides) (Birla et al., 2019), and *Ginkgo biloba* (ginkgolides) (Singh et al., 2019) that exhibited promising psychotropic and neuroprotective properties.

The genus *Hypericum* (Hypericaceae) consists of herbs and shrubs found commonly in temperate regions, and used as a source of natural medicines, pigments, dyes, gums, resins, and timbers (Crockett and Robson, 2011). Various species within the *Hypericum* genus, especially *Hypericum caprifoliatum* (Viana et al., 2005), *Hypericum perforatum* (Dinamarca et al., 2006; Valvassori et al., 2018), *Hypericum grandifolium* (Sánchez-Mateo et al., 2009), *Hypericum oblongifolium* (Raziq et al., 2015), *Hypericum polyanthemum* (Borsoi et al., 2018), and *Hypericum triquetrifolium* (Alzoubi et al., 2020) have been studied for their antidepressant, antioxidant, anxiolytic, antimicrobial, antiviral, anticancer, anti-inflammatory, and anti-ulcerogenic properties.

Hypericum oblongifolium Wall. (also known as basant, sheen chayi, Pendant St. John's Wort) grows at altitudes of 800-1200 m within the Western Himalayas (Raziq et al., 2015). It is widely distributed in Northern Pakistan. Its flowers are yellow with persistent-withering petals, and bloom from June to September (Arfan et al., 2010). The species is used traditionally for external wounds, hepatitis, gastric ulcers, and other gastrointestinal disorders (Butola et al., 2007). It has potent *in vitro* antiglycation and antioxidant properties (Abbas et al., 2013). It has also demonstrated antiproliferative activity on HT-29 human colon adenocarcinoma cells (Sajid et al., 2018). The species contains flavonoids, triterpenes, and xanthones (Ali et al., 2014). In this study, we reported on the isolation of a new flavonoid, identified as folecitin, from *H. oblongifolium*, and the evaluation of its protective activity against neuroinflammation using a lipopolysaccharide (LPS)-induced neurotoxicity assay in mice.

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2. Materials and methods

2.1. Plant materials

Fresh leaves of *H. oblongifolium* were collected from Thandiyani, Abbottabad, KPK, Pakistan. The plant was authenticated by Dr. Banaras Khan, Department of Botany, Post Graduate College Attock city, and a voucher specimen (Atk/102/2018) was deposited in the herbarium of College.

2.2. Extraction

The pulverized leaves (15 kg) were macerated in methanol:water (7:3) for 14 days with constant stirring using a steel rod. The resulting suspension was filtered through Whatman filter paper. The obtained solution was concentrated under vacuum to afford a first crude extract. The remaining plant material was re-extracted with a fresh mixture of methanol:water (7:3) for another 7 days. After filtration as aforementioned, the solution was concentrated under vacuum to afford a second extract. The combined crude extracts were re-suspended in distilled water (1 L), and liquid-liquid partition was carried out using solvents of increasing polarity starting with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol to afford *n*-hexane (640 g), chloroform (40 g), ethyl acetate (400 g), *n*-butanol (180 g), and aqueous (390 g) fractions.

2.3. Isolation of folecitin from H. oblongifolium

The ethyl acetate fraction (400 g) was subjected to column chromatography over silica gel mesh size 230 (Merck), eluting with *n*-hexane:ethyl acetate (2:8). A total of 16 major fractions were obtained, pooled on the basis of their similar chemical profiles on thin layer chromatography (TLC) using silica gel 60 PF_{254} plates (Merck). Fraction 14 (1.2 g) was further subjected to flash silica gel column chromatography, eluting with ethyl acetate:chloroform (1:1). Twelve major fractions were pooled based on TLC analysis. Fraction 5-9 led to the isolation of folecitin (70 mg) as yellow crystals. Folecitin was

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visualized on TLC plates using solid iodine and cerium sulfate ($CeSO_4$), followed by heating. The structure of folecitin was confirmed following analysis of its ^{1}H and ^{13}C NMR, COSY, HSQC, and HMBC spectra recorded on Bruker spectrometers (Avance Av 500, 600/150 MHz) and comparison with the literature data (Raziq et al., 2015). All chemical shifts (δ) and coupling constants (J) were measured in ppm and Hz, respectively.

2.4. Neuroprotective activity-

2.4.1. Ethical approval

All experimental animals were taken care of according to the approvals of the ethical committee of the Neuroprotective Medicine and Molecular Research Center, Ring road Peshawar, Pakistan (Ethical committee number: NMMRC/2019/Rodents/015). Animals were handled as per the scientific procedures of the UK Animals guidelines Act 1986.

2.4.2. Chemicals and reagents

Lipopolysaccharide, polyvinylidene fluoride (PVDF) membrane, phosphate buffered saline tablets, RNAwait solution, reagent for tissue protein extraction (T-PER), reagent for protein assay dye, sample buffer (2X Laemmli), trizma base, acrylamide, bis-acrylamide, sodium dodecyl sulfate (SDS), ammonium per sulfate (APS), TEMED, glycine, methanol, skim milk, KCl, NaCl, and Tween 20 were purchased from Sigma Aldrich (USA), and stored at the required temperature for further experimental purposes.

2.4.3. Maintenance of animals used

BALB/c adult male mice of 7−8 weeks old (weighing 30−32 g) were obtained from the Veterinary Research Institute, Peshawar, KPK, Pakistan. They were maintained separately in cages (Biobase, China), kept in a special room with a constant 12 h dark and 12 h light cycle at 27±3 °C, with easy access to food and water.

