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Targeting LPS-induced Neuroinflammation with Puerarin: Mechanistic Insights into the TLR4/NF-κB/NLRP3/IL-1β Pathway

Hesham A. Malak[1,2]

[1]Department of Biology, Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia, [2]Research Laboratories Centre, Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia.

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- Neuroinflammation,
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Abstract

Abstract: Puerarin (Pur), a famous isoflavonoid, can be used in the regulation of many biological and clinical conditions. However, its role in protecting against LPS-induced neuroinflammation is still unclear. This study supposed to assess the protective effect of Pur against LPS-induced memory impairment and hippocampal inflammation. The study included three groups of mice: Control group (n=6), LPS group (n=6), and LPS + Pur group. In the LPS group, there was a marked increase in the time to reach the target box (TTB) and WME and RME compared to control mice, indicating impaired spatial memory and learning. This was also reflected in the histological picture of hippocampal neuronal damage. Additionally, LPS induced a classic picture of hippocampal oxidative stress, evidenced by elevated MDA levels and depletion of GSH and GPx. Moreover, LPS caused neuroinflammation, as shown by increased protein levels of hippocampal TLR4and NF-κB, along with the consequent secretion of IL-1β, TNF-α, IL-6. Furthermore, intraperitoneal injection of LPS significantly increased the levels of pyroptotic markers (NLRP3, caspase-1, IL-1β, IL-18, GSDMD) compared to normal mice. Meanwhile, oral intake of Pur improved the cognitive functions of LPS-treated mice by decreasing memory impairment parameters. These protective effects are attributed to its preservation of hippocampal neuron structure, its antioxidant properties, and its inhibition of LPS-activated TLR4/NF-κB and NLRP3/IL-1β pathways, thereby reducing hippocampal inflammation and pyroptosis. Overall, we can conclude that Pur has the ability to mitigate the negative impact of LPS on memory function in mice through its anti-inflammatory effects. Thus, it might be used as a novel protective agent against clinical forms of LPS-induced cognitive impairment.

Corresponding author: Malak, Hesham A. Assistant Professor, Email. hamalak@uqu.edu.sa Tel. 00966536430303, Department of Biology, Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia

Introduction

Neuroinflammation offers a crucial role in the pathology of cognitive impairment and neurodegenerative disorders [1,2]**.** Injection of LPS, from the wall of gram-negative bacteria, induces significant neuroinflammation through the release of inflammatory cytokines. [3,4]. It binds to TLR4, which is expressed in many supporting glial cells such as astrocytes, microglia, and macrophages. [5]. After initiation of TLR4 by LPS, the NF-κB inflammatory signaling pathway is activated, mediating the neuroinflammatory process. [6]. Many studies have reported that LPS application induces cognitive impairment in mice through its neuroinflammatory effects. [7-9]. Microglial activation plays an important role in the neuroinflammatory response through the secretion of NO, ROS, and inflammatory chemokines. [10]. Recent studies have focused on the inflammasome complex as a regulatory factor in inflammatory diseases. [11,12]. A well-known inflammasome, NLRP3, activates caspase-1, which induces IL-1β and IL-18 production [11]. Inhibition of NLRP3 is involved in the treatment of several inflammatory diseases [13,14].

In recent years, Pueraria lobata, a famous Chinese traditional plant, has been used in many functional foods and medications [15]. The active compound pur can cross the BBB, providing many beneficial biological effects such as antioxidant, antiapoptotic, anti-inflammatory, and autophagy modulation. [16]. It is used Clinically, it is used in the prevention and treatment of many disorders such as diabetes, AD, tumors, and PD [17,18]. Puerarin's neuroprotective role has been previously examined in hydroxydopamine-induced neuronal injury, highlighting its anti-apoptotic and antioxidant characteristics [19-21]. Moreover, another study discussed the protective effect of puerarin against neuronal damage in a traumatic brain model, attributing this to its antioxidant effect [22]. Therefore, we were encouraged to investigate the elucidative effect of puerarin against LPS-induced cognitive impairment and neuroinflammation, exploring the involved mechanisms.

2. Material and Methods

2.1. Animals

In the present work, we used eighteen male ICR mice weighing between twenty-five and thirty-five grams. They were classified into three groups (n=6) and lived in polypropylene cages with unlimited supply of food and water. The study was conducted in accordance with the National Institutes of Health guidelines for the care and use of laboratory animals (NIH Publication No. 8023, revised 1996).

2.2. Experimental design

The study included three groups as follows:

1. **Control group**: This group received normal saline (10 mL/kg) via oral gavage for thirty days.
2. **LPS group**: Mice in this group received normal saline throughout the experiment but were injected intraperitoneally with a dose of 250 µg/kg on days twenty-three, twenty-four, twenty-five, and twenty-six to induce neuroinflammation [23,24].
3. **LPS + Pur group**: Animals in this group received Pur at a dose of 200 mg/kg body weight via oral gavage [25] for thirty days, along with the previously mentioned dose of LPS as in the LPS group.

During the experiment, behavioral evaluation was conducted using the Radial Arm Maze. The animals were trained from days twenty-four to twenty-six, preceded by diet restriction from day sixteen today twenty-three. Memory evaluation was then performed on days twenty-seven, twenty-eight, twenty-nine, and thirty.

