Mesenchymal Stem Cell-Derived Secretome Modulates Apoptotic And Inflammatory Markers In The Hippocampus Of Septic Mice

Rahmat Nugroho [1*], Arifin [1,2], and Ratih Arianita Agung [1,2]

Affiliation:
1. Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia, 57126
2. Department of Internal Medicine, Dr. Moewardi General Hospital, Surakarta, Indonesia, 57126
3. Department of Public Health, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia, 57126

*Correspondence: Rahmat Nugroho, Department of Internal Medicine, Faculty of Medicine, Universitas Sebelas Maret, Surakarta, Indonesia, 57126; rahmatnugrohofkuii@gmail.com

Received: 31/04/2025
Accepted: 31/05/2025
Published: 31/05/2025

ABSTRACT

Introduction: Sepsis is a global health problem that significantly increases morbidity and mortality worldwide. The inflammatory process in sepsis can cause damage to the hippocampus, leading to brain dysfunction mediated by apoptosis (Caspase-3) and neuroinflammation (TNF-α). Mesenchymal stem cells (MSCs) have potential as a regenerative therapy to reduce brain damage. This study aims to assess the therapeutic effect of MSC secretome on hippocampus damage, measured through Caspase-3 and TNF-α expression, in a mice model of sepsis.

Methods: This experimental study used a post-test-only randomized controlled trial (RCT) design. Male mice (Mus musculus Balb/C) were divided into five groups: normal control (NC), sepsis control (SC) induced with LPS, and three treatment groups induced with LPS and given MSC secretome at doses of 150 µL, 300 µL, and 600 µL. Caspase-3 and TNF-α expression levels in the hippocampus were measured using immunohistochemistry (IHC). Statistical analysis was performed using ANOVA and Kruskal-Wallis tests ($p < 0.05$).

Results: Administration of MSC secretome did not result in a statistically significant reduction in Caspase-3 ($p=0.730$) or TNF-α ($p=0.135$) levels in the hippocampus. Although average values were lower in the treatment groups compared to the sepsis control group, the differences were not statistically significant. The greatest reduction in Caspase-3 occurred in the group receiving 600 µL secretome , while the lowest average TNF-α was observed in the 600 µL dose group.

Conclusion: Mesenchymal stem cell secretome administration reduced Caspase-3 and TNF-α levels in the hippocampus of a mice sepsis model, but the impact was not statistically significant.

Keywords: Secretome; mesenchymal stem cells; Caspase-3; TNF-α; hippocampus; sepsis.

INTRODUCTION

Sepsis remains a global health challenge, significantly increasing morbidity and mortality rates worldwide and representing one of the most frequent complications in intensive care units [1]. In 2017, approximately 48.9 million cases of sepsis were reported globally, resulting in 11 million deaths [2]. In Indonesia, a study involving 14,076 sepsis patients reported a survival rate of only 41.7%, with 58.3% of patients succumbing to the condition [3]. Beyond its high mortality, sepsis imposes a substantial financial burden on healthcare systems. In the United States, sepsis care costs were estimated at 24 billion USD annually [2], while in Indonesia, the national burden is estimated at 130 million USD per 100,000 patients [3].

The systemic inflammatory cascade in sepsis can induce significant alterations in vulnerable brain regions [4,5], potentially leading to severe brain dysfunction [6]. Sepsis-associated brain dysfunction (SABD) is the most common form of encephalopathy in critically ill patients, affecting between 53% and 70% of septic individuals [7,8]. The underlying mechanisms include excessive microglial activation, disturbances in cerebral perfusion, blood-brain barrier (BBB) dysfunction, and alterations in neurotransmission. Furthermore, prolonged inflammation, severe hypoxemia, and persistent hyperglycemia may exacerbate sepsis-induced brain injury [5]. Currently, no specific treatment directly addresses the mechanisms of brain dysfunction in sepsis, and optimal therapeutic approaches remain a subject of controversy [4].

