Research Article / Araştırma Makalesi

Hexagonal Boron Nitride Nanoparticles Prevent Neurodegeneration in Septic Rat Brain

Hekzagonal Bor Nitrür Nanopartikülleri Septik Sıçan Beyninde Nörodejenerasyonu Önler

[1]Ayşe Çakır Gündoğdu, [2]Fatih Kar

[1]Kutahya Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, Kutahya,Türkiye
[2] Kutahya Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, Kutahya, Türkiye

Abstract: Sepsis, which develops with the triggering of an uncontrolled inflammatory response, causes multiple organ damage and dysfunction. Neuroinflammation occurring in sepsis causes varying degrees of deterioration in the central nervous system. Hexagonal boron nitride (h-BN) nanoparticles composed of boron and nitrogen have potential biomedical applications and are well tolerated by animals. Research has indicated that h-BN nanoparticles exhibit antioxidative characteristics. Although the anti-inflammatory properties of the boron present in them, the effectiveness of h-BN nanoparticles on systemic inflammation or neuroinflammation is unknown. Thus, the aim of this research was to investigate the potential protective benefits of h-BN nanoparticles against inflammation induced by lipopolysaccharide (LPS) in rat brains. An intraperitoneal 5 mg/kg dose of LPS was used to induce sepsis in Sprague Dawley rats. h-BN nanoparticles were given at 50 µg/kg and 100 µg/kg concentrations 24 h before LPS injection. To assess the prophylactic effect of h-BN nanoparticles in sepsis-induced neurodegeneration, besides measuring pro-inflammatory, oxidative stress, and apoptosis markers in brain tissues, the cerebral cortex and hippocampus were also examined histopathologically. Our ELISA results show that h-BN nanoparticles inhibit inflammation in the brain as evidenced by the reduction in LPS-induced increase in tumor necrosis factor-alpha (TNF-α) and interleukin-1 beta (IL-1β) levels. h-BN nanoparticles diminished the oxidative stress index and lowered cytochrome c and caspase-3 levels, components of the intrinsic apoptotic pathway. Our histopathological analyzes demonstrated that neuronal and neuroglial damage in the cerebral cortex and hippocampus was also prevented by the treatment of h-BN nanoparticles. These results implicated that h-BN nanoparticles could have a neuroprotective effect against sepsis-induced neurodegeneration through their anti-inflammatory, antioxidant, and anti-apoptotic properties.
Keywords: Apoptosis, Hexagonal Boron Nitride Nanoparticles, Lipopolysaccharide, Neuroinflammation, Oxidative Stress, Sepsis

Özet: Kontrolsüz bir inflamatuar yanıtın tetiklenmesi ile gelişen sepsis, çoklu organ hasarına ve disfonksiyona neden olmaktadır. Sepsiste meydana gelen nöroinflamasyon, merkezi sinir sisteminde değişen derecelerde bozulmalara yol açmaktadır. Bor ve nitrojenden oluşan hekzagonal bor nitrür (h-BN) nanopartikülleri potansiyel biyomedikal uygulamalara sahiptir ve hayvanlar tarafından iyi tolare edilmektedir. Çalışmalar h-BN nanopartiküllerinin antioksidatif özelliklerine sahip olduğunu bildirmiştir. İçerdiği boronun anti-inflamatuar özellikleri bilinmesine rağmen h-BN nanopartiküllerinin sistemik inflamasyon veya nöroinflamasyon üzerindeki etkinliği bilinmemektedir. Bu nedenle, bu çalışmada h-BN nanopartiküllerinin sıçan beyninde lipopolisakkarid (LPS) kaynaklı inflamasyon üzerindeki koruyucu etkilerini araştırmak amaçlanmıştır. Sprague Dawley sıçanlarında sepsis oluşturmak için 5 mg/kg dozda LPS intraperitoneal olarak uygulanmıştır. h-BN nanopartikülleri, LPS enjeksiyonundan 24 saat önce 50 µg/kg ve 100 µg/kg konsantrasyonlarda verilmiştir. h-BN nanopartiküllerinin sepsis kaynaklı nörodejenerasyondaki profilaktik etkisini belirlemek için beyin dokularında pro-inflamatuar, oksidatif stres ve apoptoz belirteçlerinin ölçülmesinin yanı sıra serebral korteks ve hipokampüs histopatolojik olarak incelenmiştir. ELISA sonuçlarımız, h-BN nanopartiküllerinin, tümör nekrozis faktör-alfa (TNF-α) ve interlökin-1 beta (IL-1β) seviyelerinde LPS'nin neden olduğu artıştaki azalma ile kanıtlandığı gibi beyindeki inflamayonu önlediğini göstermektedir. h-BN nanopartikülleri ayrıca oksidatif stres indeksini düşürmüş ve intrinsik apoptotik yolak bileşenleri olan sitokrom c ve kaspaz-3 seviyelerini azaltmıştır. Histopatolojik analizlerimiz, serebral korteks ve hipokampüsteki nöronal ve nöroglial hasarın da h-BN nanopartiküllerinin uygulanması ile önlendiğini göstermiştir. Bu sonuçlar, h-BN nanopartiküllerinin anti-inflamatuar, antioksidan ve anti-apoptotik özellikleri sayesinde sepsis kaynaklı nörodejenasyona karşı koruyucu bir etkiye sahip olabileceğine işaret etmektedir.
Anahtar Kelimeler: Apoptoz, Hekzagonal Bor Nitrür Nanopartikülleri, Lipopolisakkarid, Nöroinflamasyon, Oksidatif Stres, Sepsis

