

Evaluation of the Mixture "MagSelDex" For Neuroprotection Against Offspring Brain Injury in Endotoxin-Induced Chorioamnionitis: A Preliminary Study

Bakteriyel Endotoksin Kaynaklı Koryoamniyonit Nedeniyle Oluşan Fetal Beyin Hasarına Karşı "MagSelDex" Karışımının Nöroprotektif Etkilerinin Değerlendirilmesi: Ön Çalışma

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ABSTRACT

Objective: Chorioamnionitis resulting from preterm labor leads to concurrent damage in both the placenta and fetal brain. This study aims to explore the impact of incorporating antioxidants and anti-inflammatory agents, specifically selenium (Sel) and dexpanthenol (Dex), into the standard magnesium (Mg) regimen, in mitigating this damage.

Materials and Methods: A total of six pregnant rats were assigned to six distinct groups: control, lipopolysaccharide (LPS) (1 mg/kg, single intraperitoneal dose on day 17), Mg (60 mg/kg Mg, intraperitoneal), Mg+Sel (1 mg/kg, intraperitoneal), Mg+Dex (500 mg/kg, intraperitoneal), and Mg+Sel+Dex. On the 17th day of pregnancy, fetal brain and placenta tissues were harvested for histopathological examination and immunohistochemical evaluation of tumor necrosis factor-alpha (TNF- α) and neurofilament expression.

Results: The histopathological assessment revealed LPS-induced hemorrhage and mild inflammatory cell infiltration in the placenta, and pronounced hyperemia along with minor hemorrhage in the fetal brain. The LPS group exhibited significantly elevated TNF- α expression in both placenta and fetal brain, coupled with reduced neurofilament expression in the fetal brain. In contrast, the groups treated with Mg alone and the combined Sel and Dex therapy exhibited moderate to substantial improvement in pathological findings across both tissues. The most notable enhancement was observed in the Mg+Sel+Dex group.

Conclusion: Administration of Mg as a standalone treatment and the coadministration of Sel and Dex effectively shielded the placenta and fetal brain from LPS-triggered chorioamnionitis. However, the most prominent protective effect was observed in the Mg+Sel+Dex group.

Keywords: Brain injury, Corioamnionitis, Dexpanthenol, Magnesium, Selenium

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Ö Z E T

Amaç: Preterm doğum kaynaklı koryoamnionitin plasenta ve fetal beyinde eş zamanlı hasara neden olduğu bilinmektedir. Bu çalışma, standart magnezyum (Mg) rejimine antioksidan ve anti-inflamatuar ajanlar olan selenyum (Sel) ve dekspantenol (Dex) eklemenin, bu hasarı hafifletmedeki etkisini araştırmayı amaçlamaktadır.

Gereç ve Yöntem: Toplam altı gebe sıçan, altı ayrı gruba ayrılmıştır: kontrol, lipopolisakkarit (LPS) (1 mg/kg, 17. gün tek doz intraperitoneal uygulama), Mg (60 mg/kg, intraperitoneal), Mg+Sel (1 mg/kg, intraperitoneal), Mg+Dex (500 mg/kg, intraperitoneal) ve Mg+Sel+Dex. Gebeliğin 17. gününde fetal beyin ve plasenta dokuları histopatolojik inceleme ile tümör nekroz faktör-alfa (TNF- α) ve nörofilament (NF) ekspresyonlarının immünohistokimyasal değerlendirilmesi için toplandı.

Bulgular: Histopatolojik değerlendirme, plasentada LPS kaynaklı kanama ve hafif inflammatuar hücre infiltrasyonunu ortaya çıkardı; aynı zamanda fetal beyinde belirgin hiperemi ve hafif kanama görüldü. LPS grubunda, hem plasenta hem de fetal beyinde belirgin şekilde yükselmiş TNF- α ekspresyonu ve fetal beyinde azalmış NF ekspresyonu gözlemlendi. Bunun aksine, yalnızca Mg ile tedavi edilen gruplar ve birlikte uygulanan Sel ve Dex tedavisi, her iki dokuda da patolojik bulgularda düzelme sergiledi. En dikkate değer iyileşme Mg+Sel+Dex grubunda gözlemlendi.

Sonuç: Mg'nin tek başına uygulanması ve Sel ile Dex'in birlikte uygulanması, plasentayı ve fetal beyini LPS ile indüklenmiş koryoamnionitten etkili bir şekilde korudu. Ancak, en belirgin koruyucu etki Mg+Sel+Dex grubunda gözlemlendi.

Anahtar Kelimeler: Beyin Hasarı, Dekspantenol, Koryoamnionit, Magnezyum, Selenyum



1. Introduction

Among the primary contributors to perinatal mortality, preterm births (PTB) and subclinical intra-amniotic infections have emerged as significant causes of prematurity. Studies employing sensitive methods to analyze amniotic fluid have revealed infection and/or inflammation markers within the amnion in a substantial proportion, at least 40-50%, of preterm pregnancies [1]. In cases of premature rupture of membranes, often triggered by inflammation within the chorioamniotic membranes, activation of matrix metalloproteinases ensues, leading to membrane impairment and rupture [2, 3].

Definitive solutions remain elusive for several perinatal challenges, particularly cerebral palsy [4]. Inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) increase permeability, enabling inflammatory agents to traverse into the fetal circulation and fetal brain [5]. A schematic representation of the plausible mechanism underlying placental and neuronal damage induced by TNF- α in conjunction with lipopolysaccharide (LPS) is given in Figure 1.