2.3. Behavioral Assessment Using the Radial Arm Maze (RAM)

In this study, the Radial Arm Maze (RAM) was used to evaluate the memory and spatial learning of mice. The maze consisted of eight arms, each measuring 48 × 12 centimeters, connected to a central platform with a diameter of 32 centimeters. The procedures followed were based on previous studies [23,26]. Behavioral assessments were conducted in three phases: food restriction, training, and memory evaluation. Of the eight arms, the first, third, fourth, sixth, and seventh arms were baited with food, while the second, fifth, and eighth arms were non-baited. A seven-day diet restriction, from days 16 to 23 of drug treatment, was implemented to create a food motivation. During the training phase, which spanned from day 24 to day 26, food was scattered throughout the maze on the first day, allowing the animals to explore for five minutes. On the following days, only the baited arms contained food. During the test phase, from day 27 to day 30 of the treatment time, memory parameters such as the time taken to consume all five baits (TTB), working memory errors (WME), and reference memory errors (RME) were recorded over five minutes of exploration. WME was defined as the total number of re-entries into baited arms that had already been visited, while RME referred to the total number of entries into non-baited arms [26].

2.4. Determination of inflammatory and pyroptotic markers

After the animals were sacrificed, their brains were removed, homogenized, and then centrifuged at 4000 rpm for ten minutes. The resulting supernatant was examined for TLR4 (Cat# MBS2702402), MyD88 (Cat# MBS2533515), NF-κB (Cat# MBS2023542), TNF-α (Cat# BMS607-3), IL-1β (Cat# BMS6002), IL-6 (Cat# KMC0061), NLRP3 (Cat# MBS7606270), caspase-1 (Cat# E-EL-M0201), IL-18 (Cat# BMS618-3), and GSDMD (Cat# ab233627) using ELISA kits, following the manufacturers' instructions.

2.5. Pathological Evaluation

Following the mice authentication, the brains were removed and cut coronally to make sections of the hippocampus. These sections were then fixed, embedded in paraffin blocks, and cut into four-micron sections on glass slides for H&E staining and examination under a light microscope [27].

2.6. Assessment of the Oxidative Stress Biomarkers

Malonaldehyde (MDA), a well-known lipid oxidation marker, was assayed in hippocampal homogenate in accordance with [28]. Additionally, GSH and GPx were measured following the procedures of [29,30].

2.7. Statistical Analysis

One-way analysis of variance (ANOVA) was performed in our study using GraphPad Prism (version 8.0.2), followed by Tukey's test to compare the results between different study groups. A p-value of less than 0.05 was considered significant.

3. Results

3.1. Mitigative effect of Pur against LPS-induced memory impairment with hippocampal histological improvement

As shown in Figure 1, the LPS group showed a significant ($p < 0.05$) increase in the TTB time over four days, indicating a clear deterioration in the memory of the mice [Fig. 1A]. Additionally, LPS-treated mice displayed a marked increase in WME compared to control animals, indicating an impairment of working memory [Fig. 1B]. The LPS group also revealed a significant increase in RME numbers compared to control mice over the four days [Fig. 1C], reflecting deterioration in refractory memory. Conversely, co-treatment with Pur and LPS significantly ($p < 0.05$) decreased TBB, WME, and RME compared to the LPS group, indicating that Pur had a positive impact on the memory of mice affected by intraperitoneal injection of LPS.

Histological examination of hippocampal sections (CA2, CA3) in the LPS group showed marked neuronal degeneration in the form of neuronal apoptosis and shrinkage, with obvious vacuolations and vascular congestion [Fig. 2C, D] compared to the control group [Fig. 2A, B]. However, oral intake of 200 mg/kg of Pur with LPS displayed significant histological improvement, with a decrease in the number of damaged neurons and vacuolations [Fig. 2E, F]. The clear improvement in neuronal histology by Pur supports its positive effect on memory.