ORCID ID of the authors: AÇG. 0000-0002-2466-9417, FK. 0000-0001-8356-9806

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Correspondence: Ayşe Çakır GÜNDOĞDU- Department of Histology and Embryology, Faculty of Medicine, Kutahya Health Sciences University, Kutahya,Türkiye e-mail: aysecakirgundogdu@gmail.com

1. Introduction

Sepsis is a condition distinguished by a systemic inflammatory reaction to an infection, which may also result in neuroinflammation. In sepsis, the inflammatory response is triggered by the release of pro-inflammatory cytokines and other immune system molecules that are capable of crossing the blood-brain barrier and activating inflammatory processes in the brain (1). This action might result in the stimulation of microglia, the principal immune cells within the brain. This, in turn, can trigger the discharge of more pro-inflammatory cytokines, reactive oxygen species (ROS), and other molecules that have the potential to harm and disrupt the functioning of neurons (2). Being exposed to lipopolysaccharide (LPS), which is an element of the external layer of gram-negative bacterial membranes, has the potential to prompt the immune system to activate and induce the secretion and release of pro-inflammatory cytokines, including TNF-α, IL-1β, and IL-6. These cytokines can subsequently cause neuroinflammation and neuronal damage (3). Due to the fact that LPS-induced neuroinflammation can lead to a complex series of pathological changes that can ultimately result in neuronal damage, synaptic dysfunction, and cognitive deficits, it has been connected to various neurological conditions, including Alzheimer's disease, Parkinson's disease, and sepsis-associated encephalopathy (4). Therefore, animal models, such as rats and mice, have been used to study the mechanisms of LPS-induced neurotoxicity and to evaluate potential treatments. However, since LPS-induced neurotoxicity is a complex process that involves multiple molecular pathways, more investigations are required to gain a complete comprehension of its mechanisms and to devise effective treatments.

Boron is a trace mineral that is necessary for the metabolism of plants, animals, and humans. A growing body of research suggests that boron can inhibit both acute and chronic inflammatory responses and the resulting oxidative stress associated with inflammation (5). In fact, it has been found that boron can reduce inflammation in mice with rheumatoid arthritis. The mechanism behind this anti-inflammatory activity of boron is thought to involve its ability to regulate systemic inflammation markers (6). In addition, it has been reported that boron-containing compounds (BCC) other than boron element can regulate inflammatory processes by acting on natural killer (NK) cells, dendritic cells and macrophages. Furthermore, these compounds play a modulating role in innate and adaptive immunity by regulating the production of cytokines (7).