2.4.4. Experimental groups

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Animals were arbitrarily divided into 4 experimental groups (n = 6). The control group-1 was administered with normal saline (C). Group-2 was intraperitoneally (i.p.) injected with LPS (250 µg/kg) daily for three consecutive weeks (LPS). Group-3 was injected i.p. with LPS (250 µg/kg/day) for three weeks plus folecitin (30 mg/kg) for the last two weeks of the experiment (LPS+F). Group-4 was injected i.p. with folecitin (30 mg/kg) alone for the last two weeks of the experiment (F).

2.4.5. Western blot

Western blotting was performed according to the method of Badshah et al. (2016) with minor modifications. The mice brains were collected as quickly as possible and then homogenized in a T-PER solution. The levels of proteins in all 4 groups were measured using a BioRad protein assay. For each of the 4 groups, 30 µg of the protein content was run on a 15-20% SDS–PAGE. After completing the electrophoresis process, all the proteins were shifted to a PVDF membrane (Santa Cruz Biotech, USA) over trans-blot (Bio-Rad). Different primary monoclonal antibodies (Santa Cruz, CA, USA) such as Caspase-3, Bcl-2-associated X protein (BAX), Beta-secretase-1 (BACE-1), Poly (ADP-ribose) polymerase-1 (PARP-1), Amyloid beta (Aβ), Synaptophysin (SYP), postsynaptic density protein 95 (PSD-95), and beta actin as well as HRP-conjugated secondary antibodies (Santa Cruz, CA, USA) were smeared. All results were visualised in a dark room on X-ray films.

2.4.6. Behavioral tests-

Two well-known behavioral tests, the Morris water maze (MWM) and the Y-maze, were conducted in order to investigate the neuroprotective effect of folecitin on learning and remembrance behaviors in adult mice.

2.4.6.1. MWM test

The MWM test was performed to study the four-dimensional learning (spatial learning) and commemoration in adult mice (Rehman et al., 2017) . The MWM apparatus was made of

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a circular water tank of about 1.8 m in diameter and 0.6 m height filled with normal tap water (25-28 °C). Skimmed milk powder (1 kg) was added to make the water appearance opaque. A grey escape plastic platform (35.5 cm in height) was located 1 cm underneath the water surface near the middle of any one of the four corners of the maze apparatus. This unique stage could be withdrawn to the bottom of the tank or raised to its normal place on the maze apparatus during behavioral learning and testing. A second stand (36.5 cm in height) black in colour was raised 2 cm over the water layer during the initial 3 days of training. The MWM was encircled by white curtains with fixed patterns in order to provide a configuration of three-dimensional signals. Observations were made using a video tracing system (HVS Image Analyzing VP-112) with an in-built software.

All the experimental mice were trained for 3 days as per the protocol of Shah et al. (2016). All the mice were allowed to receive 3 training trials per day for 5 successive days (60s per trial). On each training test, an animal was released freely in the MWM from one of four equally spread out starting positions round the border of the water tank. If any animal did not locate the escape stage within 80s on any training session, it was guided and placed for 30s on the platform. After resting for 2 days, all the animals were subjected to the probe test. The tested animals were forced to search for the hidden stage. The time consumed in the particular quadrant by every animal was recorded.

2.4.6.2. Y-maze test

The Y-maze test consisted of 3 arms of 60 cm length, 12 cm diameter (width), and 22 cm height at an angle of 120° from each other (Shah et al., 2016; Rehman et al., 2017). For the first 2 days, the animals were trained for 10 min so that they could adapt to their new surroundings. Then, for the next 3-4 days of the test, all the mice were allowed to search and explore the Y-maze for 8 min by keeping them in the center of the arms. A build-in camera with software was used to record the movements of mice, total arm entrances, and successive

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number of triplets. The percentage (%) of repetitions was calculated as per the equation below:

$$\text{Repetition (\%)} = (\text{consecutive sets of triplets/total number of arm entries}_{-2}) \times 100$$

The changes and repetition (%) were associated positively with spatial functioning memory.

2.5. Statistical analysis

Results obtained were calculated as group mean±SEM and analysed using one-way or two-way analysis of variance (ANOVA) followed by suitable *post hoc* tests. All the statistical investigations were conducted using the GraphPad Prism-5 software. *P* values less than 0.05 were considered significant.

3. Results

3.1. Effect of folecitin on LPS-induced neurodegeneration

Different apoptotic indicators such as BAX, caspase-3, and PARP-1 protein expressions were analyzed with the Western blotting technique. Results revealed that a 3-week administration of LPS induced widespread neurodegeneration in the brain of adult mice. LPS induced an increase in the pro-apoptotic BAX protein expression ($P<0.001$), and also triggered Caspase-3 protein expression ($P<0.001$). Finally, LPS induced neuronal DNA fragmentation by increasing PARP-1 protein expression ($P<0.001$). Interestingly, the administration of folecitin during the last 2 weeks of the experiment significantly inhibited pro-apoptotic-BAX ($P<0.001$), followed by low levels of caspase-3 ($P<0.01$) and PARP-1 ($P<0.001$) proteins (**Fig. 1)**.