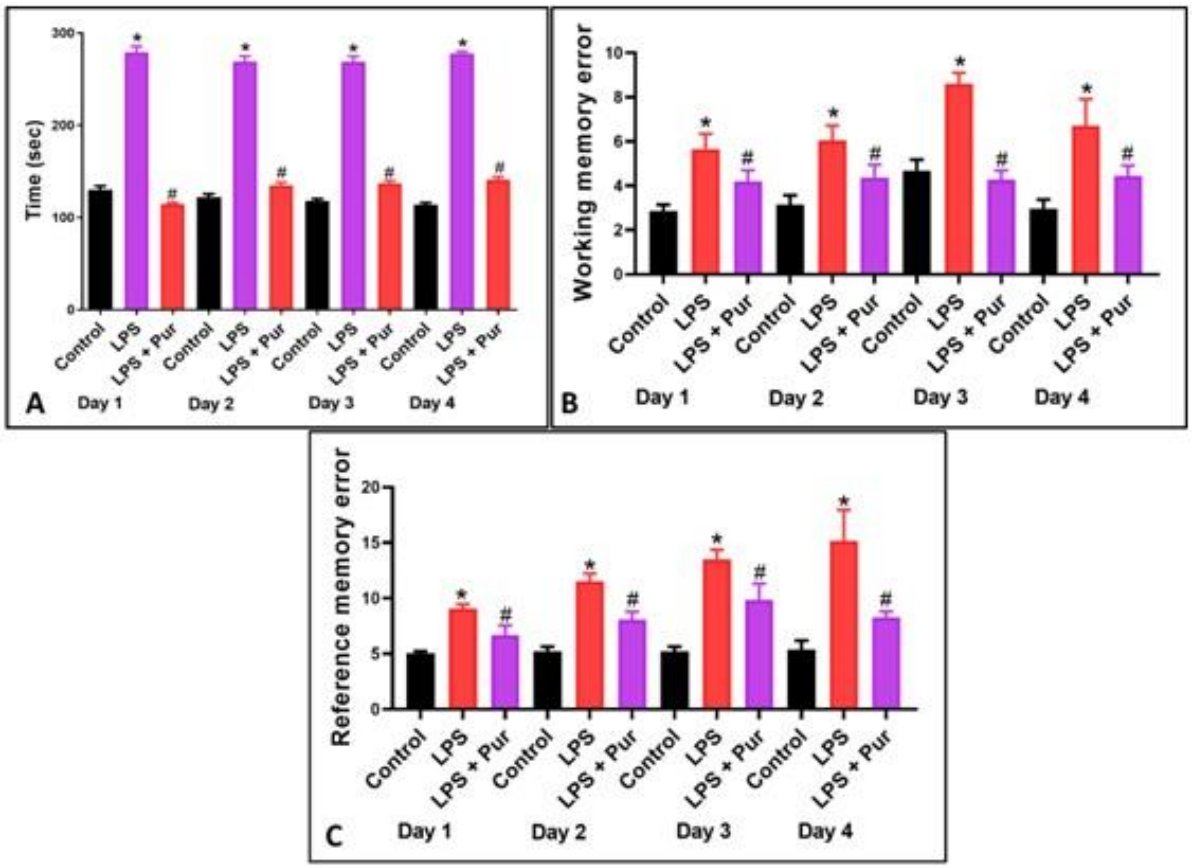

Fig. 1. Impact of Pur on behavioral testes (A)(TTB)the time which taken to consume in the five baits, (B) WME, (C) RME. All numbers represented as mean±SD. *p<0.05 significant to control, #$p$significant to LPS.

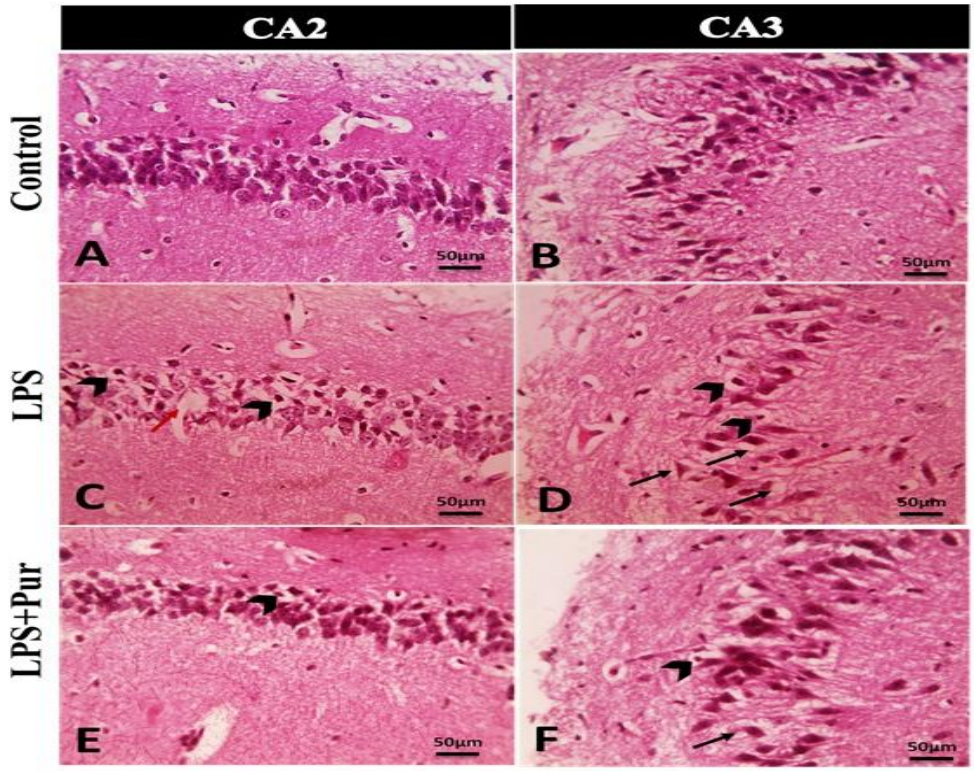

Fig.2. H&E hippocampal sections revealed a normal pyramidal neuron in CA3&CA2 in control mice (A,B). Hippocampal sections from LPS group showing marked neural shrinkage &apoptosis (arrowheads) with dilated blood vessels (red arrows) in CA2 (C), neural shrinkage &apoptosis (arrowheads) and vacuolation (black arrow) in CA3 (D). Hippocampal sections from LPS+ Pur group showing mild neural shrinkage & apoptosis (arrowheads) and vacuolation (black arrow) in CA2&CA3 (E,F). X: 400 bar 50.

3.2. Antioxidant effect of Pur on LPS-induced hippocampal oxidative stress

As shown in Figure 3, LPS-treated mice exhibited upsurge in hippocampal homogenate MDA, with a decline in the levels of antioxidants GSH and GPx compared to control mice. Meanwhile, Group III, treated with both LPO and Pur, showed a reduction in MDA and rise in GSH and GPx, confirming the powerful antioxidant function of Pur.

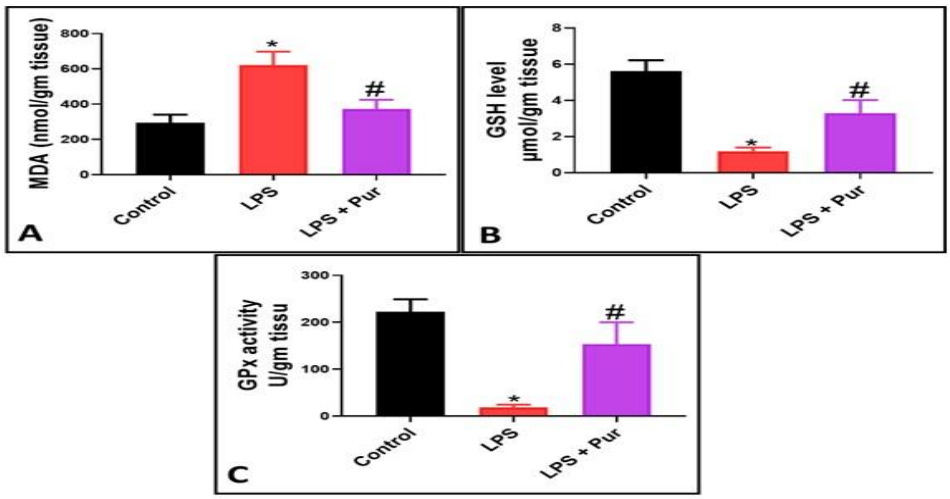

Fig. 3. Modulatory effect of Pur on (A)MDA, (B) GSH, (C) GPx. All results expressed as mean±SD. **p*<0.05 vs normal mice, #*p*vs LPS mice.