Boron nitride (BN) is a heat-resistant substance composed of boron and nitrogen that can take on various crystal structures based on the level of pressure and temperature it's exposed to. These structures include hexagonal, rhombohedral, diamond-like cubic, and wurzite. However, at ordinary room temperature, the most stable form of BN is the hexagonal structure. The hexagonal form of BN is distinguished by its arrangement of hexagonal layers containing alternating boron and nitrogen atoms, much like the structure of graphene (8). Hexagonal boron nitride (h-BN) nanoparticles have unique properties, such as high thermal conductivity, high mechanical strength, and high chemical stability, which make them useful in a wide range of applications including electronics, optics, and energy storage (9). Moreover, studies of the use of h-BN nanoparticles have also shown that it has potential biomedical applications in drug delivery, bioimaging, tissue engineering, and cancer therapy. Recent studies evaluated the biocompatibility, toxicity, and biodistribution of h-BN nanoparticles and the results suggest that they are generally well-tolerated by animal models, including rats and mice, and have low toxicity at appropriate doses (10). h-BN nanoparticles systemically increase antioxidant capacity in rats (11). It has been shown that this compound significantly inhibits neurotoxic damage induced by amyloid-β in human neuroblastoma cells *in vitro* (12). Similarly, h-BN nanoparticles have been revealed o exhibit neuroprotective activity by reducing oxidative stress in an *in vitro* Parkinson's model (13). Moreover, h-BN nanoparticles were found to reduce oxidative

stress in embryonic mouse hippocampal cells (14). However, although there is ample evidence of the anti-inflammatory impact of boron, the protective or therapeutic effect of h-BN nanoparticles against neuroinflammation is unknown.

The objective of the present study to explore the potential protective benefits of h-BN nanoparticles against LPS-induced brain damage and enhance our understanding of its connection with restraining the inflammatory response, oxidative stress, and apoptosis in the septic rat brain.

2. Materials and Methods

2.1. Animals and Experimental Design

The subjects of this study were male *Sprague Dawley* rats, aged between 3-4 months and weighing between 250-300 grams. These animals were supplied by the Experimental Animal Breeding and Application Center located at Kütahya Health Sciences University (Kütahya, Turkey). The animals were kept in a controlled room with 12-hour periods of light and darkness, and the temperature was maintained at around 24 ± 1 °C. They were provided with free access to food and water without any limitations.

The rats were randomly assigned to 6 groups, each comprising 7 animals. Rats in the neuroinflammation LPS group were injected intraperitoneally (i.p.) 5 mg/kg LPS (*Escherichia coli*, serotype O111:B4, L263, Sigma Aldrich) dissolved in PBS (15). 50 µg/kg and 100 µg/kg h-BN nanoparticles (Bortek Boron Technologies and Mechatronic Inc., Eskişehir, Turkiye) dissolved in sterile saline were injected (i.p) to the animals in the treatment groups (11). 50 µg/kg and 100 µg/kg h-BN nanoparticles were administered 24 h before LPS injection to rats in the 50 + LPS and 100 + LPS groups, respectively. After 24 hours of receiving either h-BN or LPS administration, the animals were sacrificed under high-dose ketamine hydrochloride: xylazine (90:10 mg/kg) anesthesia. The control group's rats received an injection (i.p.) of 4 ml/kg of sterile saline as a vehicle and they were sacrificed 24 hours after the injection. Brain tissues of all rats were immediately harvested and cerebral hemispheres were separated. One of the hemispheres was stored at -80^{o}C for biochemical analysis, while the other was placed in fixative for histochemical analysis.

2.2. Enzyme-linked Immunosorbent Assay (ELISA)

The brain tissue samples from all the groups were examined for oxidative stress markers, pro-inflammatory proteins, and apoptosis-related proteins using ELISA assay following the guidelines in the commercially purchased kit protocols. Briefly, tissues preserved at -80°C were homogenized in normal saline at 4°C, centrifuged at 3500 rpm for 10 minutes and supernatants were removed. The samples were incubated with biotinylated antibodies and a solution of streptavidin-horseradish peroxidase, which was then left to incubate for one hour. The concentration was measured by recording the absorbance at 450 nm after the addition of the provided chromogen solution and stop solution.

To assess oxidative stress in the brain tissues, the total antioxidant status (TAS) and total oxidant status (TOS) were quantified using the respective kits from Rel Assay® Diagnostics, located in Gaziantep, Turkey. The oxidative stress index was determined by using the formula: OSI = [TOS (µmol H2O2 Eq/L) divided by TAS (µmol Trolox Eq/L)] multiplied by 100. In addition, to evaluate the contribution of h-BN nanoparticles in inflammatory processes, the levels of TNF-α (E0764Ra) and IL-1β (E0119Ra) were quantified using the corresponding kits from BTLAB, USA. Finally, the measurement of cytochrome c (E1939Ra) and caspase-3 (E1648Ra) levels were performed to assess the ability of h-BN nanoparticles to prevent apoptosis by the kits purchased from BTLAB, USA.

