ZITA MEDICAL MANAGEMENT

www.hsmc.gr/en/pharmakeftiki-journal/

PHARMAKEFTIKI, 37, 2S, 2025 | 420-424 RESEARCH

https://doi.org/10.60988/p.v37i2S.244

Huperzine A *versus* epicatechin: a comparative study of their potential protective effect on lipopolysaccharide-induced lung injury in mice

Zeena Thamir Tariq[1], Ahmed R. Abu-Raghif[2], Abdulla K. Raheem[3], Hayder Ridha-Salman[3,*], Alaa Hamza Abbas[3], Qassim A. Zigam[3], Weaam J. Abbas[3], Maytham Ahmed AbdulAemah[3], Hasanain A. M. Abbas[3], Ali Jihad Hemid Al-Athari[3], Elaf Mahmood Shihab[4], Salim K. Hajwal[5], Ahmed Abbas Al-Khafaji[3], Mahdi Hamzah Al-Taee[5]

[1]Antimicrobial Resistance Sector, Public Health Directorate, Ministry of Health, Baghdad, Iraq
[2]Department of Pharmacology, College of Medicine, Al-Nahrain University, Baghdad, Iraq
[3]Department of Pharmacology, College of Pharmacy, Al-Mustaqbal University, Hillah, Iraq
[4]Department of Pharmacology and Toxicology, College of Pharmacy, Al-Esraa University, Baghdad, Iraq
[5]Department of Nursing, College of Nursing, Al-Mustaqbal University, Hillah, Iraq

KEY WORDS:
huperzine A; epicatechin; methylprednisolone; lipopolysaccharide; cytokine storm

ARTICLE INFO:
Received: January 15, 2025
Revised: February 14, 2025
Accepted: February 19, 2025
Available online: October 10, 2025

*** CORRESPONDING AUTHOR:**
Hayder Ridha-Salman, Department of Pharmacology, College of Pharmacy, Al-Mustaqbal University, Hillah, Iraq; e-mail: hayder80.ridha@gmail.com

ABSTRACT

A cytokine storm is a severe and potentially fatal condition resulting from an excessive immune response. Epicatechin and huperzine A have demonstrated anti-inflammatory and antioxidant properties, suggesting their potential utility in mitigating tissue damage and cytokine storm severity. This study aimed to compare the protective effects of huperzine A and epicatechin in a cytokine storm-like murine model. Sixty male Swiss albino mice were randomly allocated into six groups. Except for the control group, all animals received a single intraperitoneal injection of lipopolysaccharide (LPS; 5 mg/kg) in order to induce a cytokine storm. The induction group received LPS without further intervention. The remaining groups were pre-treated for three consecutive days prior to LPS administration as follows: vehicle group (1% dimethyl sulfoxide), methylprednisolone group (50 mg/kg/day methylprednisolone), huperzine A group (0.2 mg/kg/day huperzine A), and epicatechin group (25 mg/kg/day epicatechin). The histological analysis of lung tissues and the quantification of serum cytokines – interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) – revealed that all pre-treated groups exhibited significant anti-inflammatory effects. Notably, epicatechin conferred a more pronounced protective effect than either

PHARMAKEFTIKI, 37, 2S, 2025 | 420-424

methylprednisolone or huperzine A, as evidenced by reduced pulmonary histopathological alterations and lower serum cytokine concentrations. In conclusion, both huperzine A and epicatechin demonstrated protective efficacy against the LPS-induced cytokine storm, with epicatechin showing superior performance in attenuating systemic inflammation and lung injury.

1. Introduction

A cytokine storm is an acute, life-threatening inflammatory response characterized by an excessive activation of the immune system and the uncontrolled release of pro-inflammatory cytokines. This phenomenon is a hallmark of severe inflammatory conditions, including sepsis, autoimmune disorders, and viral infections such as coronavirus disease 2019 (COVID-19). Among the key mediators, interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) play essential roles in immune regulation, but can drive hyperinflammation during cytokine storms, leading to extensive tissue damage, multi-organ failure, and increased mortality[1].

Huperzine A, an alkaloid extracted from the Chinese herb *Huperzia serrata*, is a potent acetylcholinesterase inhibitor known for its neuroprotective properties. It modulates the cholinergic anti-inflammatory pathway, reduces oxidative stress, and inhibits D-galactose-induced expression of pro-inflammatory cytokines[2].

Epicatechin, a flavonoid found abundantly in cocoa, green tea, and various fruits, has demonstrated notable anti-inflammatory potential. It exerts its effects by modulating nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and nuclear factor erythroid 2-related factor 2 signalling pathways, both of which are central to the regulation of inflammatory and oxidative stress responses[3].