3.2. Effect of folecitin on LPS-induced amyloid beta (Aβ) production

The 3-week administration of LPS significantly increased beta-secretase activity with significant ($P<0.001$) up-regulation of the BACE-1 protein expression (which produces fragments of Aβ precursor protein to cut into toxic Aβ fragments). Similarly, the expression of Aβ protein (both oligomers and monomers) was significantly ($P<0.001$) increased after

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LPS administration. In contrast, treatment with folecitin not only significantly (P<0.05) inhibited BACE-1 expression but also significantly (P<0.001) decreased both oligomers and monomers in the brain homogenates mixture of adult mice (**Fig. 2)**.

3.3. Effect of folecitin on neuronal synapse

LPS administration suppressed both pre-SYP and post-SYP proteins (P<0.001), and PSD-95 (P<0.05). The administration of folecitin markedly improved the protein expressions of both pre- and post-SYP proteins (P<0.01, P<0.001) (**Fig. 3)**.

3.4. Effect of folecitin on stimulation of phosphorylated-Akt (p-Akt) to activate Nrf-2 /HO-1

LPS administration significantly suppressed the protein expressions of p-AKT (P<0.001), nuclear factor erythroid 2–related factor 2 (Nrf-2) (P<0.01), and HO-1 (P<0.001), similar to the control group. In contrast, the administration of folecitin for two weeks stimulated p-Akt protein significantly (P<0.001). It was accompanied by the stimulation of Nrf-2 (P<0.001) and HO-1 (P<0.01) protein expression in the brain homogenates of mice (**Fig. 4)**.

3.5. Effect of folecitin on behavior and LPS-induced memory deficits

The MWM and Y-maze tests were employed to check the memory improving ability of folecitin. In the MWM test, LPS-treated mice displayed significantly (P<0.001) higher mean latencies from day 1 to day 5. There was a gradual decrease in the mean escape latency from the start to the end of experiment, suggesting that these mice had impaired memory. There was a little decrease in the mean latency on a daily basis from day 1 to day 5. On the other hand, the mice treated with LPS+folecitin showed better memory from day 1 to day 5. The performance of these mice was better with reduced mean escape latencies from day 1 to day 5 compared to LPS-treated mice (P<0.05, P<0.001). The experimental mice treated with folecitin alone showed escape latencies similar to the control group from the beginning to the end of the test, suggesting that they have no memory deficiency (**Fig. 5A)**. On day 9, in the

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probe test, the control animal spent more time as compared to the folecitin treated animals. Among the other 2 groups, the mice treated with LPS+folecitin had spent more time (P<0.001) in the marked quadrant, similar to the LPS-treated mice (**Fig. 5B)**. The short term memory was investigated by performing Y-maze test. The control animals showed higher percentage of spontaneous alteration, while the LPS-treated animals displayed less percentage of spontaneous alteration (P<0.001). Similarly, animals treated with LPS+folecitin showed a significantly (P<0.01) higher percentage of spontaneous alteration, similar to the LPS-treated mice (**Fig. 5C**).

4. Discussion

Alzheimer's disease is one of the main neurodegenerative diseases, and a leading reason of death and disability (Weuve et al., 2014). In aging individuals, AD leads to a gradual impairment in cognition and neuronal dysregulation of communication. There are many evidences of involvement of neuroinflammation, apoptosis, and oxidative stress in the pathogenesis of AD and its associated neurodegenerative diseases (Gan and Johnson, 2014). In particular, it has been reported that the microglial activation plays an important role in triggering excessive oxidative stress when high levels of reactive oxygen species (ROS) are present (Cobb and Cole, 2015). Intracellular ROS in microglial cells causes inflammation and ultimately leads to neuronal cell death (Bedard and Krause, 2007). The level of endogenous ROS can be controlled by antioxidant molecules and antioxidative enzymes *via* the Nrf-2)/antioxidant response element signal pathways (Pajares et al., 2017).

Studies have shown that the LPS-stimulation in the brain impairs learning and memory (Sell et al., 2001; Swiergiel and Dunn, 2007; Badshah et al., 2016). This was also observed in the present study. The MWM test results showed that mice receiving LPS had more mean escape time latencies and travelled longer distance to find the escape platform, as compared to the control group. Our probe trial results also demonstrated that animals receiving LPS

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failed to find the exact location of the submerged platform, which is why they spent less time in the target quadrant. On the other hand, animals receiving LPS+folecitin displayed a good recognition pattern, and consumed more time in the target quadrant.

Our study also demonstrated that folecitin significantly reduced the expression of apoptotic markers such as BAX, PARP-1, and Caspase-3 proteins against LPS in the mature mice brain. Moreover, here we hypothesized that folecitin could activate Nrf-2/HO-1 against LPS-induced oxidative stress. Our results indicated that folecitin increased the nuclear translocation of Nrf-2/HO-1, and its production to inhibit LPS-induced oxidative stress. Among different genes involved in the antioxidative process, HO-1 has been recognised to exert strong protection via various mechanisms. The Akt signaling pathway which regulates various functions such as apoptosis, cell proliferation, and cellular protection, is known to modulate Nrf-2 (Sparkman et al., 2005; Yu et al., 2015; de Oliveira et al., 2015). Crosstalk between the PI3K/Akt and Nrf2 signaling pathways effectively protect cells against inflammatory and oxidative damage (Padiya et al., 2014; Reddy et al., 2015; Badshah et al., 2016). Results showed that folecitin induced a significant augmentation of Akt protein phosphorylation which had a direct effect on the Nrf-2-mediated antioxidant activity. An overview of the mechanisms of action of folecitin is illustrated in **Fig. 6.**

5. Conclusion

Administration of LPS to the brain of adult mice induced oxidative stress-mediated neurodegeneration as well as neuronal synapse and memory dysfunction. Folecitin abrogated the LPS-induced oxidative stress and neurodegeneration by reducing the amyloidogenic Aβ production and improving associated neuronal synapse and memory impairment. Moreover, folecitin stimulated the p-Akt signaling pathway to activate HO-1/Nrf-2. Owing to its antioxidant potential and ability to improve memory deficits, folecitin might be a promising lead in designing new drugs for neurotoxin-triggered neurodegenerative disorders.