2.3. Hematoxylin and Eosin (H&E) Staining

The brains of the rats were promptly collected and then immersed in a 10% neutral formalin solution to fix them. After being subjected to standard tissue processing, the tissues were embedded in paraffin wax. 4 µm thick

sections were obtained by a microtome (Thermo-Fisher Scientific, Germany) from paraffin blocks. Sections were deparaffinized in xylene followed by rehydration with a gradient ethanol concentration from 100 to 70% and stained with Hematoxylin and Eosin (H&E). Stained sections dehydrated with ascending gradient of ethanol (70 to 100%), cleared in xylene and mounted with entellan. Subsequently, they were examined using a light microscope (Nikon Eclipse 80i, Nikon, Germany) at a magnification of x200.

2.4. Statistical Analysis

SPSS 21.0 (SPSS, Chicago, IL, USA) and GraphPad 7 Prism (GraphPad Software, Inc., San Diego, CA USA) were used for statistical analysis. One-way analysis of variation (ANOVA) was carried out, then Tukey's test was employed as a post-hoc analysis for multiple comparisons. The mean ± standard error of the mean (SEM) was used to present all values. A *p*-value lower than 0.05 was regarded as statistically significant.

3. Results

3.1. h-BN nanoparticles inhibit the inflammatory responses in the brain induced by sepsis.

To evaluate the role of h-BN nanoparticles in inflammatory responses, pro-inflammatory marker levels were measured in the brain by ELISA. It was observed that LPS administration led to a considerable elevation in the levels of both TNF-α and IL-1β, as compared to the control rats ($p < 0.001$ and $p < 0.01$, respectively) (Fig.1a,b). When 50 µg/kg and 1000 µg/kg h-BN nanoparticles were applied, TNF-α levels were not different versus control ($p > 0.05$), but were lower than those in rats received LPS ($p < 0.001$). Prophylactic h-BN administration led to a significant decrease in TNF-α levels compared to LPS at both doses ($p < 0.05$ and $p < 0.01$, respectively) (Fig.1a). The IL-1β levels were significantly lower in the group administered 50 µg/kg h-BN nanoparticles in comparison with LPS group ($p < 0.05$), nanoparticles at 100 µg/kg concentration did not cause any significant change ($p > 0.05$). Moreover, both 50 µg/kg and 100 µg/kg h-BN nanoparticles significantly attenuated the LPS-induced upregulation of IL-1β ($p < 0.05$) (Fig.1b).

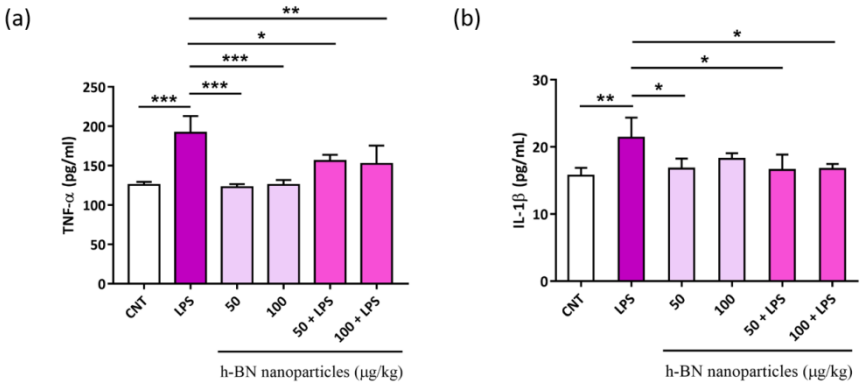

Figure 1. Alterations in pro-inflammatory cytokine levels in the brain tissue of rats treated with h-BN nanoparticles. (a) Tumor necrosis factor alpha (TNF-α) and (b) interleukin-1 beta (IL-1β) levels. Values were presented as the mean ± SEM, n=7. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.001$.