3.3. Impact of Pur on TLR4/NF-κB pathway in LPS-induced neuroinflammation

Intraperitoneal administration of LPS significantly ($p < 0.05$) increased the protein levels in the TLR4 pathway, including TLR4, MyD88, NF-κB, TNF-α, IL-1β (Fig. 4A-E), and IL-6 (Fig. 4F), compared to the control group. On the other hand, treatment with Pur and LPS produced a remarkable decrease in these inflammatory markers compared to the LPS group. From these results, we can conclude that Pur has an anti-inflammatory effect against neuroinflammation.

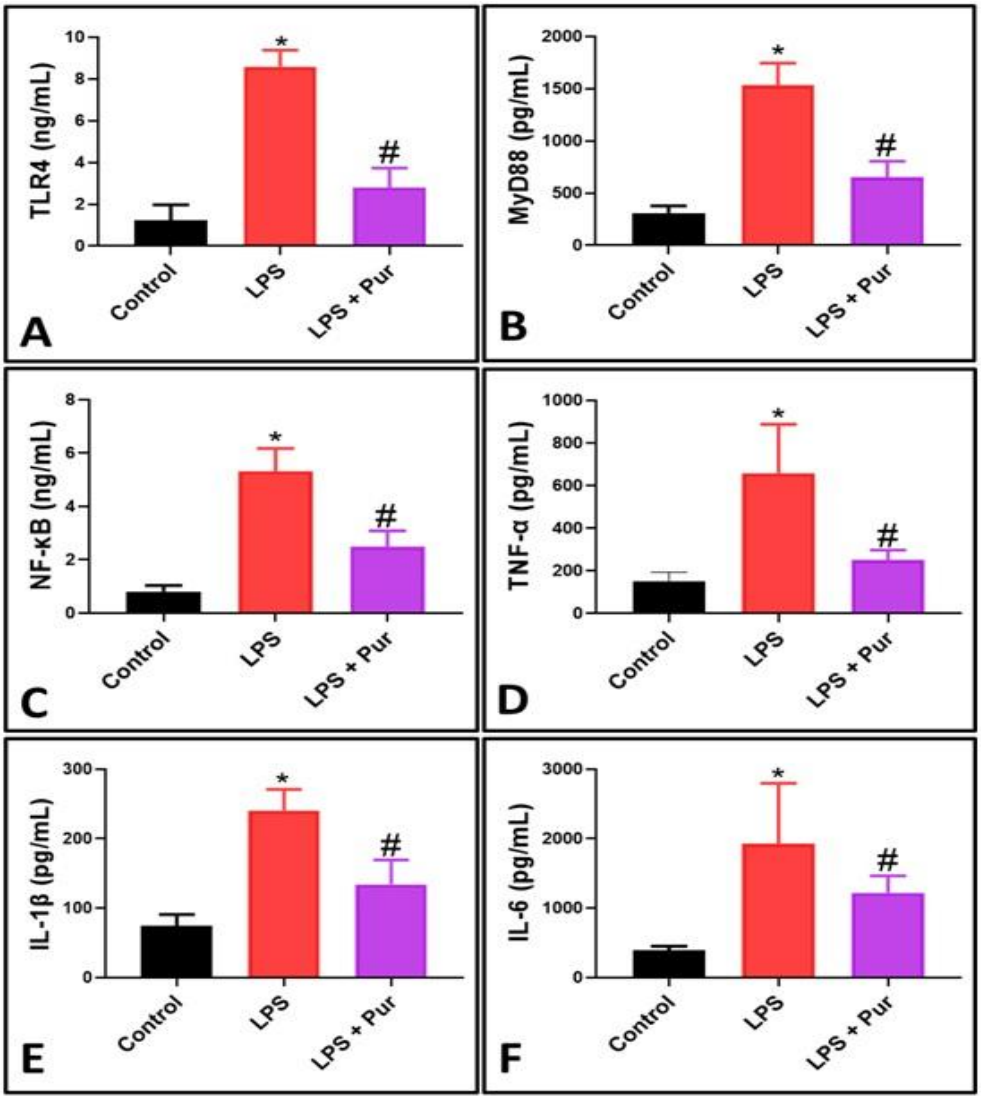

Fig. 4. Downregulatory impact of Pur on (A)TLR4, (B) MyD88 (C) NF-κB, (D) TNF-α, (E) IL-1β, (F) IL-6. All data represented as mean±SD. *p<0.05 indicates significancy to control animals, #$p$indicates significancy to LPS animals.

3.4. Negative effect of Pur on NLRP3/IL-1β pyroptotic pathway

LPS injection in Group II markedly elevated the ELISA levels of the NLRP3 activation cascade, including increased NLRP3 (Fig. 5A), Caspase-1 (Fig. 5B), IL-18 (Fig. 5C), and GSDMD (Fig. 5D), compared to control animals. However, combined treatment with LPS and Pur reversed these effects, inhibiting the process of pyroptosis in the mice hippocampus and preventing the inflammatory form of cellular death.