Apoptosis is a pivotal factor in organ-specific cell death during sepsis. Caspase-3 acts as the key effector responsible for morphological and biological changes in apoptotic cells [9]. Inhibiting proapoptotic molecules, such as Caspase-3, and reducing the expression of Bax and Bim has been shown to attenuate dysfunction and reduce mortality in lipopolysaccharide (LPS)-induced models [9]. Additionally, Tumor Necrosis Factor-alpha (TNF-α) plays a critical role in sepsis-associated brain dysfunction by promoting neuroinflammation, disrupting the BBB, and causing neuronal damage [10]. Although targeting Caspase-3 and TNF-α holds therapeutic potential, further research is required to fully elucidate their roles and develop appropriate interventions [9,10].

Mesenchymal stromal cells (MSCs) have shown promise as a regenerative treatment for brain trauma due to their diverse characteristics [11]. Mitochondrial transfer from MSCs can reduce apoptosis in recipient cells and enhance survival by regulating the Bax/Bcl-2 protein ratio and downregulating Caspase-3 expression [11]. Previous studies demonstrated that MSCs protect BBB integrity, reduce astrogliosis and neuroinflammation, and improve cognitive outcomes [12]. Furthermore, MSCs have been shown to effectively reduce serum levels of inflammatory mediators, including TNF-α, interleukin-6, and CRP [13]. This study aims to explore the therapeutic potential of the mesenchymal stem cell secretome on sepsis-induced brain damage by analyzing its effects on Caspase-3 and TNF-α expression in a mouse sepsis model.

METHODS

Study Design and Ethical Approval

This research utilized an experimental Randomized Controlled Trial (RCT) with a post-test-only control group design using a mouse sepsis model. The ethical procedure for this study was approved by the Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret, under recommendation number 1.605/VI/HREC/2024.

Animals and Setting

The study was conducted from April to May 2024. Animal maintenance and sepsis induction were carried out at the Food and Nutrition PAU Laboratory, Gadjah Mada University, Yogyakarta, while tissue processing,

immunohistochemical staining, and result reading were performed at the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sebelas Maret, Surakarta.

The study population consisted of male mice of the Mus musculus Balb/C strain obtained from the Faculty of Veterinary Medicine, Gadjah Mada University. The inclusion criteria were male mice, aged 3–4 months, weighing 20–30 grams. Mice that died before the study concluded were excluded.

Experimental Procedures

The experimental animals were divided into five groups, each consisting of five mice. The groups were defined as follows:

a. Normal Control (NC): Received 0.9% NaCl.
b. Sepsis Control (SC): Induced with Lipopolysaccharide (LPS).
c. Treatment Group 1 (KP1): Induced with LPS and administered 150 µL MSC secretome.
d. Treatment Group 2 (KP2): Induced with LPS and administered 300 µL MSC secretome.
e. Treatment Group 3 (KP3): Induced with LPS and administered 600 µL MSC secretome.

Sepsis was induced via intraperitoneal injection of LPS. The secretome was administered on the third day after the onset of sepsis.