Paradoxically, the administration of systemic maternal antibiotics to expectant mothers at preterm delivery risk has been linked to increased rates of cerebral palsy. This can be attributed to amplified brain damage due to cytokine release subsequent to antibiotic treatment [6, 7]. Presently, magnesium (Mg) stands as the sole neuroprotective agent. Evidence suggests that Mg exerts its neuroprotective effects by suppressing cerebral inflammation [8]. Limited literature highlights that Mg treatment may regress the elevated TNF- α levels, safeguarding fetal brain tissue from harm [9]. Moreover, clinical studies have demonstrated that antenatal Mg reduces the incidence of cerebral palsy among newborns [10].

Despite these effects, the feasibility of recommending magnesium sulfate for individuals at risk of preterm birth remains a topic of debate. Its effectiveness is not 100%, as depicted in obstetric literature and practices [10-14]. Consequently, there is a demand for safe compounds capable of impeding the inflammatory cascade at early stages, thereby preventing cases of preterm birth and associated cerebral palsy.

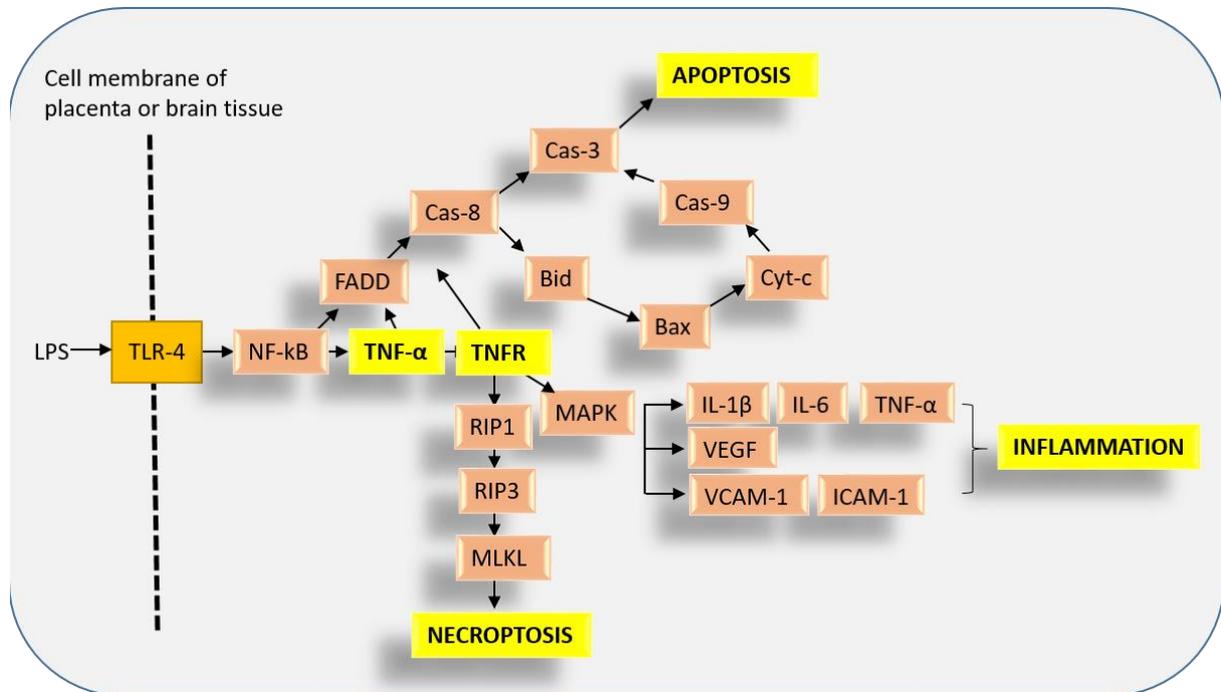


Figure 1. The possible mechanism of TNF- α signaling-induced placental and neuronal damage.

LPS: Lipopolysaccharide, TLR-4: Toll-like receptor, NF- κ B: Nuclear factor kappa beta, FADD: Fas Associated Via Death Domain, Cas: Caspase, Cyt-c: Cytochrome c, Bax: Bcl-2-associated X protein, TNF- α : Tumor necrosis factor alpha, RIP: Receptor interacting protein kinase, MLKL: Mixed lineage kinase domain-like pseudokinase, MAPK: Mitogen-activated protein kinase, IL-1 β : Interleukin-1 beta, IL-6: Interleukin-6, VEGF: Vascular endothelial growth factor, VCAM-1: Vascular cell adhesion molecule 1, ICAM-1: Intercellular adhesion molecule-1.

Selenium (Sel) is a vital trace element characterized by its antioxidant and anti-inflammatory attributes. Sel plays a pivotal role in the composition of glutathione (GSH)-peroxidases, which rank as the most potent antioxidant enzymes safeguarding cells against oxidative harm and diverse ailments stemming from such damage [15]. Research has indicated a decline in serum Sel levels during pregnancy [16]. It is established that Sel through the reduction of TNF- α levels, mitigates brain tissue damage for a multitude of reasons [17].

Dexpanthenol (Dex) is an active biologically-alike analog of pantothenic acid. Pantothenic acid exerts its antioxidant effects by augmenting the synthesis of reduced GSH and associated peroxidase enzymes, which constitute the paramount defensive mechanisms against oxidative stress and lipid peroxidation [18]. While the impact of Dex on this pathway has not been extensively explored, numerous investigations have demonstrated pantothenic acid's inhibitory influence on nuclear factor kappa beta (NF- κ B) [19]. Thus, insights into the cellular-level actions of Dex, which has recently gained attention due to its anti-inflammatory and antioxidant properties, remain somewhat limited.