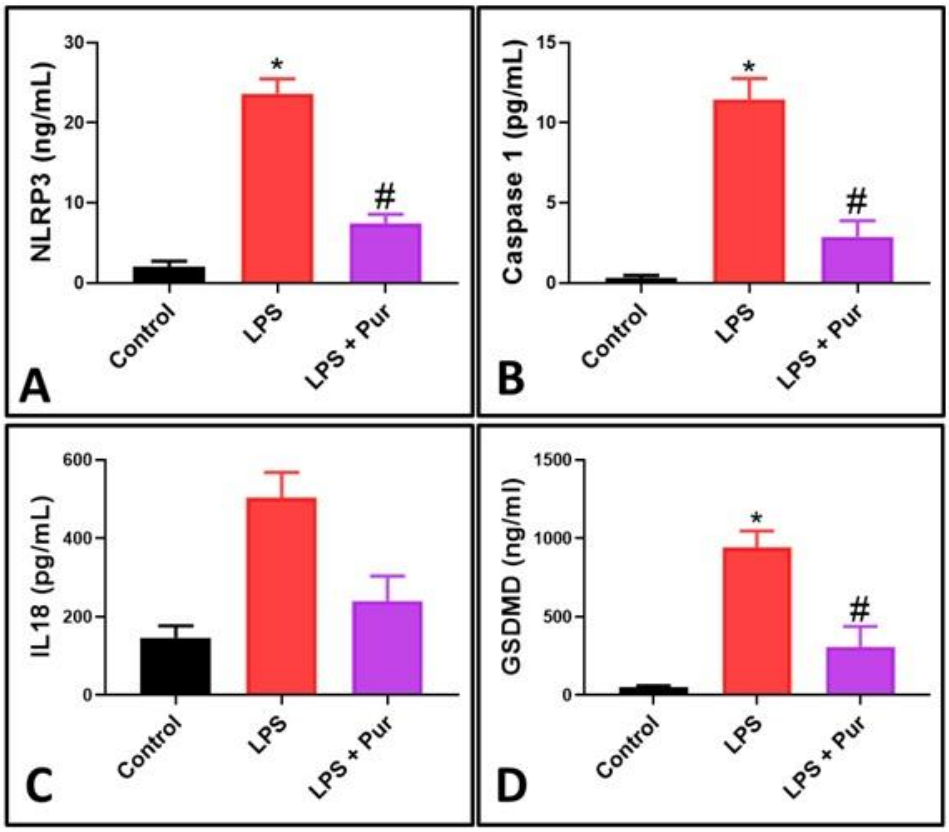

Fig. 5. Inhibitory effect of Pur on NLRP3 inflammasomes sequence (A)NLRP3, (B) Caspase-1 (C) IL-18, (D) GSDMD. All study results displayed as mean±SD. *p<0.05 significant to control animals, #$p$significant to LPS animals.

Discussion

Cognitive impairment and neurodegenerative disorders are strongly linked to neuroinflammation. The exact pathogenesis of diseases such as MS, PD, and AD remains unclear, which is why effective treatments are still lacking. Therefore, animal models of neuroinflammation are essential for examining the association between neuroinflammation, cognitive decline, and neurodegenerative diseases [31]. The present work demonstrated that LPS-induced neuroinflammation affects spatial memory and learning in mice, with notable histological changes in the hippocampus. These cognitive changes in the LPS group are attributed to hippocampal oxidative damage and activation of neuronal inflammation via the TLR4/NF-κB pathway, leading to a surge in inflammatory cytokine secretion. Additionally, LPS induced a pattern of inflammatory neuronal cell death through the stimulation of NLRP3 inflammasomes. Fortunately, co-treatment with Pur significantly improved memory in the mice by reversing the aforementioned parameters.

RAM test was constructed to examine the working memory and refractory memory in experimental mouse study [32]. Recent research has reported that LPS administration is associated with microglial activation, which is linked to neuronal damage in the mouse hippocampus, significantly affecting memory [33]. The findings of our study support this previous data, as the LPS group disclosed a marked increase in TTB, WME, and RME, reflecting impairments in memory and

spatial learning. These cognitive deficits were accompanied by hippocampal neuronal pyknosis and vacuolation when compared to the control group. These results are consistent with previous studies [24,34]. Conversely, the intake of Pur markedly improved the memory of the mice by reducing working and reference memory errors and decreasing hippocampal neuronal pyknosis. These findings are consistent with the study by Cui et al. [35], which reported that Pur administration in cases of chronic alcohol toxicity improved spatial memory in the WMT. This improvement was evidenced by a decrease in escape latency and distance, an increase in platform crossing times, and total swimming distance, along with a significant decline in the number of microglia in the cerebral cortex and hippocampal dentate gyrus of mice. Overall, these findings suggest that the positive effect of Pur on memory in LPS-induced memory impairment is due to its ameliorative effect on the morphology of neurocytes.

Previous studies have documented that LPS injection induces brain oxidative stress [36]. It is well known that dementia in older individuals results from oxidative stress [37]. Our findings align with previous data, showing that intraperitoneal injection of LPS induces severe hippocampal oxidative stress by increasing MDA levels and decreasing the antioxidants GSH and GPx compared to control mice homogenates, consistent with [38]. Meanwhile, coadministration of LPS with Pur significantly reversed hippocampal oxidative injury by decreasing MDA and increasing GSH and GPx, in agreement with [24].