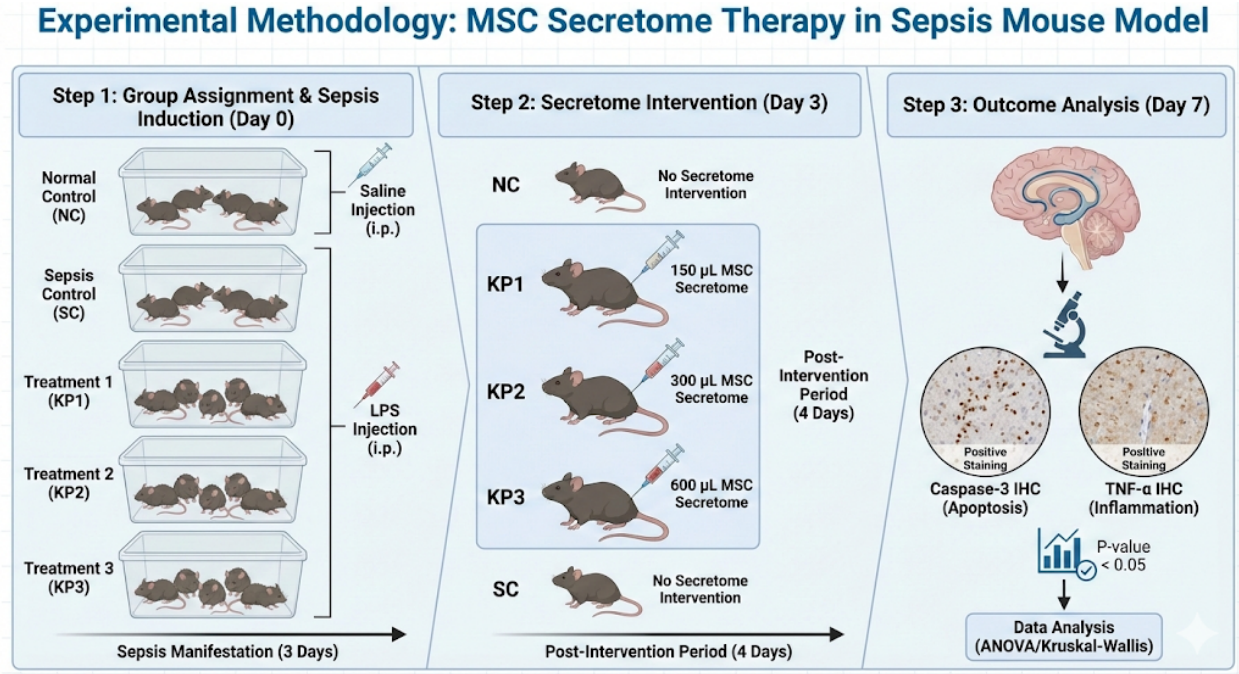


Figure 1. Schematic workflow of the experimental study design.

Figure 1 shows Male Mus musculus Balb/C mice were randomly divided into five groups (n=5 per group): Normal Control (NC), Sepsis Control (SC), and three treatment groups (KP1, KP2, and KP3). Sepsis was induced in all groups except the NC group through an intraperitoneal injection of Lipopolysaccharide (LPS). On the third day after sepsis manifestation, the treatment groups received varying doses of Mesenchymal Stem Cell (MSC) secretome: 150 µL (KP1), 300 µL (KP2), or 600 µL (KP3). The NC and SC groups did not receive the secretome intervention. Following the treatment period, hippocampal tissues were harvested and processed for immunohistochemistry (IHC) to evaluate the expression levels of Caspase-3 (an apoptotic marker) and TNF-α (an inflammatory marker). The resulting data were statistically analyzed using One-Way ANOVA or Kruskal-Wallis tests to determine the therapeutic impact of the secretome

Data Collection and Analysis

Following the intervention, Caspase-3 and TNF-α expression levels in the hippocampus were measured using immunohistochemistry. Statistical analysis was performed using SPSS version 23.00 for Windows. The Shapiro-Wilk test was used to assess data normality. If the data were normally distributed, the One-Way ANOVA parametric test was employed; if not, the Kruskal-Wallis nonparametric test was applied. Post-hoc tests were conducted if significant differences were found between groups, with statistical significance determined at $p < 0.05$.