The principal objective of this study was to ascertain whether the protective efficacy of magnesium (Mg) therapy against fetal brain damage ensuing from LPS-induced chorioamnionitis can be amplified through the adjunct incorporation of Sel and Dex. Furthermore, the study aims to present an exhaustive framework that encompasses Western blot and PCR analyses, encompassing the comprehensive outcomes derived from this investigation.

2. Material and Method

Ethical Approval

This study was conducted using Wistar Albino female rats at 10-12 weeks of pregnancy. The rats were sourced from the Süleyman Demirel University Experimental Animals Laboratory. The entire experiment adhered to the animal research guidelines established by the National Institutes of Health. The local animal experiments ethics committee of Süleyman Demirel University (Isparta, Türkiye) granted approval for the experimental protocol, with the reference number 16.06.2022/05-65.

Animal Selection and Experimental Setup

Six mature pregnant Wistar Albino female rats, weighing between 300 and 350 grams, were accommodated in Euro type-4 cages. The animals were maintained under stable environmental conditions, including a constant temperature of 22 ± 2 °C, humidity levels of 55-60%, and a light-dark cycle of 12 hours each. During their stay, the rats received standard care encompassing feeding, housing, and general well-being. Both female and male rats shared the same environment, with the estrous cycle of female rats monitored via daily vaginal smear samples. Alternatively, pregnancy strips were utilized. The day of conception was identified as day 0 for rats with confirmed smears. The six pregnant rats were assigned to different groups, each containing one rat. The experimental groups are illustrated in Figure 2.

Control (n=1): On days 15-17 of pregnancy, 1 ml of saline (PS) was administered intraperitoneally (ip) from both the right and left inguinal regions. One hour after the PS administration on day 17, an additional 1 ml of PS was administered ip from the right inguinal area.

LPS (n=1): On days 15-17 of pregnancy, 1 ml of PS was administered ip from both the right and left inguinal regions. One hour after the PS administration on day 17, 1 mg/kg of LPS was administered ip from the right inguinal area [20].

Mg (n=1): On days 15-17 of pregnancy, 60 mg/kg of MgSO₄ was administered ip from the right inguinal area, and 1 ml of PS was administered from the left inguinal area [21]. One hour after the drug administration on day 17, 1 mg/kg of LPS was administered ip from the right inguinal area.

Mg+Sel (n=1): On days 15-17 of pregnancy, 60 mg/kg of MgSO₄ was administered ip from the right inguinal area, and 1 mg/kg of Sel was administered from the left inguinal area [22]. One hour after the drug administration on day 17, 1 mg/kg of LPS was administered ip from the right inguinal area.

Mg+Dex (n=1): On days 15-17 of pregnancy, 60 mg/kg of MgSO₄ was administered ip from the right inguinal area, and 500 mg/kg of Dex was administered from the left inguinal area [23]. One hour after the drug administration on day 17, 1 mg/kg of LPS was administered ip from the right inguinal area.

Mg+Sel+Dex (n=1): On days 15-17 of pregnancy, a combined preparation (MagSelDex) containing 60 mg/kg of MgSO₄, 1 mg/kg of Sel, and 500 mg/kg of Dex was administered ip from the right inguinal area. Simultaneously, an equal volume of PS was administered ip from the left inguinal area. One hour after the drug administration on day 17, 1 mg/kg of LPS was administered ip from the right inguinal area.

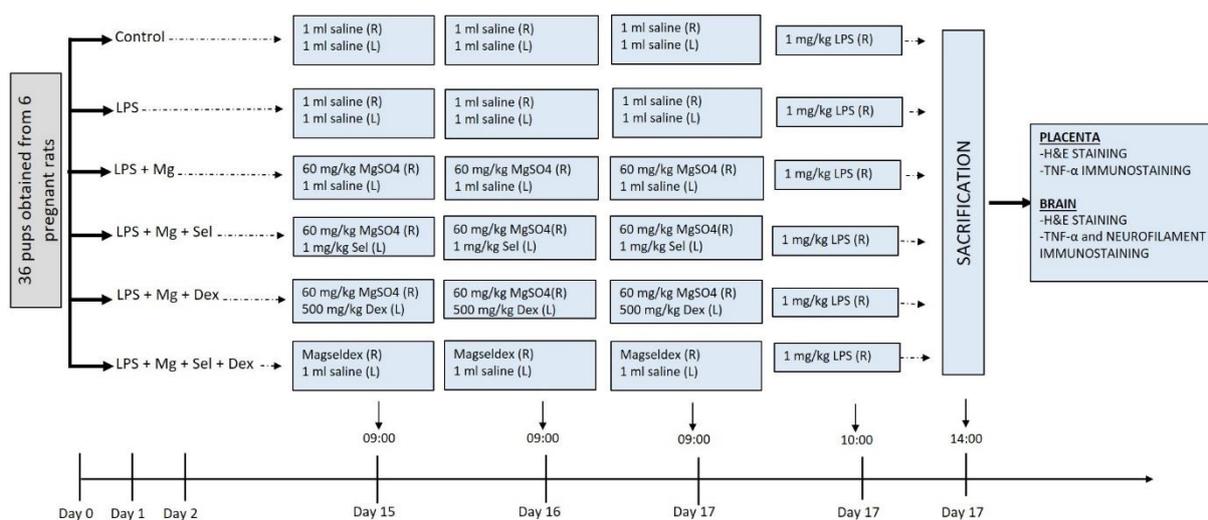


Figure 2. Experimental procedure of the study.