3.2. h-BN nanoparticles reduce LPS-induced oxidative stress in the brain

The ELISA assay was employed to measure TAS and TOS levels in brain tissue to assess the impact of h-BN nanoparticles on oxidative stress. The results indicated a significant reduction in the TAS level in the LPS group when compared to the control group ($p < 0.001$). The administration of h-BN nanoparticles alone significantly increased the TAS level at both doses used ($p < 0.001$ for 50 µg/kg and $p < 0.05$ for 100 µg/kg) versus the LPS group. The prophylactic injection of 50 µg/kg h-BN nanoparticles did not produce a significant change in TAS levels when compared to the group that received LPS ($p > 0.05$). However, the levels of TAS were prominently lower in the 100 µg/kg dose group than in the LPS group ($p < 0.05$) (Fig.2a). In contrast to the antioxidant status, the administration of LPS caused a substantial rise in brain TOS levels versus the untreated rats ($p < 0.01$). The treatment of h-BN nanoparticles prior to LPS injection, at both 50 and 100 µg/kg dosages, reversed the TOS increase ($p < 0.05$ and $p < 0.0001$), but the prophylactic benefit was more pronounced at the 100 µg/kg dosage (Fig.2b). OSI values calculated based on these data were also statistically significant, reflecting that LPS triggered oxidative stress ($p < 0.0001$) and h-BN nanoparticles showed antioxidative activity ($p < 0.0001$) at both concentrations used (Fig.2c).

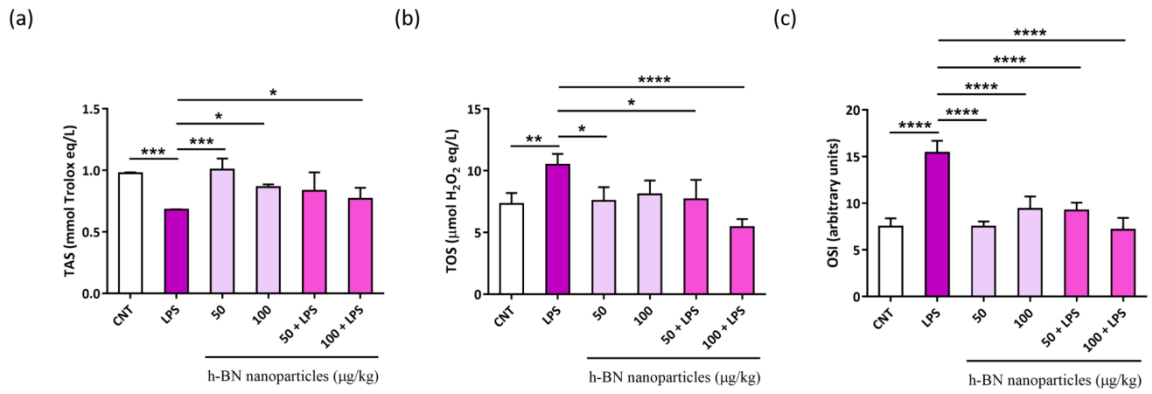

Figure 2. Alterations in indicators of oxidative stress in the brain tissue of rats treated with h-BN nanoparticles. The levels of (a) total antioxidant status (TAS), (b) total oxidant status (TOS) and (c) the calculated oxidative stress index (OSI). Values were presented as the mean ± SEM, n=7. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ and ****$p < 0.001$.

3.3. h-BN nanoparticles attenuate apoptosis triggered by LPS in the brain

In order to investigate whether h-BN nanoparticles have anti-apoptotic activity on LPS-induced neurodegeneration, caspase-3, and cytochrome-c levels, which are markers of the extrinsic apoptotic pathway, were measured in brain tissues. The application of LPS led to a significant rise in the levels of both cytochrome c and caspase-3 ($p < 0.0001$ and $p < 0.001$ respectively). Prophylactically administered 50 µg/kg h-BN nanoparticles decreased the concentration of cytochrome c ($p < 0.0001$) and caspase-3 ($p < 0.001$) compared to the LPS group. Similarly, cytochrome c ($p < 0.0001$) and caspase-3 ($p < 0.05$) concentrations were found to be reduced when 100 µg/kg h-BN nanoparticles were applied before LPS (Fig.3).