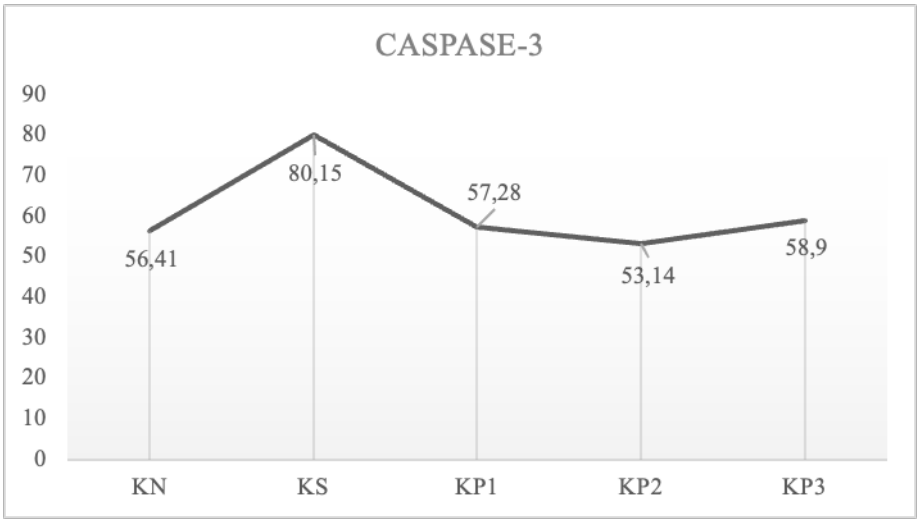
RESULTS

Effect of Secretome Administration at Various Treatment Doses on Hippocampus Caspase-3 IHC

The differences in hippocampal Caspase-3 IHC following secretome administration are shown in Table 1. The data show that the highest average Caspase-3 value was found in the sepsis control group (KS) at 80.15 ± 20.91. The lowest average Caspase-3 value was observed in treatment group 2 (KP2) at 53.14 ± 41.41. The average hippocampus IHC Caspase-3 values are plotted in Graph 1.

Table 1. Differences in Hippocampus Caspase-3 IHC Based on Secretome Administration

Group	IHC Caspase-3 (mean ± standard deviation)
KN	56.41 ± 34.57
KS	80.15 ± 20.91
KP1	57.28 ± 32.11
KP2	53.14 ± 41.41
KP3	58.90 ± 29.69



Graph 1. Average Hippocampus Caspase-3 IHC Values

The Shapiro-Wilk and Levene's tests indicated that the Caspase-3 hippocampus values were normally distributed and that the data were homogeneous ($p > 0.05$). Subsequently, the data were analyzed using a parametric One-Way ANOVA. One-Way ANOVA can be performed if the data meet the assumptions of normality and homogeneity of variance. Since the Caspase-3 data were normally distributed and homogeneous, a one-way ANOVA was conducted (Table 2).

Table 2. One-Way ANOVA for Caspase-3

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	2337.986	4	584.497	0.508	0.730
Within Groups	23009.478	20	1150.474		
Total	25347.464	24			

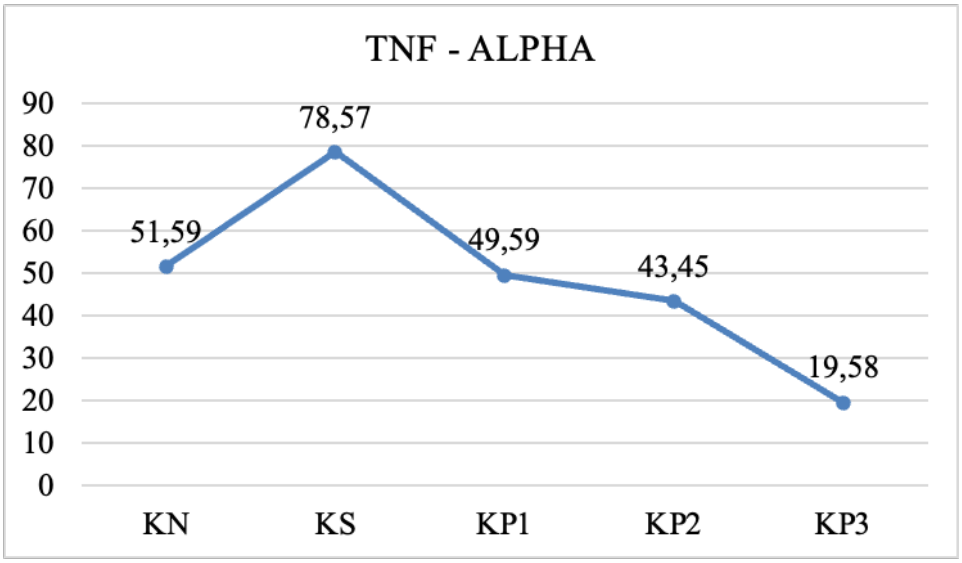
The One-Way ANOVA test was conducted to examine the effect of secretome administration on Caspase-3 levels. Table 2 shows a p-value of 0.730 ($p > 0.05$), indicating no significant difference in the mean Caspase-3 levels between the groups.