MgSO₄: Magnesium sulfate, Sel: Selenium, Dex: Dexamethasone, LPS: Lipopolysaccharide, TNF- α : Tumor necrosis factor-alpha, H&E: Hematoxylin Eosin, R: Right, L: Left.

Animal Euthanasia and Sample Collection

Subsequent to the final day of drug administration, euthanasia of all rats was performed using the surgical exsanguination method. This process involved abdominal incision under intraperitoneal (ip) anesthesia utilizing 90 mg/kg ketamine (Keta-Control, Doğa Ilac, Türkiye) and 10 mg/kg xylazine (Xylazine Bio %2, Bioveta, Czechia). A total of 36 placental tissues and fetal brains, harvested from 6 amniotic sacs taken from pregnant rats, were preserved in 10% formaldehyde for subsequent histopathological and immunohistochemical analyses, specifically targeting TNF- α and neurofilament (NF) expressions.

Chemicals

LPS 048K4126 was procured from Sigma Aldrich, USA. Injectable magnesium sulfate solution (Osel®) was sourced from a local pharmacy (Osel, Turkey). Selenium powder was acquired from Sigma Aldrich, USA. Injectable dexamethasone solution (Bepanthen®, Bayer, Turkey) was purchased from a pharmacy.

Preparation of Combined Formulation of Magnesium and Selenium

For administration to pregnant Wistar Albino rats, a solution combining Mg and Sel was formulated. Sel powder, weighing 1 mg/kg Sel based on each animal's weight, was dissolved in physiological saline [22]. Employing a commercial injection solution containing 1.5 g MgSO₄ in each 10 ml ampoule, 60 mg/kg Mg solutions were prepared for each pregnant rat [21]. The two solutions were thoroughly mixed, filled into ampoules, sealed using an ampoule sealing machine (YH® RF-1), and subjected to wet heat sterilization at 121°C for 15 minutes.

Preparation of Combined Formulation of Magnesium and Dexamethasone

A combined solution of Mg and Dex was devised. Using a weight-based approach, 60 mg/kg Mg solutions were prepared for each pregnant rat, employing a commercial injectable solution containing 1.5 g MgSO₄ in each 10 ml ampoule. Similarly, a solution containing 500 mg/kg Dex was prepared for each pregnant rat, relying on the commercial injectable solution containing 500 mg of Dex in each 2 ml ampoule [23]. The two solutions were mixed, loaded into ampoules, sealed, and then sterilized using wet heat at 121°C for 15 minutes.

Preparation of Combined Formulation of Magnesium, Selenium, and Dexpanthenol

For the comprehensive Mg, Sel, and Dex formulation, the first solution was crafted by employing the commercial injectable solution containing 1.5 g MgSO₄ in each 10 ml ampoule. This was used to formulate 60 mg/kg Mg per pregnant Wistar Albino rat, proportionate to each animal's weight. A second solution was devised for the combined solution, drawing from the commercial injectable solution containing 500 mg of Dex in each 2 ml ampoule, resulting in a solution of 500 mg/kg Dex for each pregnant rat. Additionally, Sel powder, weighing 1 mg/kg Sel based on each animal's weight, was dissolved in physiological saline. The three solutions were blended, loaded into ampoules, sealed, and then subjected to wet heat sterilization at 121°C for 15 minutes.

Histopathological Analyses

During necropsy, placental and fetal brain samples were meticulously collected and preserved in 10% buffered formalin for subsequent histopathological evaluation. Using an automated tissue processor, tissue samples were processed, and 5 µm sections were obtained from paraffin blocks using a rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). These sections were stained with hematoxylin-eosin (HE), dehydrated with xylene, and cover-slipped for microscopic examination. An impartial expert pathologist from another institution conducted blinded histopathological evaluations using a light microscope.

Immunohistochemical Examinations

Fetal brain and placenta samples, mounted on polylysine-coated slides, underwent immunostaining for TNF-α (Recombinant Anti-TNF alpha antibody [RM1005] (ab307164), 1/100 dilution), and fetal brain samples for NF (Anti-160 kD Neurofilament Medium antibody [NF-09] – Neuronal Marker (ab7794), 1/100 dilution), adopting the streptavidin-biotin technique. Both primary and secondary antibodies were procured from Abcam (Cambridge, UK). Following a 60-minute incubation with primary antibodies, sections were subjected to immunohistochemical staining using biotinylated secondary antibodies and streptavidin-alkaline phosphatase conjugate. EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) served as the secondary antibody, and diaminobenzidine (DAB) acted as the chromogen. Negative controls were established by replacing the primary antiserum with the antigen dilution solution. All tests were executed on blinded samples. Immunohistochemical scores were computed and analyzed using ImageJ software (version 1.48, National Institutes of Health, Bethesda MD). Microphotography was facilitated using the Database Manual Cell Sens Life Science Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical Analyses

Histopathological scores of the groups were subjected to statistical analyses employing the One-way ANOVA Duncan test from the SPSS-22.00 software package. The threshold of significance was established at $P < 0.05$.

3. Results

Histopathological Findings

Examination of placenta from the control group unveiled no pathological anomalies. Conversely, LPS application resulted in placental hemorrhage and marginal inflammatory cell infiltration. Treatment with Mg displayed moderate improvement, notably reducing infiltration and hemorrhage. The Mg-Dex and Mg-Sel groups revealed substantial recovery from LPS-induced placental lesions. The most pronounced reduction was observed in the Mg+Sel+Dex group, as shown in Figure 3.