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Authors' contributions

Conceptualization, U.F., S.A.S., and T.K.; Methodology, U.F., S.A.S.; Software, U.F. and S.A.S.; Validation, T.K., R.U. and N.R.; Formal Analysis, T.K., R.U., N.R., and M.S.; Investigation, U.F. and S.A.S.; Resources, U.F.; Data Curation, T.K. and A.K.; Writing – Original Draft Preparation, U.F., S.A.S., and T.K.; Writing – Review & Editing, U.F., A.K., M.U.K.S., and V.S.; Visualization, A.K., M.U.K.S., and V.S.; Supervision, T.K. and M.U.K.S.; Project Administration, T.K.; Funding Acquisition, A.A., G.E-S.B., and N.K.

Conflict of interest

The authors declare that they have no conflict of interest.

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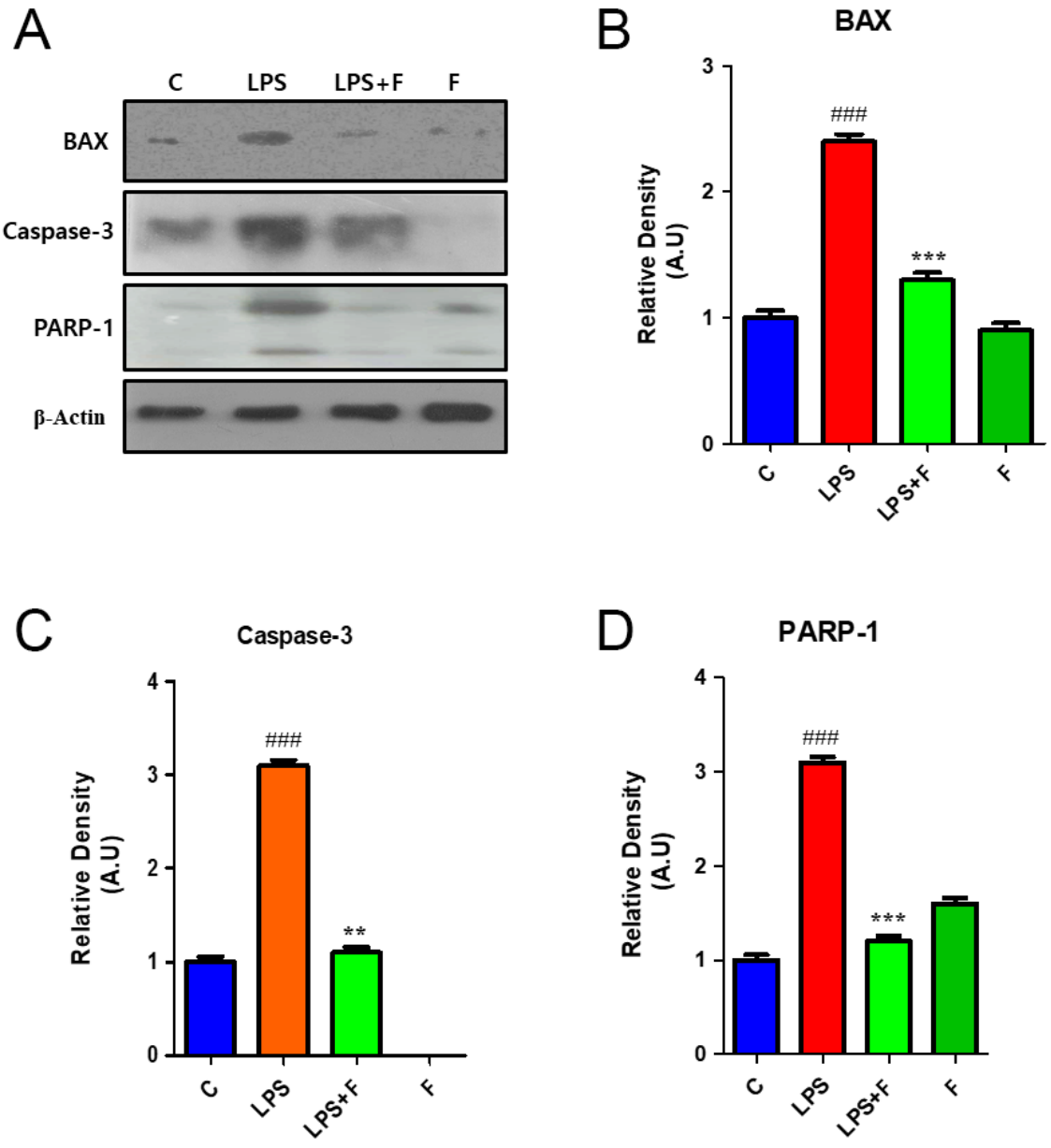

Fig. 1: Reduction of apoptotic neurodegeneration by folecitin. **(A)** Immunoblots of BAX, PARP-1, and Caspase-3 in the brain homogenates of the adult mice for the experimental groups including C, LPS, LPS+F, and F alone. Histogram of **(B)** BAX, **(C)** Caspase-3, **(D)** and PARP-1. β-actin was used as a loading control (reference control). The bands were analysed using the Image J software, and the density histograms (expressed in A.U) compared to the control were organized using GraphPad Prism. Values are presented as the mean±SEM for the indicated proteins (n = 6 mice per group).