This study investigates and compares the protective effects of epicatechin and huperzine A against cytokine storm-induced lung injury in mice, focusing on their capacity to prevent histopathological damage and modulate serum cytokine levels.

2. Methodology

Huperzine A, epicatechin, and methylprednisolone were procured from Haibo Chemicals, Inc. (China). Lipopolysaccharide (LPS) was obtained from Sigma Aldrich Chemical Co., Ltd. (USA). Dimethyl sulfoxide (DMSO) was sourced from Chemlab NV (Belgium), and chloroform (99%) was manufactured by LobaChemie Pvt. Ltd. (India). Enzyme-linked immunosorbent assay (ELISA) kits for TNF-α, IL-1β, and IL-6 (mouse-specific) were supplied by Xinlong Technology Co., Ltd. (China).

In order to prepare the LPS solution, 10 mg of freeze-dried LPS powder were dissolved in 10 mL of normal saline and mixed for 15 min. Solutions of huperzine A, epicatechin, and methylprednisolone were prepared by dissolving the compounds in 1% DMSO, followed by dilution with distilled water so as to achieve the required volume.

The experimental work was conducted at the Al-Nahrain University between January and June 2023. Sixty healthy male Swiss albino mice (aged 7–8 weeks; weight 25–30 g) were acclimatized for one week in a well-ventilated, pathogen-free environment with ad libitum access to food and water.

Mice were randomly assigned to six groups (n=10 per group): (i) control group (healthy, untreated mice), (ii) induction group (received a single intraperitoneal injection of LPS at 5 mg/kg, without further treatment), (iii) vehicle group (received 1% DMSO intraperitoneally once daily for three days, followed by an LPS injection), (iv) methylprednisolone group (pre-treated with methylprednisolone