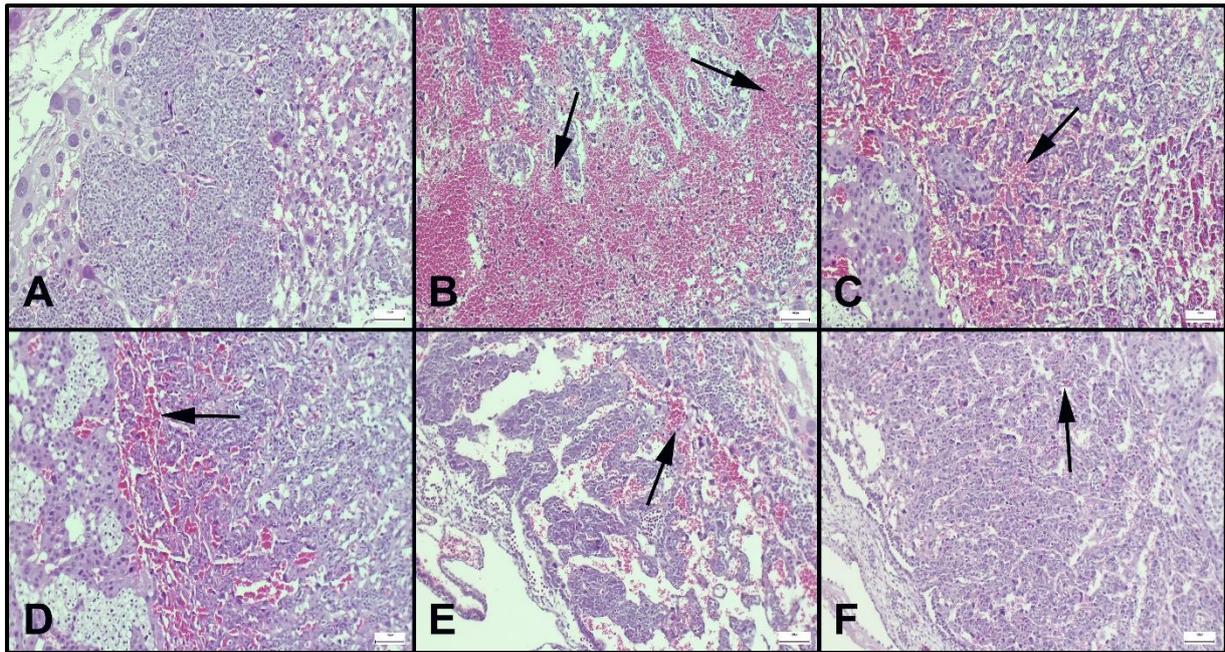


Figure 3. Histopathological appearance of the placenta in the experimental groups.

(A) Normal placental histology in the control group. (B) Marked hemorrhage (arrows) in the LPS group. (C) Moderate decrease in hemorrhage (arrow) in the Mg group. (D) Marked decrease in hemorrhage (arrow) in placenta in the Mg+Dex group. (E) Similar decrease in hemorrhage (arrow) in placenta in the Mg+Sel group. (F) Almost normal appearance and markedly decrease in hemorrhage (arrow) in placenta in the Mg+Sel+Dex group, HE, Scale bars= 100µm.

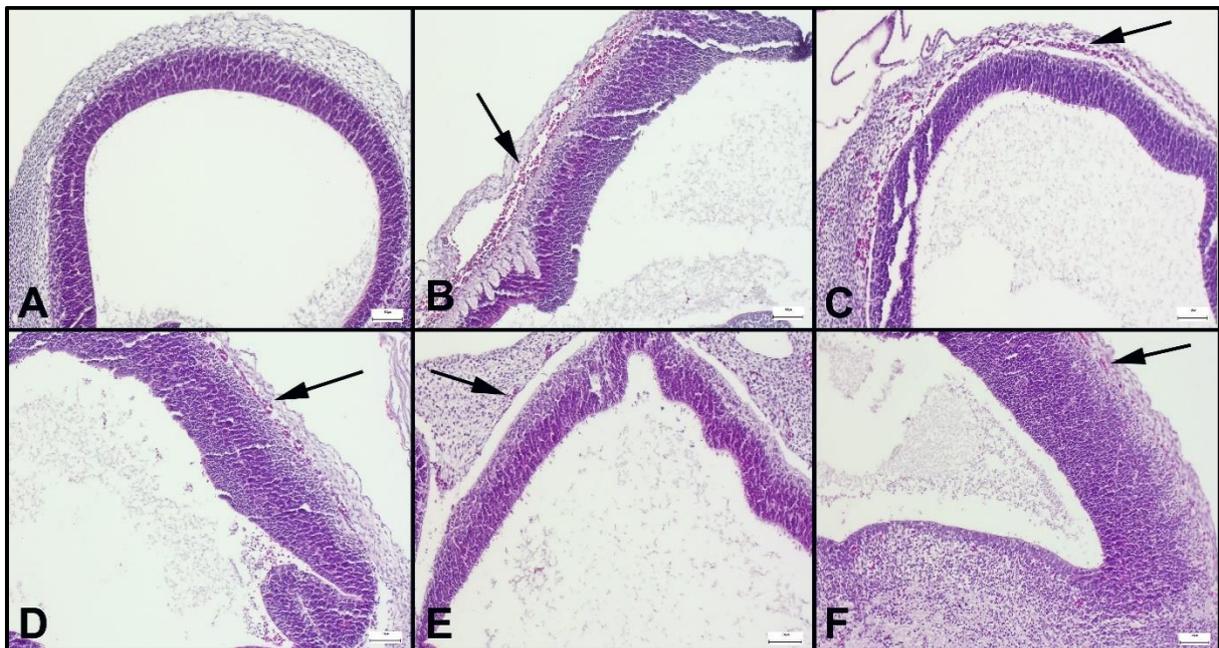


Figure 4. Representative histopathological microphotos of the brains.