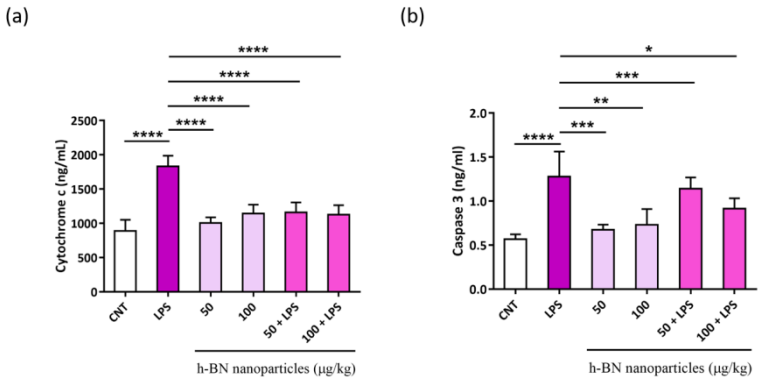

Figure 3. Alterations in apoptotic markers in the brain tissue of rats treated with h-BN nanoparticles. (a) Cytochrome c and (b) caspase-3 levels. Values were presented as the mean ± SEM, n=7. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 and *****p* < 0.001.

3.4. h-BN nanoparticles attenuates LPS-induced neurodegeneration in cerebral cortex and hippocampus

Histopathological assessments revealed that the brain cortex of the control group had a normal structure in terms of neurons, neuroglial cells, and neuropil. Rats treated with 50 and 100 mg/kg h-BN nanoparticles exhibited similar cortical architecture to that of the control group rats. However, rats treated with LPS had neurons with condensed nuclei, and neuronal shrinkage was a common finding. Neuroglial cells were also shrunken and showed deep staining. In addition, hemorrhage and capillary dilation were evident. Prophylactic administration of h-BN nanoparticles at a concentration of 50 µg/kg prior to LPS significantly prevented LPS-induced cortical degeneration, with only a few neurons and glial cells exhibiting shrinkage. On the other hand, a 100 µg/kg concentration of h-BN nanoparticles was found to be more efficient in preventing the degenerative effects of LPS compared to the 50 mg/kg concentration (Fig.4).

The normal morphology of neurons in the CA1 and CA3 regions of the hippocampus was observed in both the control group and rats treated with 50 and 100 µg/kg h-BN nanoparticles. Conversely, LPS treatment resulted in a majority of neurons with darkly stained pycnotic nuclei, and focal inflammatory cell infiltration was also detected in some areas. The administration of h-BN nanoparticles at a concentration of 50 µg/kg significantly mitigated neuronal damage. Furthermore, the injection of 100 µg/kg h-BN nanoparticles almost completely prevented neuronal degeneration and maintained the hippocampal structure similar to that of the control group rats. No inflammatory cell infiltration was observed in rats treated with nanoparticles at either dosage (Fig.5).

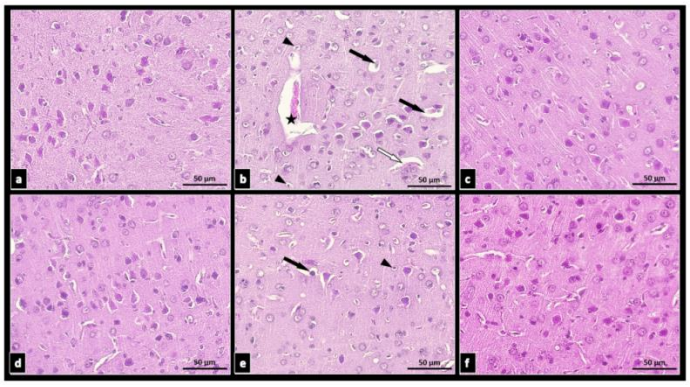

Figure 4. Histopathological appearance of the cerebral cortex. (a) Normal structure of cortical tissue of rats in the control group. (b) Lipopolysaccharide (LPS) administrated rats showed neuronal shrinkage (dark arrows), shrunken neuroglial cells (arrowhead), hemorrhage (star), and dilatation in capillaries (white arrow). (c,d) Rats treated with h-BN nanoparticles at 50 and 100 µg/kg concentrations respectively revealed normal cortical morphology. (e) 50 µg/kg h-BN nanoparticles treated rats prior to LPS administration showed attenuated neuronal degeneration with shrinkage in only some neurons (dark arrow) and neuroglial cells (arrowhead). (f) 100 µg/kg h-BN nanoparticles treated rats prior to LPS administration exhibited normal cortical architecture. Hematoxylin and Eosin (H&E).