Oxidative stress and neuroinflammation are involved in many neurodegenerative diseases, significantly impacting patients' lives [39]. Administration of LPS tends to bind to Toll-like receptors (TLRs) on the surface of astrocytes and microglia, inducing a neuroinflammatory response [40]. TLR4 is also found on the surface of Schwann cells, oligodendrocytes, and neurons in response to DAMPs [41]. Activation of TLR4 leads to downstream signaling and activation of the inflammatory transcription factor NF-κB, resulting in the release of inflammatory interleukins like IL-6, IL-1β, and TNF-α, which cause neuronal damage and glial activation [42].Our study findings align with these facts, as the LPS group showed a significant elevation in ELISA levels of TLR4, MyD88, NF-κB, and inflammatory cytokines. These results are concomitant with the work by El-Sahar et al. [43]. However, cotreatment with Pur markedly reduced the protein levels of inflammatory biomarkers. This is in line with previous work by Ren et al. [44], who reported that a combination of Pur with catalpol, borneol, and gastrodin, as natural products, mitigates streptozotocin-induced Alzheimer's disease by inhibiting microglial activation through downregulation of the TLR4 inflammatory cascade. Additionally, the study by Wang et al. [45] discussed the protective effects against experimental lung, liver, and kidney sepsis through NF-κB downregulation.

Activation of neurotoxic astrocytes has been shown to result from microglial NLRP3 inflammasomes through the upregulation of the NF-κB inflammatory cascade [46]. Lipopolysaccharide (LPS) activates toxic astrocytes by triggering NLRP3 inflammasomes, followed by downstream instigation of caspase-1 and the release of the inflammatory cytokine IL-1β

[47]. In our study, the LPS group showed a significant upgrade in the ELISA levels of NLRP3, caspase-1, IL-18, IL-1β, and GSDMD as pyroptotic markers compared to control mice, which is supported by previous research [48]. However, cotreatment with Pur significantly reversed these pyroptotic markers, as confirmed by the work of Zhou et al. [49].

Conclusions

Taken together, puerarin demonstrated a neuroprotective effect against LPS-induced memory impairment and hippocampal histological alterations. This effect is attributed to its antioxidant properties, suppression of neuronal inflammatory reactions by inhibiting the TLR4/NF-κB pathway, and downregulation of the hippocampal pyroptotic process. Interestingly, the present study suggests the potential neuroprotective power of puerarin in LPS-induced neuroinflammation and cognitive decline.

List of abbreviations	
Pur	Puerarin
LPS	Lipopolysaccharide
TTB	The time to reach the target box
WME	Working memory error
RME	Relative memory error
MDA	Malonaldehyde
GSH	Reduced glutathione
GPx	Glutathione peroxidase
TLR4	Tall like receptor 4
NF-κB	Nuclear factor kappa beta
MyD88	Myeloid differentiation primary response 88
IL-1β	Interleukin-1 beta
TNF-α	Tumor necrosis factor-alpha
IL-6	Interleukin-6
NLRP3	NOD-like receptor protein 3
NO	Nitric oxide
ROS	Reactive oxygen species
BBB	Blood brain barrier
AD	Alzheimer's disease
PD	Parkinsons disease
MS	Multiple sclerosis

References

1. **Allison, D. J. & Ditor, D. S.** The common inflammatory etiology of depression and cognitive impairment: a therapeutic target. J Neuroinflammation. 2014;11: 1–12.
2. **Nguyen, M. D., Julien, J. P. & Rivest, S.** Innate immunity: The missing link in neuroprotection and neurodegeneration? Nat Rev Neurosci. 2002;3: 216–227.
3. **Erickson, M.A., Banks, W.A.** Cytokine and chemokine responses in serum and brain after single and repeated injections of lipopolysaccharide: multiplex quantification with path analysis. Brain Behav Immun. 2011 Nov;25(8):1637-48.
4. **Parajuli, B., Sonobe, Y., Kawanokuchi, J., Doi, Y., Noda, M., Takeuchi, H., Mizuno, T., Suzumura, A.** GM-CSF increases LPS-induced production of proinflammatory mediators via upregulation of TLR4 and CD14 in murine microglia. J Neuroinflammation. 2012 Dec 13;9:268.
5. **Marinelli, C., Di Liddo, R., Facci, L. et al.** Ligand engagement of toll-like receptors regulates their expression in cortical microglia and astrocytes. J Neuroinflammation 2015;12:244 .
6. **Gárate, I., García-Bueno, B., Madrigal, J.L., Caso, J.R., Alou, L., Gómez-Lus, M.L., Leza, J.C.** Toll-like 4 receptor inhibitor TAK-242 decreases neuroinflammation in rat brain frontal cortex after stress. J Neuroinflammation. 2014 Jan 11;11:8.