(A) Normal brain histology in the control group. (B) Marked hyperemia (arrows) in the LPS group. (C) Decrease in hyperemia (arrow) in the Mg group. (D) Marked decrease in hyperemia (arrow) in fetal brain in the Mg+Dex group. (E) Similar decrease in hyperemia (arrow) in brain in the Mg+Sel group. (F) Only slight hyperemia (arrow) in brain in the Mg+Sel+Dex group, HE, Scale bars= 100µm.

At the histopathological examination of the fetal brain samples, normal tissue histology was observed in the control group. LPS group showed marked hyperemia and slight hemorrhage in the fetal brains. Mg decreases the pathological findings in fetal brains. Similarly, Mg+Dex and Mg+Sel treatments markedly decreased in these groups. Combined treatment with Mg+Sel+Dex were more effective for the reduced LPS-induced lesions in brains (Figure 4).

Immunohistochemical Findings

TNF- α Immunohistochemistry:

Evaluation of placental and fetal brain sections revealed negative expressions in the control group, while marked expressions were evident in the LPS group. The administration of Mg reduced the expressions, and a substantial decrease was observed in the Mg+Dex and Mg+Sel groups. Most notably, the Mg+Sel+Dex group displayed the most significant decrease in expressions (Figure 5, Figure 6).

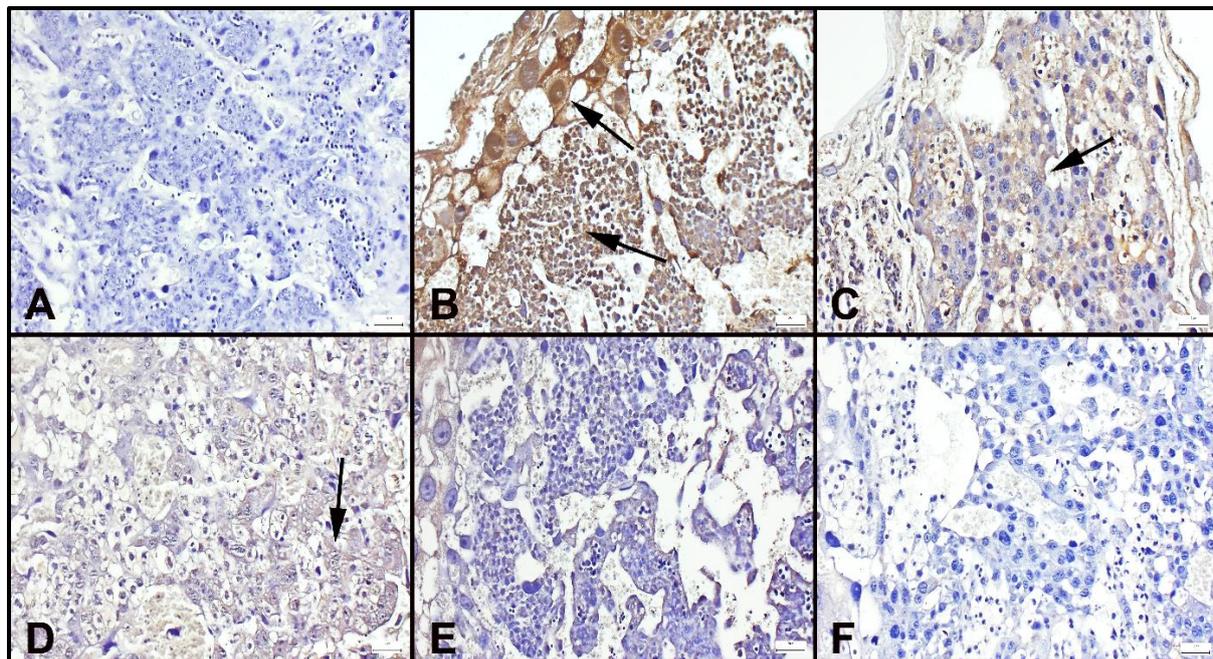


Figure 5. TNF- α immunohistochemical findings in the placenta.

(A) Negative expression in control group. (B) Marked expression (arrows) in the LPS group. (C) Decreased expression (arrow) in Mg group. (D) Marked decrease (arrow) in Mg+Dex group. (E) More marked decrease in Mg+Sel group. (F) Almost negative expression in Mg+Sel+Dex group, Streptavidin Biotin Peroxidase method, Scale bars= 50 μ m.