C – control, LPS – lipopolysaccharide, F – folecitin, A.U – arbitrary units, $^{###}P < 0.001$ as compared to C group, $^{**}P < 0.01$, and $^{***}P < 0.001$ as compared to LPS group.

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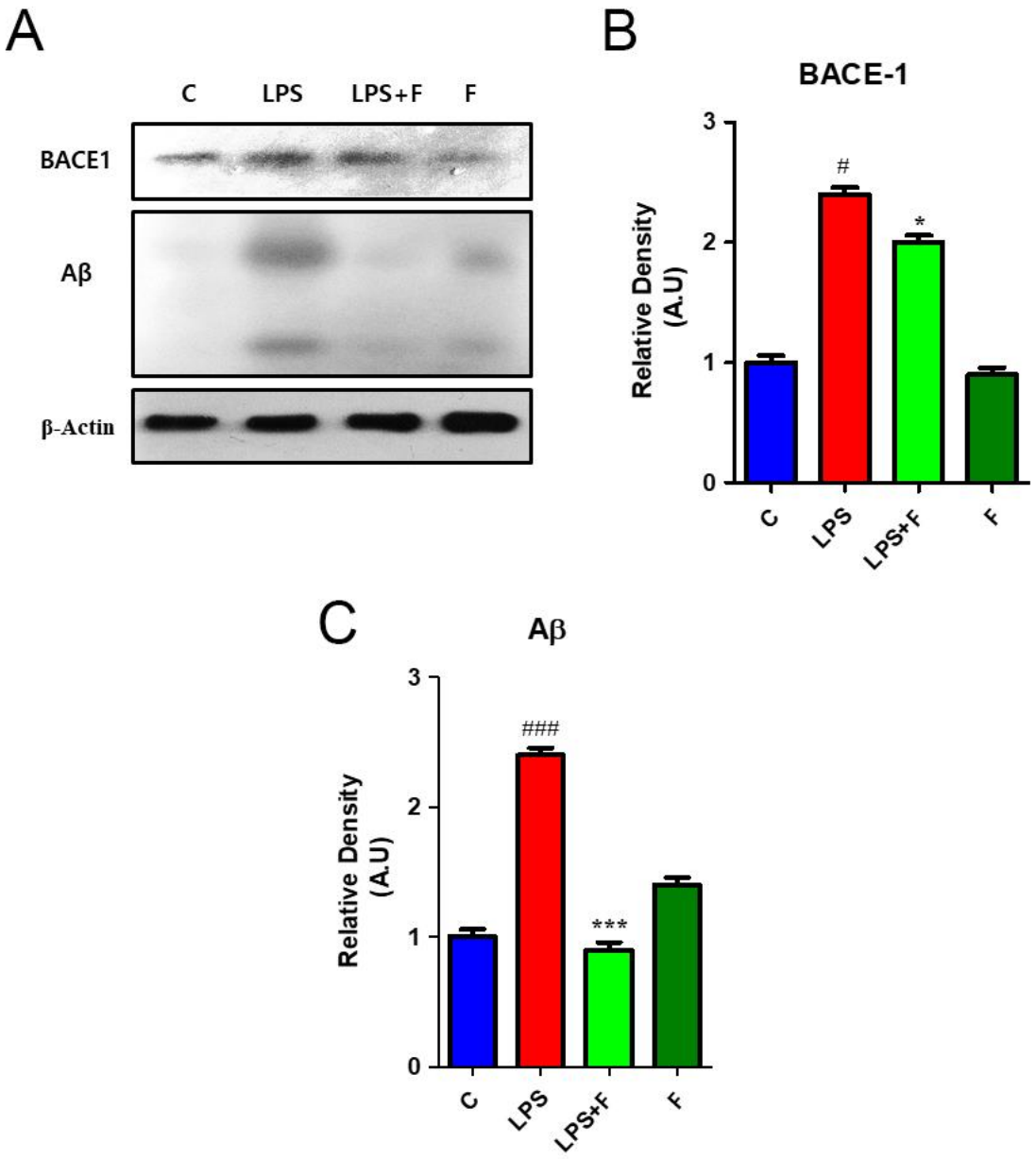

Fig. 2: Reduction of Aβ production by folecitin via inhibiting BACE-1 expression. (**A**) Immunoblots of BACE1 and Aβ in the brain homogenates of the adult mice for the experimental groups including C, LPS, LPS+F, and F alone. Histogram of **(B)** BACE1 and **(C)** Aβ. β-actin was used as a house keeping loading control (reference control). The bands were analysed using the Image J software, and the density histograms (expressed in A.U) compared to the control were organized using GraphPad Prism. Values are presented as the mean±SEM for the indicated proteins (n = 6 mice per group).

C – control, LPS – lipopolysaccharide, F – folecitin, A.U - arbitrary units, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ as compared to C group, $^{*}P < 0.05$, $^{***}P < 0.001$ as compared to LPS group.