7. **Qin, L., Wu, X., Block, M.L., Liu, Y., Breese, G.R., Hong, J.S., Knapp, D.J., Crews, F.T.** Systemic LPS causes chronic neuroinflammation and progressive neurodegeneration. Glia. 2007 Apr 1;55(5):453-62.
8. **Batista, C.R.A., Gomes, G.F., Candelario-Jalil, E., Fiebich, B.L., de Oliveira, A.C.P.** Lipopolysaccharide-Induced Neuroinflammation as a Bridge to Understand Neurodegeneration. Int J Mol Sci. 2019 May 9;20(9):2293.
9. **Zhao, J., Bi, W., Xiao, S., Lan, X., Cheng, X., Zhang, J., Lu, D., Wei, W., Wang, Y., Li, H., Fu, Y., Zhu, L.** Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. Sci Rep. 2019 Apr 8;9(1):5790.
10. **Gao, H.M., Hong, J.S.** Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. Trends Immunol. 2008 Aug;29(8):357-65.
11. **Schroder, K., Tschopp, J.** The inflammasomes. Cell. 2010 Mar 19;140(6):821-32.
12. **Davis, B.K., Wen, H., Ting, J.P.** The inflammasome NLRs in immunity, inflammation, and associated diseases. Annu Rev Immunol. 2011;29:707-35.
13. **Dempsey, C., Rubio, A.A., Bryson, K.J., Finucane, O., Larkin, C., Mills, E.L., Robertson, A.A., Cooper, M.A., O'Neill, L.A., Lynch, M.A**. Inhibiting the NLRP3 inflammasome with MCC950 promotes non-phlogistic clearance of amyloid-b and cognitive function in APP/PS1 mice. Brain Behav Immun. 2017; 61:306–316.
14. **Dang, R., Zhou, X., Tang, M., Xu, P., Gong, X., Liu, Y., Jiao, H., Jiang, P.** Fish oil supplementation attenuates neuroinflammation and alleviates depressive-like behavior in rats submitted to repeated lipopolysaccharide. Eur J Nutr. 2018 Apr;57(3):893-906.
15. **Zhang, L.** Pharmacokinetics and drug delivery systems for puerarin, a bioactive flavone from traditional Chinese medicine. Drug Deliv. 2019;26: 860–869.
16. **Wang, S.Q., Zhang, S.M., Wang, S.P., Gao, P., Dai, L.** A comprehensive review on Pueraria: Insights on its chemistry and medicinal value. Biomed Pharm. 2020; 131: 110734.
17. **Zhou, Y.X., Zhang, H., Peng, C. Puerarin:** A review of pharmacological effects. Phytother. Res. 2014, 28, 961–975.
18. **Xu, H., Wang, E., Chen, F., Xiao, J.B., Wang, M.F.** Neuroprotective Phytochemicals in Experimental Ischemic Stroke: Mechanisms and Potential Clinical Applications. Oxidative Med. Cell. Longev. 2021;2021: 1–45.
19. **Zhu, G., Wang, X., Chen, Y., Yang, S., Cheng, H., Wang, N., Li, Q.** Puerarin protects dopaminergic neurons against 6-hydroxydopamine neurotoxicity via inhibiting apoptosis and upregulating glial cell line-derived neurotrophic factor in a

rat model of Parkinson's disease. Planta Med. 2010 Nov;76(16):1820-6.

20. **Li, R., Liang, T., Xu, L., Zheng, N., Zhang, K., Duan, X.** Puerarin attenuates neuronal degeneration in the substantia nigra of 6-OHDA-lesioned rats through regulating BDNF expression and activating the Nrf2/ARE signaling pathway. Brain Res. 2013 Jul 26; 1523:1-9.
21. **Li, R., Zheng, N., Liang, T., He, Q., Xu, L.** Puerarin attenuates neuronal degeneration and blocks oxidative stress to elicit a neuroprotective effect on substantia nigra injury in 6-OHDA-lesioned rats. Brain Res. 2013;1517:28–35.
22. **Wang, J-W., Wang, H-D., Cong, Z-X., Zhou, X-M., Xu, J-G., Jia, Y., Ding, Y.** Puerarin ameliorates oxidative stress in a rodent model of traumatic brain injury. J Surg Res. 2014;186:328–337.
23. **Delcourt, J.**, **Miller, N.Y.**, **Couzin, I.D.**, **Garnier, S.** Methods for the effective study of collective behavior in a radial arm maze. Behav. Res. Methods. 2018;50: 1673–1685.
24. **Rahim, N.S.**, **Lim, S.M.**, **Mani, V.**, **Hazalin, N.A.M.N.**, **Majeed, A.B.A.**, **Ramasamy, K.** Virgin Coconut Oil-Induced Neuroprotection in Lipopolysaccharide-Challenged Rats is Mediated, in Part, Through Cholinergic, Anti-Oxidative and Anti-Inflammatory Pathways. J. Diet. Suppl. 2020;18: 655–681.
25. **Wang, L.**, **Wang, T.**, **Wen, S.**, **Song, R.**, **Zou, H.**, **Gu, J.**, **Liu, X.**, **Bian, J.**, **Liu, Z.**, **Yuan, Y.** Puerarin Prevents Cadmium-Induced Neuronal Injury by Alleviating Autophagic Dysfunction in Rat Cerebral Cortical Neurons. Int J Mol Sci. 2023 May 5;24(9):8328.
26. **Mani, V.**, **Jaafar, S.M.**, **Azahan, N.S.M.**, **Ramasamy, K.**, **Lim, S.M.**, **Ming, L.C.**, **Majeed, A.B.A.** Ciproxifan improves cholinergic transmission, attenuates neuroinflammation and oxidative stress but does not reduce amyloid level in transgenic mice. Life Sci. 2017;180: 23–35.
27. **Bancroft, J.**, **Stevens, A.** Theory and practice of histological techniques. Churchil Livingstone, Edinburgh (1996).
28. **Mihara, M.**, **Uchiyama, M.** Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal Biochem. 1978; 86:271–278.
29. **Ellman, G.L.** Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82:70–77.
30. **Paglia, D.E.**, **Valentine, W.N.** Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med. 1967; 70:158–169.
31. **Noh, H.**, **Jeon, J.** & **Seo, H.** Systemic injection of LPS induces region-specific neuroinflammation and mitochondrial dysfunction in normal mouse brain. Neurochem Int. 2014;69: 35–40.
32. **Mei, J.**, **Kohler, J.**, **Winter, Y.**, **Spies, C.**, **Endres, M.**, **Banneke, S.**, **Emmrich, J.V.** Automated radial 8-arm maze: A voluntary and stress-free behavior test to

assess spatial learning and memory in mice. Behav. Brain Res. 2019;381: 112352.