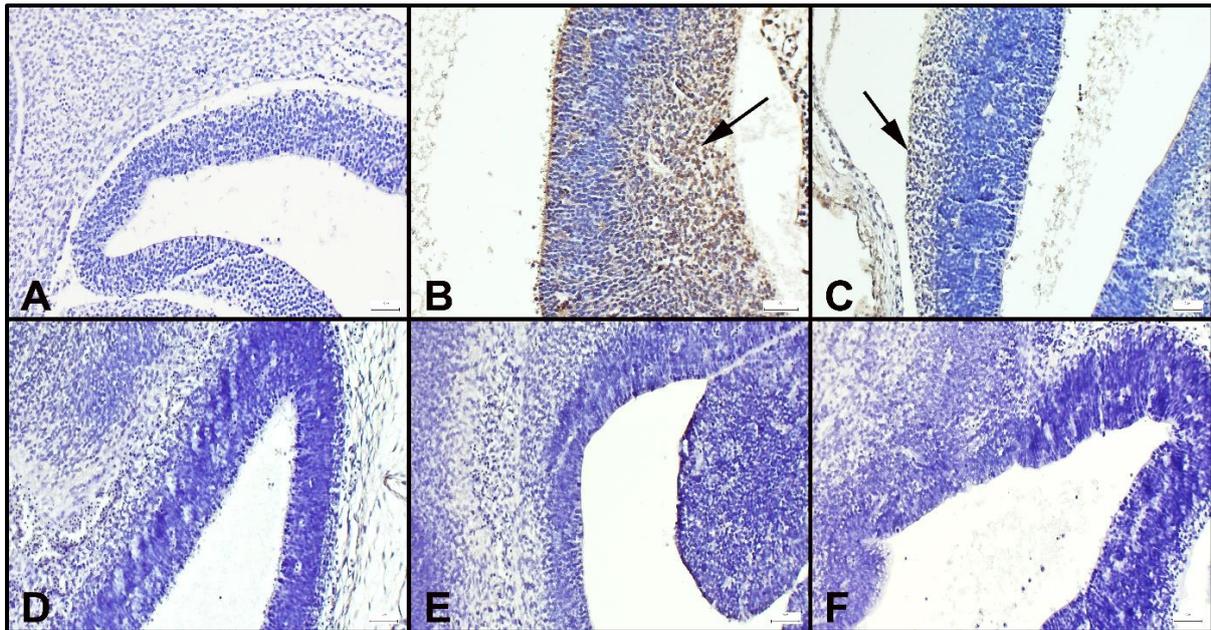


Figure 6. TNF- α immunohistochemical findings in the fetal brains.

(A) Negative expression in control group. (B) Marked expression (arrows) in the LPS group. (C) Decreased expression (arrow) in Mg group. (D) Marked decrease in Mg+Dex group. (E) More marked decrease in Mg+Sel group. (F) Almost negative expression in Mg+Sel+Dex group, Streptavidin Biotin Peroxidase method, Scale bars= 50 μ m.

NF Immunohistochemistry:

Immunostaining of NF in fetal brain sections displayed marked expression in the control group. In the LPS group, a significant decrease in expression was noted. Conversely, the Mg, Mg+Dex, and Mg+Sel groups exhibited increased expressions. The most pronounced expression was observed in the Mg+Sel+Dex group (Figure 7).

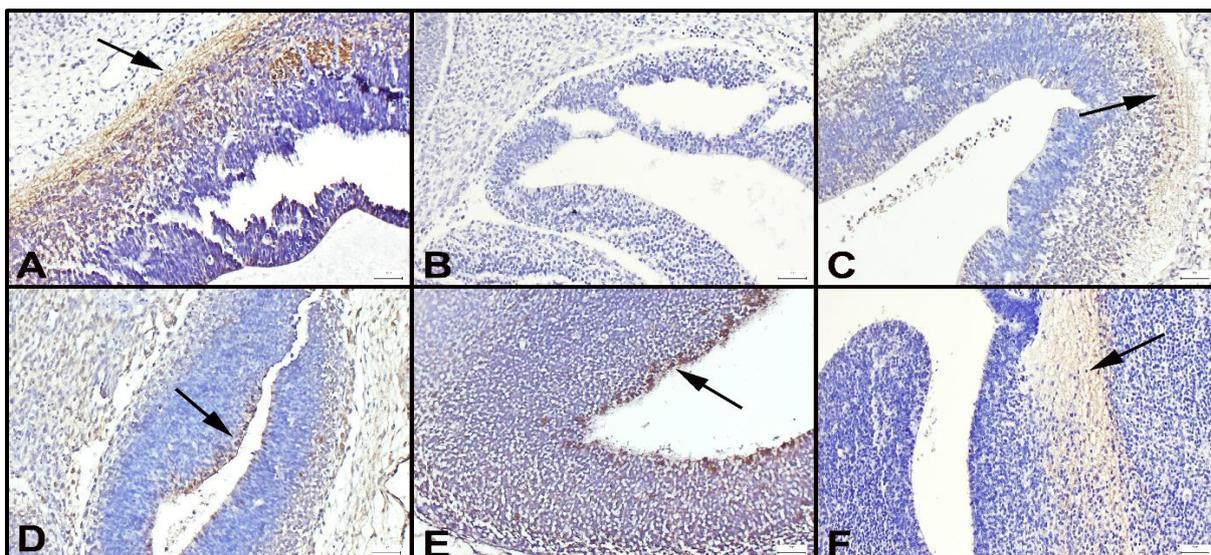


Figure 7. NF immunohistochemical findings in the fetal brains.

(A) Marked expression (arrow) in control group. (B) Marked decrease in expression in the LPS group. (C) Increased expression (arrow) in Mg group. (D) Marked increase in Mg+Dex group. (E) More marked increase (arrow) in Mg+Sel group. (F) Almost normal expression (arrow) in Mg+Sel+Dex group, Streptavidin Biotin Peroxidase method, Scale bars= 50 μ m.

Statistical comparisons of immunohistochemical scores across the groups are shown in Table 1.