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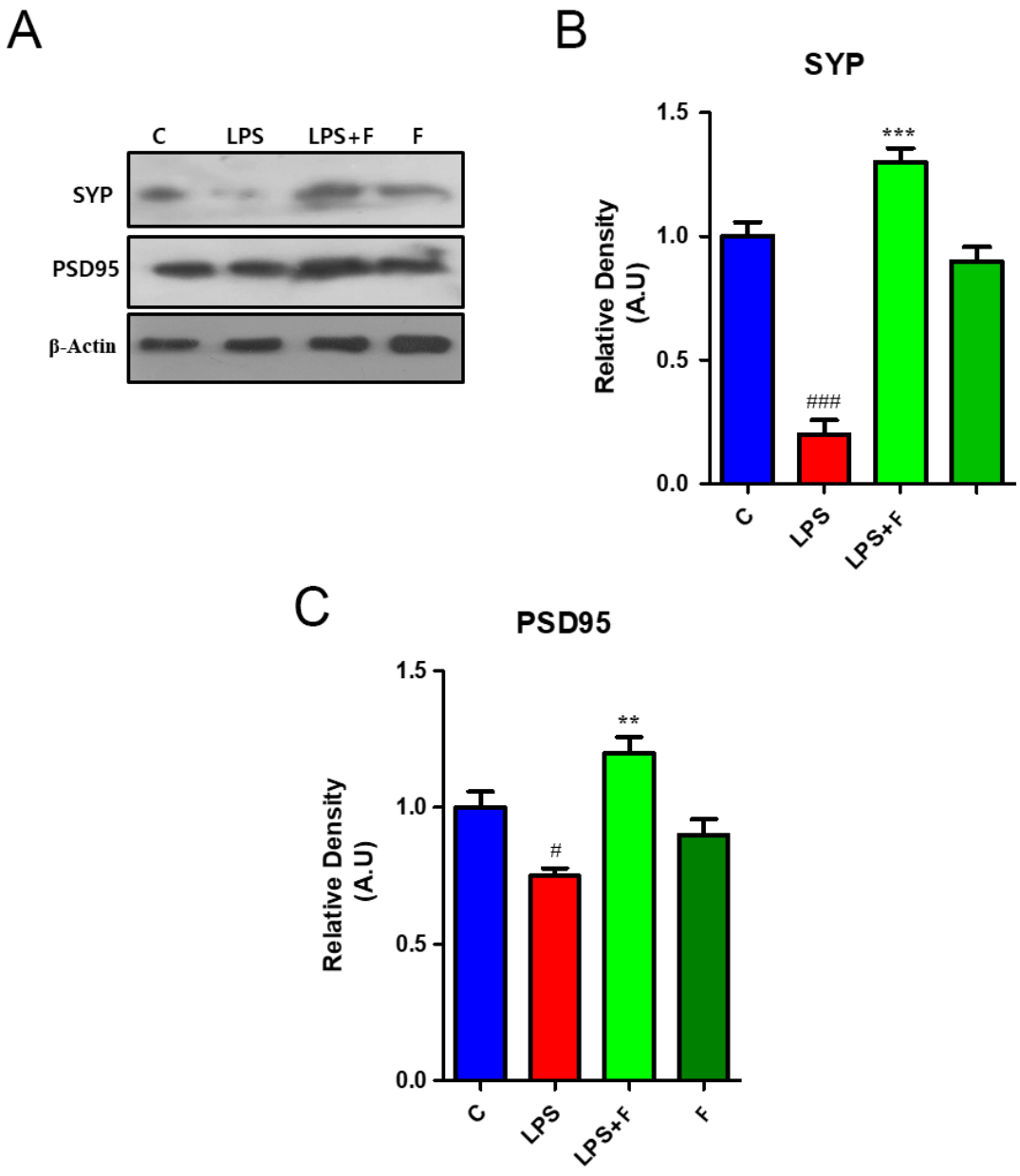

Fig. 3: Enhancement of both pre-and post-SYP expression. **(A)** Immunoblots of SYP and PSD95 proteins in the brain homogenates of male adult albino mice for the experimental groups including C, LPS, LPS+F, and F alone. Histograms of **(B)** SYP and **(C)** PSD-95. Values are presented as the mean±SEM for the indicated proteins (n = 6 mice per group).

C – control, LPS – lipopolysaccharide, F – folecitin, A.U – arbitrary units, $^{\#}P < 0.05$, $^{\#\#\#}P < 0.001$ as compared to C group, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to LPS group.