33. **Kongsui, R.**, **Sriraksa, N.**, **Thongrong, S.** The Neuroprotective Effect of Zingiber cassumunarRoxb. Extract on LPS-Induced Neuronal Cell Loss and Astroglial Activation within the Hippocampus. BioMed Res. Int. 2020;2020: 4259316.
34. **Mohd Azahan, N.S.**, **Mani, V.**, **Ramasamy, K.**, **Lim, S.M.**, **Johari James, R.M.**, **Alsharidah, M.**, **Alhowail, A.**, **Abdul Majeed, A.B.** Mahanimbine-induced neuroprotection via cholinergic system and attenuated amyloidogenesis as well as neuroinflammation in lipopolysaccharides-induced mice. Pharmacog. Mag. 2020;16: 57–63.
35. **Cui, S.Q.**, **Wang, Q.**, **Zheng, Y.**, **Xiao, B.**, **Sun, H.W.**, **Gu, X.L.**, **Zhang, Y.C.**, **Fu, C.H.**, **Dong, P.X.**, **Wang, X.M.** Puerarin protects against damage to spatial learning and memory ability in mice with chronic alcohol poisoning. Braz J Med Biol Res. 2015 Jun;48(6):515-22.
36. **Tyagi, E.**, **Agrawal, R.**, **Nath, C.**, **Shukla, R.** Effect of melatonin on neuroinflammation and acetylcholinesterase activity induced by LPS in rat brain. Eur J Pharmacol. 2010;640:206–210.
37. **Ton, A.M.M.** et al. Oxidative stress and dementia in Alzheimer's patients: effects of synbiotic supplementation. Oxidative Med Cell Longev 2020:2638703.
38. **Thingore, C.**, **Kshirsagar, V.**, **Juvekar, A.** Amelioration of oxidative stress and neuroinflammation in lipopolysaccharide-induced memory impairment using Rosmarinic acid in mice. Metab Brain Dis. 2021 Feb;36(2):299-313.
39. **Adams, K.L.**, **Castanon-Cervantes, O.**, **Evans, J.A.**, **Davidson, A.J.** Environmental circadian disruption elevates the IL-6 response to lipopolysaccharide in blood. J. Biol. Rhythms. 2-13;28 (4): 272–277.
40. **Skrzypczak-Wiercioch, A.**, **Sałat, K.** Lipopolysaccharide-induced model of neuroinflammation: mechanisms of action, research application and future directions for its use. Molecules. 2022;27 (17): 5481.
41. **Tang, S.**, **Li, C.**, **Suo, Z.**, **Xu, Y.**, **Wei, K.**, **Zhao, L.**, et al. Neuroprotective effects of savinin on LPS-induced neuroinflammation in vivo via regulating MAPK/NF-κB pathway and NLRP3 inflammasome activation. Molecules 28 (4), 1575 (2023).
42. **Liu, T.**, **Zhang, L.**, **Joo, D.**, **Sun, S.C.** NF-κB signaling in inflammation. Signal Transduct. Target. Ther. 2017;2 (1): 1–9.
43. **El-Sahar, A.E.**, **Shiha, N.A.**, **El Sayed, N.S.**, **Ahmed, L.A.** Alogliptin Attenuates Lipopolysaccharide-Induced Neuroinflammation in Mice Through Modulation of TLR4/MYD88/NF-κB and miRNA-155/SOCS-1 Signaling Pathways. Int J Neuropsychopharmacol. 2021 Feb 15;24(2):158-169.
44. **Ren, H.**, **Tang, L.**, **Yuan, Z.**, **Liu, Y.**, **Zhou, X.**, **Xiao, X.**, **Wu, X.**, **Chen, W.**, **Chen, Y.**, **Wang, H.**, **Xue, Q.**, **Xu,**

X. Combined administration of catalpol, puerarin, gastrodin, and borneol modulates the Tlr4/Myd88/NF-κB signaling pathway and alleviates microglia inflammation in Alzheimer's disease. Front Pharmacol. 2024 Oct 31; 15:1492237.

45. **Wang, L.**, **Liang, Q.**, **Lin, A.**, **Chen, X.**, **Wu, Y.**, **Zhang, B.**, **Zhang, Y.**, **Min, H.**, **Wen, Y.**, **Song, S.**, **Gao, Q.** Puerarin Increases Survival and Protects Against Organ Injury by Suppressing NF-κB/JNK Signaling in Experimental Sepsis. Front Pharmacol. 2020 May 7;11:560.
46. **Li, S.**, **Fang, Y.**, **Zhang, Y.**, **Song, M.**, **Zhang, X.**, **Ding, X.**, **Yao, H.**, **Chen, M.**, **Sun, Y.**, **Ding, J.**, et al. Microglial NLRP3 inflammasome activates neurotoxic astrocytes in depression-like mice. Cell Rep. 2022;41: 111532.
47. **Neher, J.J.**, **Neniskyte, U.**, **Zhao, J.W.**, **Bal-Price, A.**, **Tolkovsky, A.M.**, **Brown, G.C.** Inhibition of microglial phagocytosis is sufficient to prevent inflammatory neuronal death. J. Immunol. 2011;186: 4973–4983.
48. **Valenzuela-Arzeta, I.E.**, **Soto-Rojas, L.O.**, **Flores-Martinez, Y.M.**, **Delgado-Minjares, K.M.**, **Gatica-Garcia, B.**, **Mascotte-Cruz, J.U.**, **Nava, P.**, **Aparicio-Trejo, O.E.**, **Reyes-Corona, D.**, **Martínez-Dávila, I.A.**, **Gutierrez-Castillo, M.E.**, **Espadas-Alvarez, A.J.**, **Orozco-Barrios, C.E.**, **Martinez-Fong, D.** LPS Triggers Acute Neuroinflammation and Parkinsonism Involving NLRP3 Inflammasome Pathway and Mitochondrial CI Dysfunction in the Rat. Int J Mol Sci. 2023 Feb 27;24(5):4628.
49. **Zhou, S.**, **Li, Y.**, **Hong, Y.**, **Zhong, Z.**, **Zhao, M.** Puerarin protects against sepsis-associated encephalopathy by inhibiting NLRP3/Caspase-1/GSDMD pyroptosis pathway and reducing blood-brain barrier damage. Eur J Pharmacol. 2023 Apr 15;945:175616.