Effect of Secretome Administration at Various Treatment Doses on Hippocampus TNF-α IHC

The differences in hippocampal TNF-α IHC following secretome administration are shown in Table 3. The data show that the highest average TNF-α value was observed in the negative control group (KS), at 78.57 ± 25.88. The lowest average TNF-α value was observed in treatment group 3 (KP3) at 19.58 ± 33.07. The average hippocampus IHC TNF-α values are plotted in Graph 2.

Table 3. Differences in Hippocampus TNF-α IHC Based on Secretome Administration

Group	IHC TNF-α (mean ± standard deviation)
KN	51.59 ± 33.44
KS	78.57 ± 25.88
KP1	49.59 ± 44.34
KP2	43.45 ± 33.78
KP3	19.58 ± 33.07



Graph 2. Average Hippocampus TNF-α IHC Values

The normality and homogeneity tests for TNF-α showed that the hippocampus TNF-α values were not normally distributed ($p < 0.05$) but were homogeneous ($p > 0.05$). Since the two conditions for performing the One-Way ANOVA test were not met, the non-parametric Kruskal-Wallis test was conducted to assess the effect of secretome administration on TNF-α levels (Table 4).

Table 4. Kruskal-Wallis Test for TNF-α

	TNF- α
Kruskal-Wallis	7.023
df	4
Asymp. Sig.	0.135

DISCUSSION

This study investigated the therapeutic potential of Mesenchymal Stem Cell (MSC) secretome in mitigating hippocampal damage in a sepsis mouse model. Our results showed a trend of decreased expression of Caspase-3 and TNF-α in groups treated with MSC secretome, particularly at the highest dose (600 µL). However, these reductions did not reach statistical significance ($p > 0.05$).

The decrease in Caspase-3 expression in the treatment groups, although not significant, aligns with the known anti-apoptotic properties of MSC-derived factors. MSC secretomes contain various bioactive molecules and exosomes that can modulate the Bax/Bcl-2 ratio, thereby inhibiting the activation of pro-apoptotic caspases [11]. The lack of statistical significance in our study might be attributed to the timing of administration or the specific dosage range used. Previous studies have suggested that the neuroprotective effects of MSC-derived therapies are highly dependent on the therapeutic window and the severity of the initial inflammatory insult [14].

Similarly, TNF-α levels in the hippocampus decreased following secretome administration. TNF-α is a primary mediator of neuroinflammation in sepsis-associated brain dysfunction (SABD), leading to microglial activation and disruption of the blood-brain barrier [10]. The reduction observed in the KP3 group (600 µL) suggests a dose-dependent immunomodulatory effect, with higher concentrations of secretome more effectively neutralizing the pro-inflammatory environment within the central nervous system [13,15].

Several factors may have contributed to the non-significant results. First, the route of administration (intraperitoneal) may affect the bioavailability of the secretome reaching the brain compared to intravenous or intranasal routes [16]. Second, the observation period post-intervention might have been insufficient to capture the full regenerative impact of the secretome. Lastly, unlike some studies that combined MSC therapy with antibiotics, this study focused solely on the secretome, which might yield different results in the absence of antimicrobial synergy [17].