Table 1. Comparisons of immunohistochemical scores

	TNF- α Placenta	TNF- α Brain	NF Brain
Control	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	2.50 \pm 0.57 ^a
LPS	2.75 \pm 0.50 ^b	1.75 \pm 0.50 ^b	0.25 \pm 0.25 ^b
Mg	1.75 \pm 0.50 ^c	1.75 \pm 0.50 ^c	1.50 \pm 0.57 ^c
Mg+Dex	1.50 \pm 0.57 ^c	1.25 \pm 0.50 ^c	1.25 \pm 0.50 ^c
Mg+Sel	1.25 \pm 0.50 ^b	1.25 \pm 0.50 ^c	1.25 \pm 0.50 ^c
Mg+Sel+Dex	0.50 \pm 0.28 ^a	0.25 \pm 0.25 ^a	0.25 \pm 0.25 ^a
p value	<0.001	<0.001	<0.001

Data expressed as mean \pm standard deviation (SD) and for evaluation One-way ANOVA test was used. The differences between the groups carrying different letters in the same row are statistically significant, $p < 0.001$.

4. Discussion and Conclusion

The current findings indicate that LPS causes significant hemorrhage and infiltration of inflammatory cells in the placenta. While TNF- α expressions increased in the LPS group, Mg treatment led to a decrease in expressions. NF expressions decreased upon LPS treatment. Administration of Mg, Mg+Dex, Mg+Sel, and Mg+Sel+Dex resulted in an amelioration of these findings. The most pronounced alleviation was observed in the combined Mg+Sel+Dex group. Additionally, the combined treatment also led to an increase in NF expressions in fetal brain samples.

Preterm birth (PTB), defined as pregnancy termination before the 37th week of gestation, stands as a leading cause of neonatal mortality and ranks as the second most common cause of death among children under five globally [24]. Besides the elevated mortality rate, preterm infants face a heightened risk of early and late complications compared to term infants [25]. Consequently, PTB has become a pivotal health indicator in contemporary countries.

In experimental models addressing PTB, one approach to creating preterm birth is through the application of lipopolysaccharide (LPS), a constituent of gram-negative bacterial membranes [26]. LPS is known to activate intracellular pathways, particularly tumor necrosis factor-alpha (TNF- α), via binding to its receptor Toll-like receptor-4 on cell membranes [27]. Activation of this pathway triggers not only inflammatory reactions but also apoptotic and necrotic responses.

The systemic inflammatory response induced by LPS can heighten the permeability of the placenta, a vital vascular structure connecting mother and fetus, allowing inflammatory cytokines circulating in the mother's blood to infiltrate the fetal bloodstream and affect fetal brain tissues [28].

The observation of hemorrhages and inflammatory neutrophilic cell infiltration in placental tissues, as evidenced by histopathological analysis, demonstrates the establishment of an experimental model. Similarly, elevated TNF- α levels indicated by immunohistochemical analysis highlight the presence of acute-phase damage and inflammation.

The aforementioned mechanism's impact on brain tissue attests to the potential influence of systemic inflammation triggered by LPS on fetal brain tissues. Hemorrhagic areas and inflammatory cell infiltrations observed in fetal brain tissues align with the notion that fetal brain tissue inflammation accompanies placental inflammation.

Conventional antibiotic therapies have limited efficacy in preventing this inflammatory response in tissues. Despite widespread application, the effect of Mg in routine treatment is suboptimal and insufficient in reducing morbidity and mortality rates [29, 30]. These circumstances underscore the need for novel treatment modalities or the incorporation of supplementary medications into existing regimens. Notably, antioxidant and anti-inflammatory agents have gained prominence in this context. Such agents typically need to traverse the blood-brain barrier.

The enhanced anti-inflammatory activity observed in groups receiving additional Sel and Dex alongside Mg treatment, particularly the notable effect in the combined therapy group, suggests their potential synergistic use. Such agents likely utilize TNF- α -mediated pathways and exert cumulative suppression. Additionally, the ability of Sel and Dex, which employ diverse intracellular pathways, to reach the primary target—high anti-inflammatory activity—provides promising alternatives.

White matter injury stands as the predominant form of preterm brain injury. Oligodendrocytes and their precursors, critical to neuronal myelination, exhibit heightened sensitivity to inflammation and oxidative stress [31]. While fetal brain development initiates in early pregnancy, the final trimester represents a vital period for neuronal organization and myelination. During this phase, oligodendrocyte maturation and neuronal axon myelination can be compromised by unfavorable microenvironments resulting from hypoxia and/or inflammation [32].

In conclusion, the administration of Mg, Sel, and Dex agents mitigated placental and fetal brain damage stemming from LPS-induced chorioamnionitis. The most pronounced effects were observed with the triple combination. Building upon these findings, our study team aims to delve further by investigating the behavior of each active substance in both pathological and non-pathological scenarios. Exploration of mitochondrial and endoplasmic reticulum stress, pivotal apoptotic pathways, NF- κ B and Mitogen-activated protein kinase pathways central to inflammation, and necroptosis pathways is planned. Additionally, scrutiny of brain-derived growth factor, cAMP response element-binding protein, glial fibrillary acidic protein, and comparable neurotrophic factors will aid in assessing neuroprotection and neurogenesis.

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

The entire experiment adhered to the animal research guidelines established by the National Institutes of Health. The local animal experiments ethics committee of Süleyman Demirel University (Isparta, Türkiye) granted approval for the experimental protocol, with the reference number 16.06.2022/05-65.

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