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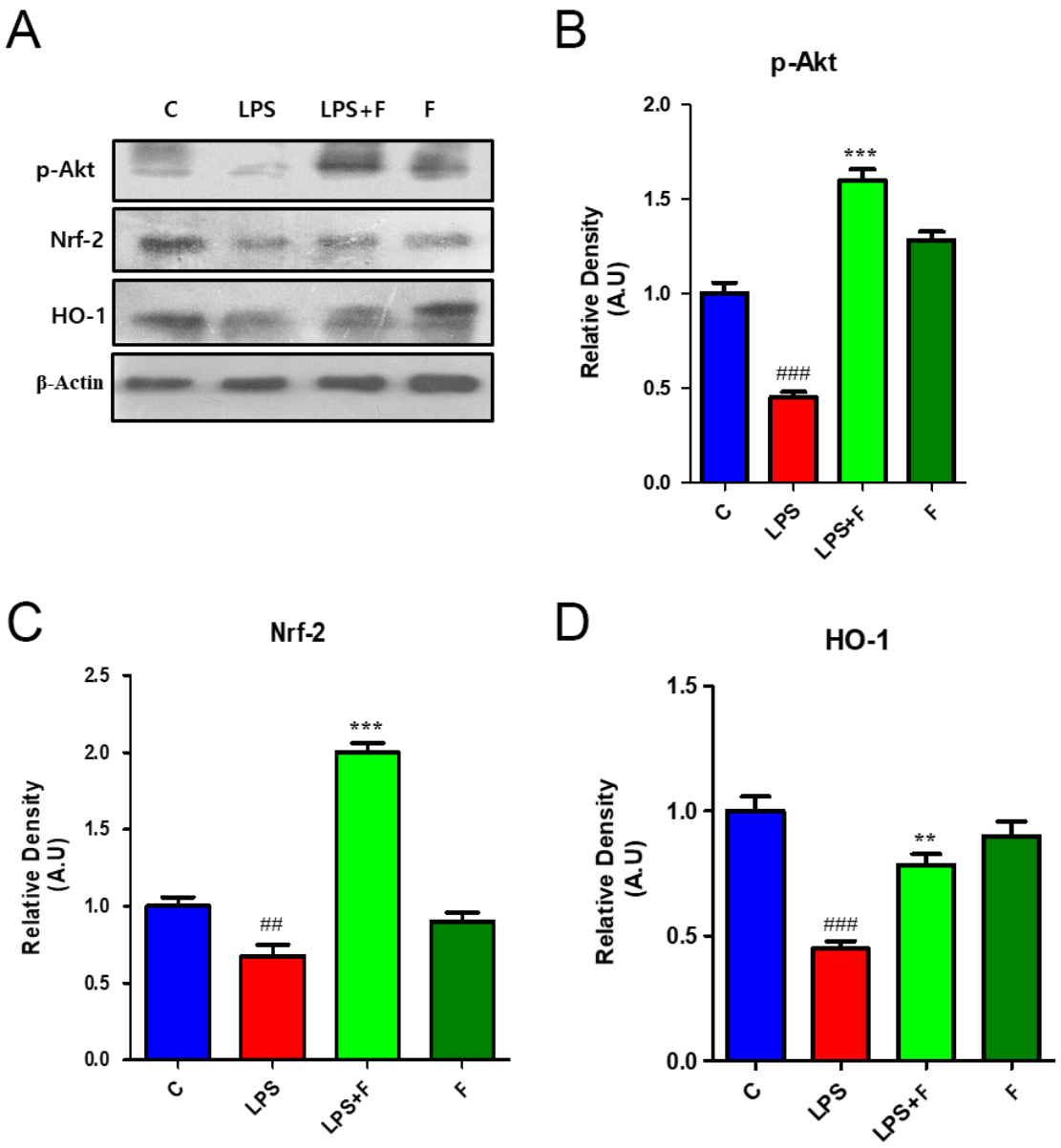

Fig. 4: Stimulation of p-Akt, Nrf-2, and HO-1 expression by folecitin. **(A)** Immunoblots of p-Akt, HO-1, and Nrf-2 proteins for the experimental groups including C, LPS, LPS+F, and F alone. Histogram of **(B)** p-Akt, **(C)** Nrf-2, and **(D)** HO-1 proteins. β-actin was used as a loading control (reference control). The bands were analysed using the Image J software, and the density histograms (expressed in A.U) compared to the control were organized using GraphPad Prism. Values are presented as the mean±SEM for the indicated proteins (n = 6 mice per group).

C – control, LPS – lipopolysaccharide, F – folecitin, A.U – arbitrary units, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ as compared to C group, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to LPS group.