Despite the lack of statistical significance, this research provides preliminary evidence that MSC secretome has the potential to attenuate hippocampal markers of apoptosis and inflammation. Future studies should explore higher dosage gradients, alternative delivery routes, and longer observation intervals to further elucidate the neuroprotective efficacy of the MSC secretome in sepsis.

CONCLUSIONS

The administration of mesenchymal stem cell secretome showed a potential trend in reducing hippocampal Caspase-3 and TNF-α levels in a sepsis mouse model, particularly at a dose of 600 µL. However, these reductions were not statistically significant compared to the sepsis control group. These findings suggest that while the MSC secretome has immunomodulatory potential, further research involving optimized dosages, alternative administration routes, or larger sample sizes is necessary to fully elucidate its therapeutic efficacy in preventing sepsis-associated brain dysfunction.

Author Contributions

Conceptualization, R.N. methodology, R.N. and A.; validation, R.A.A; formal analysis, R.N.; investigation, R.N.; resources, A.; data curation, R.N.; writing—original draft preparation, R.N.; writing—review and editing, R.N., A., and R.A.A.; supervision, A. All authors have read and agreed to the published version of the manuscript.

Funding

This research received no external funding.

Institutional Review Board Statement

The animal study protocol was approved by the Health Research Ethics Committee of the Faculty of Medicine, Universitas Sebelas Maret (protocol code 1.605/VI/HREC/2024 and date of approval).

Data Availability Statement

The data presented in this study are available on request from the corresponding author. The data are not publicly available due to privacy restrictions.

Acknowledgments

We thank the staff of the Food and Nutrition PAU Laboratory, Gadjah Mada University, and the Anatomical Pathology Laboratory, Faculty of Medicine, Universitas Sebelas Maret, for their technical assistance during the study.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Rababa, M.; Hamad, D.B.; Hayajneh, A.A. Sepsis assessment and management in critically Ill adults: A systematic review. PLoS ONE 2022, 17, e0270711. https://doi.org/10.1371/journal.pone.0270711
2. Rudd, K.E.; Johnson, S.C.; Agesa, K.M.; Shackelford, K.A.; Tsoi, D.; Kievlan, D.R.; Colombara, D.V.; Ikuta, K.S.; Kissoon, N.; Finfer, S.; et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: Analysis for the Global Burden of Disease Study. Lancet 2020, 395, 200–211. https://doi.org/10.1016/s0140-6736(19)32989-7
3. Purba, A.K.R.; Mariana, N.; Aliska, G.; Wijaya, S.H.; Wulandari, R.R.; Hadi, U.; Hamzah; Nugroho, C.W.; van der Schans, J.; Postma, M.J. The burden and costs of sepsis and reimbursement of its treatment in a developing country: An observational study on focal infections in Indonesia. Int. J. Infect. Dis. 2020, 96, 211–218. https://doi.org/10.1016/j.ijid.2020.04.075
4. Sekino, N.; Selim, M.; Shehadah, A. Sepsis-associated brain injury: Underlying mechanisms and potential therapeutic strategies for acute and long-term cognitive impairments. J. Neuroinflamm. 2022, 19, 1–16. https://doi.org/10.1186/s12974-022-02464-4
5. Sonneville, R.; Verdonk, F.; Rauturier, C.; Klein, I.F.; Wolff, M.; Annane, D.; Chretien, F.; Sharshar, T. Understanding brain dysfunction in sepsis. Ann. Intensive Care 2013, 3, 15. http://www.annalsofintensivecare.com/content/3/1/15
6. Czempik, P.F.; Pluta, M.P.; Krzych, Ł.J. Sepsis-associated brain dysfunction: A review of current literature. Int. J. Environ. Res. Public Health 2020, 17, 5852. https://doi.org/10.3390/ijerph17165852
7. Sonneville, R.; de Montmollin, E.; Poujade, J.; Garrouste-Orgeas, M.; Souweine, B.; Darmon, M.; Mariotte, E.; Argaud, L.; Barbier, F.; et al. Potentially modifiable factors contributing to sepsis-associated encephalopathy. Intensive Care Med. 2017, 43, 1075–1084. https://doi.org/10.1007/s00134-017-4807-z
8. Savitri, L.; Sandhika, W.; Widodo, A.D.W. Perbedaan Ekspresi Caspase 3 pada Limpa Tikus Model Sepsis yang Diinfeksi Escherichia coli ESBL dan dengan yang Diinfeksi Klebsiella pneumoniae Carbapenemase. In Seminar Nasional Multidisiplin; Universitas Airlangga: Surabaya, Indonesia, 2019.