PHARMAKEFTIKI, 37, 2S, 2025 | 420-424

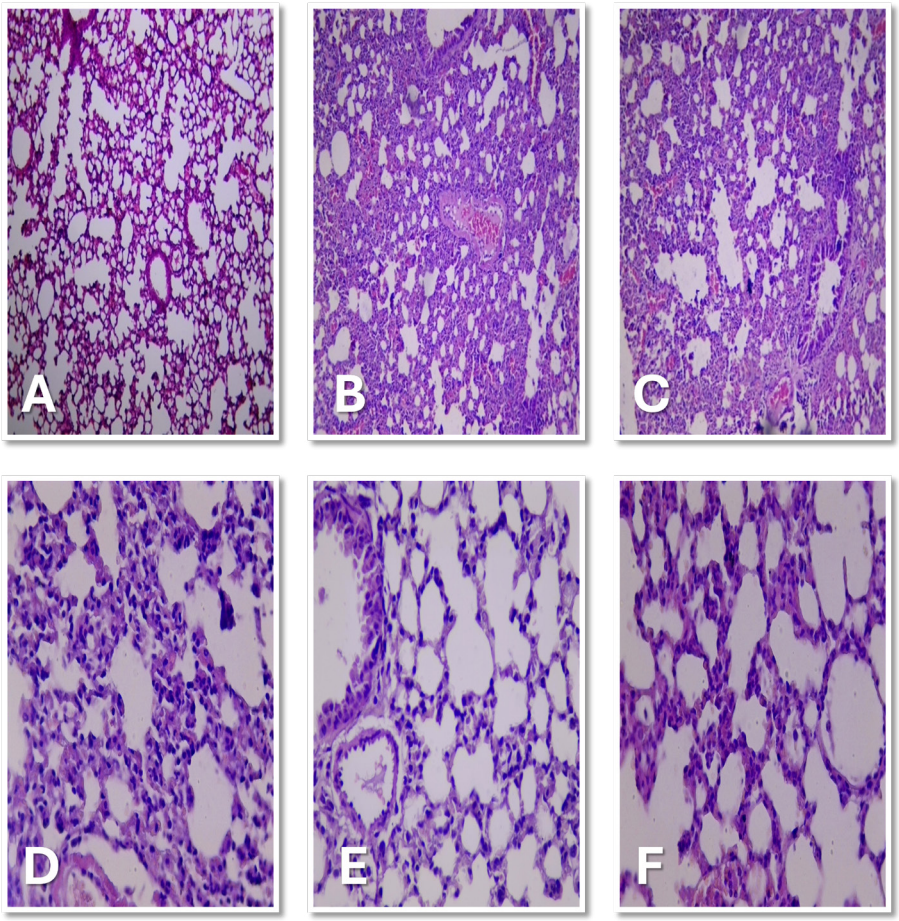

Figure 1. *Representative haematoxylin-eosin sections of lung tissues from the mice of this study. (A): The lung sections for the mice of the control group show normal alveoli surrounded by septa, no congestion or inflammatory cell infiltration (20x). (B): The lung sections for the mice of the induction group show severe interstitial inflammatory cell infiltration, congestion of alveolar capillary, diffuse alveolar damage, with thickening of the alveolar wall with hyaline membrane (20x). C: The lung sections for the mice of the vehicle group show severe interstitial inflammatory cell infiltration, congestion and haemorrhage of the alveolar capillary, with diffuse alveolar destruction (20x). (D): The lung sections for the mice of the methylprednisolone group show moderate inflammatory cell infiltration with focal destruction of the alveolar septa and emphysematous changes, (40x). (E): The lung sections for the mice of the huperzine A group show moderate inflammatory cell infiltration with mild destruction of the alveoli (40x). (F): The lung sections for the mice of the epicatechin group show mild inflammatory cell infiltration with mild destruction of the alveoli (40x).*

at 50 mg/kg/day, intraperitoneally, for three days, followed by an LPS injection), (v) huperzine A group (pre-treated with huperzine A at 0.2 mg/kg/day, intraperitoneally, for three days, followed by an LPS injection), and (vi) epicatechin group (pre-treated with epicatechin at 25 mg/kg/day, intraperitoneally, for three days, followed by an LPS injection).

All animals were sacrificed on day six. Blood samples were collected *via* jugular venipuncture under light chloroform anaesthesia and centrifuged at

PHARMAKEFTIKI, 37, 2S, 2025 | 420-424

3,000 rpm for 20 min. Serum was stored in Eppendorf tubes at -20°C. Lung tissues were preserved in 10% formalin for histological examination. Serum cytokine levels (IL-1β, IL-6, TNF-α) were quantified using ELISA kits according to the manufacturer's instructions. Histological slides were evaluated blindly by a histopathologist using a semi-quantitative scoring system (0: normal; 1: mild; 2: moderate; 3: severe).

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS). Results were expressed as mean ± standard deviation. Group comparisons were conducted using two-tailed *t*-tests and analysis of variance (ANOVA). Statistical significance was defined at a *p*-value <0.05.

Ethical approval was granted by the Institutional Review Board of the Al-Nahrain University College of Medicine on 3 August 2022 (document: IRB/170; approval number: UNCOMIRB202206161), following review of the study protocol and supporting documentation.

3. Results and Discussion

The administration of LPS (5 mg/kg) successfully induced a cytokine storm, as evidenced by elevated serum levels of TNF-α, IL-1β, and IL-6 compared to the control group. These findings are consistent with prior studies demonstrating similar immunostimulatory effects of LPS[4]. Treatment with methylprednisolone, huperzine A, or epicatechin resulted in a significant reduction in serum cytokine levels relative to the induction group. Among these, epicatechin exhibited the most pronounced anti-inflammatory effect.

Huperzine A demonstrated robust anti-inflammatory and antioxidant activity. It attenuated levels of IL-1β, IL-6, and TNF-α and reduced oxidative stress by activating the cholinergic anti-inflammatory pathway *via* α7 nicotinic acetylcholine receptors. This activation suppressed NF-κB signalling and enhanced interleukin-10 expression. Additionally, huperzine A inhibited inducible nitric oxide synthase (iNOS) and cyclooxygenase-2 (COX-2), reduced reactive oxygen species (ROS)-mediated NF-κB activation, and upregulated antioxidant enzymes including catalase, superoxide dismutase, and glutathione peroxidase. These mechanisms collectively prevented activation of the NOD-, LRR- and pyrin domain-containing protein 3 inflammasome and mitigated inflammation[5].

Epicatechin counteracted the LPS-induced inflammation by downregulating Toll-like receptor 4 expression, inhibiting mitogen-activated protein kinase (MAPK) and NF-κB signalling, and neutralizing ROS. It also suppressed COX-2 and iNOS activity, thereby reducing nitric oxide and prostaglandin E2 production[6].

Histological analysis revealed marked lung damage in the induction and vehicle groups, including proteinaceous exudates, severe inflammatory cell infiltration, alveolar congestion and haemorrhage, and destruction of the alveolar septa. Diffuse alveolar damage with emphysematous changes was evident compared to the control group (Figure 1). In contrast, the methylprednisolone, huperzine A, and epicatechin groups exhibited significantly improved histopathological scores ($p<0.05$). As illustrated in Figure 1, these treatment groups exhibited reduced histological alterations, with only mild interstitial inflammatory cell infiltration and alveolar capillary obstruction. Epicatechin conferred superior protection, yielding the lowest histology scores among the treatment groups.

Huperzine A has also been shown to mitigate LPS-induced lung injury by decreasing levels of high mobility group box 1 protein in the airways and circulation, thereby reducing inflammation and tissue damage[7]. Epicatechin has further suppressed the macrophage migration inhibitory factor and directly interacted with phosphorylated p38 within the MAPK pathway, thereby modulating neutrophil chemotaxis and limiting tissue injury[8].