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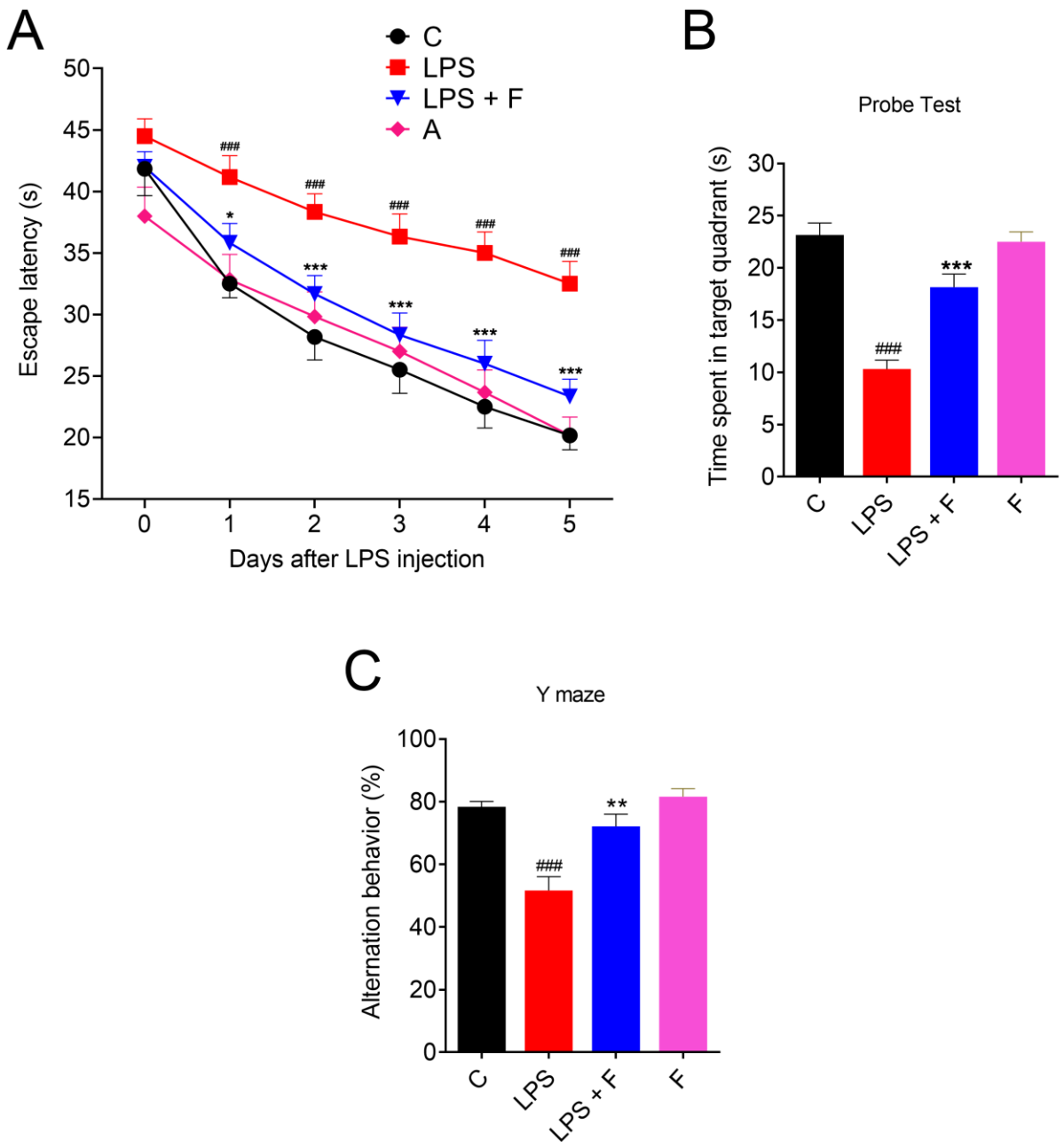

Fig. 5: Improvement of LPS-induced memory impairment by folecitin. **(A)** Effect of folecitin on escape latency in the MWM test. **(B)** Effect of folecitin in the probe test (time consumed in the target quadrant on day 5) showed during MWM test when the immersed platform was removed. **(C)** Effect of folecitin on alternation behavior in the Y-maze test. Values are presented as the mean±SEM (n = 6 mice per group).

C – control, LPS – lipopolysaccharide, F – folecitin, $^{###}P < 0.001$ as compared to control group, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ as compared to LPS group.

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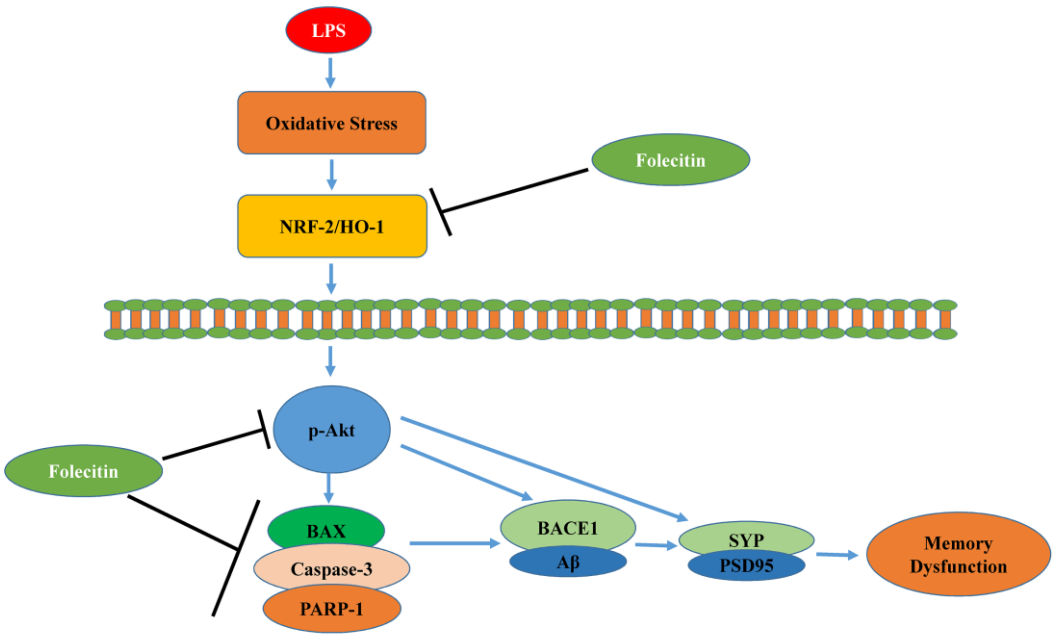

Fig. 6: Proposed mechanism of folecitin neuroprotection action against LPS-induced oxidative stress mediated memory impairment. Folecitin activated the p-Akt/HO-1/Nrf-2 signaling pathway, leading to a reduction in oxidative stress, neurodegeneration, and associated neuronal synapse and memory impairment.