9. Brites, D.; Fernandes, A. Neuroinflammation and depression: Microglia activation, extracellular microvesicles and microRNA dysregulation. Front. Cell. Neurosci. 2015, 9, 476. https://doi.org/10.3389/fncel.2015.00476
10. Zhang, K.; Jiang, Y.; Wang, B.; Li, T.; Shang, D.; Zhang, X. Mesenchymal Stem Cell Therapy: A Potential Treatment Targeting Pathological Manifestations of Traumatic Brain Injury. Oxid. Med. Cell. Longev. 2022, 2022, 4645021. https://doi.org/10.1155/2022/4645021
11. Silva, A.Y.O.; Amorim, É.A.; Barbosa-Silva, M.C.; Lima, M.N.; Oliveira, H.A.; Granja, M.G.; Oliveira, K.S.; Fagundes, P.M.; Neris, R.L.S.; Campos, R.M.P.; et al. Mesenchymal Stromal Cells Protect the Blood-Brain Barrier, Reduce Astrogliosis, and Prevent Cognitive and Behavioral Alterations in Surviving Septic Mice. Crit. Care Med. 2020, 48, e290–e298. https://doi.org/10.1097/CCM.0000000000004219
12. Petryk, N.; Shevchenko, O. Mesenchymal stem cells anti-inflammatory activity in rats: Proinflammatory cytokines. J. Inflamm. Res. 2020, 13, 293–301. https://doi.org/10.2147/JIR.S256932
13. Baez-Jurado, E.; Hidalgo-Lanussa, O.; Barrera-Bailón, B.; Sahebkar, A.; Ashraf, G.M.; Echeverria, V.; Barreto, G.E. Secretome of Mesenchymal Stem Cells and Its Potential Protective Effects on Brain Pathologies. Mol. Neurobiol. 2019, 56, 6902–6927. https://doi.org/10.1007/s12035-019-1570-x
14. Santamaria, G.; Brandi, E.; Vitola, P.L.; Grandi, F.; Ferrara, G.; Pischiutta, F.; Vegliante, G.; Zanier, E.R.; Re, F.; Uccelli, A.; et al. Intranasal delivery of mesenchymal stem cell secretome repairs the brain of Alzheimer's mice. Cell Death Differ. 2021, 28, 203–218. https://doi.org/10.1038/s41418-020-0592-2
15. Ngulum, M.N.; Arifin; Wijaya, B.B.; Sunggoro, A.J. Pengaruh Secretome Sel Punca Mesenkimal Terhadap Tingkat Kerusakan Parenkim Hepar Ditinjau Dari Ekspresi Interleukin-6 Dan Caspase 3 Pada Mencit Model Sepsis. Thesis, Universitas Sebelas Maret, Surakarta, Indonesia, 2024.
16. Sari, M.I. Efek Pemberian Sekretom Sel Punca Mesenkimal terhadap Ekspresi NF-kB, Kadar TNF-α, IL-6, IL-10, IL-4 dan Ekspresi PD-1, PD-L1 serta Survival Rate Tikus Model Sepsis. Thesis, Universitas Sumatera Utara, Medan, Indonesia, 2023.