4. Conclusion

Both huperzine A and epicatechin demonstrated protective effects against the LPS-induced cytokine storm induced in mice, as evidenced by the reduced serum levels of IL-1β, IL-6, and TNF-α and the improved lung histopathology. Notably, epicatechin

PHARMAKEFTIKI, 37, 2S, 2025 | 420-424

exhibited superior efficacy in attenuating systemic inflammation and preserving pulmonary architecture.

Acknowledgements

The authors would like to express their appreciation to the College of Pharmacy of the Al-Nahrain University for providing all the facilities needed for the completion of this work.

Conflicts of interest
None exist.

ORCIDs

0009-0005-2835-2942 (Z.T. Tariq); 0000-0003-4514-3892 (A.R. Abu-Raghif); 0000-0001-8181-3879 (A.K. Raheem); 0000-0003-1594-4883 (H. Ridha-Salman); 0000-0002-1382-8291 (A.H. Abbas); 0000-0002-1425-3450 (Q.A. Zigam); 0000-0003-0223-2924 (W.J. Abbas); 0000-0002-3333-9778 (M.A. AbdulAemah); 0009-0009-8319-8123 (H.A.M. Abbas); 0000-0003-2530-293X (A.J.H. Al-Athari); 0000-0002-9390-0440 (E.M. Shihab); 0000-0002-9793-2247 (S.K. Hajwal); 0009-0005-4030-1451 (A.A. Al-Khafaji); 0009-0005-3266-5693 (M.H. Al-Taee)

References

1. Abed-Mansoor A.F., Abu Raghif A.R. Attenuated effects of rivastigmine in induced cytokine storm in mice. *J. Emerg. Med. Trauma Acute Care* 2022(3), 12, 2022. DOI: 10.5339/jemtac.2022.ismc.12
2. Li C, Shi S. Neuroprotective effect of huperzine A on d-galactose-induced hearing dysfunction. *Ear Nose Throat J.* 100(3s), 269s–276s, 2021. DOI: 10.1177/0145561319864570
3. Granado-Serrano A.B., Martín M.A., Haegeman G., Goya L., Bravo L., Ramos S. Epicatechin induces NF-kappaB, activator protein-1 (AP-1) and nuclear transcription factor erythroid 2p45-related factor-2 (Nrf2) *via* phosphatidylinositol-3-kinase/protein kinase B (PI3K/AKT) and extracellular regulated kinase (ERK) signalling in HepG2 cells. *Br. J. Nutr.* 103(2), 168–179, 2010. DOI: 10.1017/S0007114509991747
4. Aal-Aaboda M.S., Abu Raghif A.R., Hadi N.R. Renoprotective potential of the ultra-pure lipopolysaccharide from Rhodobacter sphaeroides on acutely injured kidneys in an animal model. *Arch. Razi Inst.* 76(6), 1755–1764, 2021. DOI: 10.22092/ari.2021.356202.1803
5. Raheem A.K., Abu-Raghif A.R., Zigam Q.A. Cilostazol protects against sepsis-induced kidney impairment in a mice model. *J. Med. Chem. Sci.* 6(5), 1193–1203, 2023. DOI: 10.26655/JMCHEMSCI.2023.5.25
6. Prince P.D., Fischerman L., Toblli J.E., Fraga C.G., Galleano M. LPS-induced renal inflammation is prevented by (-)-epicatechin in rats. *Redox Biol.* 11, 342–349, 2017. DOI: 10.1016/j.redox.2016.12.023
7. Su J., Chen K., Sang X., Feng Z., Zhou F., Zhao H., *et al.* Huperzine a ameliorates sepsis-induced acute lung injury by suppressing inflammation and oxidative stress *via* α7 nicotinic acetylcholine receptor. *Int. Immunopharmacol.* 141, 112907, 2024. DOI: 10.1016/j.intimp.2024.112907
8. Kim D., Haynes C.L. The role of p38 MAPK in neutrophil functions: single cell chemotaxis and surface marker expression. *Analyst* 138(22), 6826–6833, 2013. DOI: 10.1039/c3an01076g

HOW TO CITE:
Tariq Z.T., Abu-Raghif A.R., Raheem A.K., Ridha-Salman H., Abbas A.H., Zigam Q.A., Abbas W.J., AbdulAemah M.A., Abbas H.A.M., Al-Athari A.J.H., Shihab E.M., Hajwal S.K., Al-Khafaji A.A., Al-Taee M.H. Huperzine A *versus* epicatechin: a comparative study of their potential protective effect on lipopolysaccharide-induced lung injury in mice. *Pharmakeftiki* 37(2s), 420-424, 2025. https://doi.org/10.60988/p.v37i2S.244