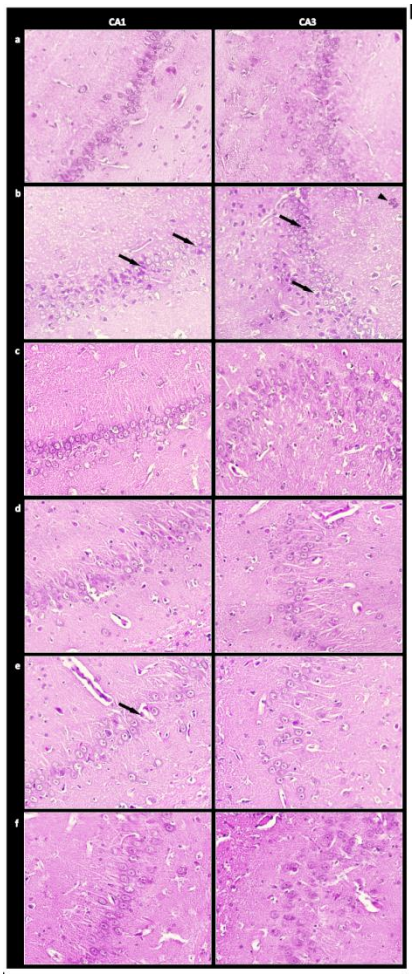

Figure 5. Histopathological appearance of the CA1 (left column) and CA3 (right column) regions in the hippocampus. (a) Normal morphology of hippocampal tissue of rats in the control group. (b) Lipopolysaccharide (LPS) administrated rats showed neurons with darkly stained pycnotic nuclei (arrows), and focal inflammatory cell infiltration (arrowhead). (c,d) Rats treated with h-BN nanoparticles at 50 and 100 µg/kg concentrations respectively exhibited the normal structure of both CA1 and CA3 regions. (e) 50 µg/kg h-BN nanoparticles treated rats prior to LPS administration showed attenuated neuronal degeneration with pyknosis in only some neurons (arrow). (f) 100 µg/kg h-BN nanoparticles treated rats prior to LPS administration revealed normal cortical architecture. Hematoxylin and Eosin (H&E).

4. Discussion

Our current research reveals, for the first time, that h-BN nanoparticles protect against LPS-induced neurodegeneration and alleviate inflammatory responses in the brain in a rat model of acute systemic inflammation. Furthermore, our analyzes revealed that this beneficial action of h-BN nanoparticles was facilitated by inhibiting oxidative stress and suppressing apoptosis.

Neuroinflammation, which is one of the main components of pathology in many neurological and neurodegenerative diseases, is a serious condition that can be triggered by sepsis. Leukocytes and proinflammatory cytokines produced in peripheral inflammation reach the central nervous system (CNS) by passing through the blood-brain barrier (BBB). Here it triggers the

inflammatory cells to generate and discharge additional cytokines. This resulting neuroinflammatory response causes synaptic disruption and death of neuronal and neuroglial cells (3, 16). Experimental sepsis induced by injecting LPS into the bloodstream in animals is a widely used model for investigating pathophysiology of sepsis and to test potential therapeutic interventions. When LPS enters the bloodstream, it activates a systemic inflammatory response, promoting the release of cytokines, such as TNF-α, IL-1β, and IL-6 (17). Therefore, the use of agents that reduce the generation of pro-inflammatory cytokines is considered a useful approach for the prevention or treatment of sepsis-associated neurological damage.

In this study, we employed h-BN nanoparticles to alleviate neuroinflammation triggered by LPS in the brain. Our findings revealed that the h-BN nanoparticles were effective in reducing inflammation by lowering the concentration of pro-inflammatory cytokines such as TNF-α and IL-1β. To the best of our knowledge, these data are the initial evidence demonstrating the anti-inflammatory properties of h-BN nanoparticles. However, several studies in the literature have presented results that are in line with our research regarding the anti-inflammatory activity of boron. According to Naghii et al.'s (2011) research involving human subjects, the administration of boron supplements resulted in a notable reduction in the concentrations of TNF-α and IL-6 in the bloodstream within a mere 6-hour timeframe (18). Turkez et al. reported that boric acid and borax, which are BCCs, reduce IL-6, IL-1α and TNF-α concentrations in human glioblastoma cell lines (19). In a study conducted by İnce and colleagues in rats with gentamicin-induced renal damage, it was observed that boron supplementation led to reduced expressions of TNF-α, NFκB, IL-1β, and IFN-γ mRNAs in the kidneys (20). Kucukkurt et al. showed that boron decreased IL1-β and TNF-α gene expression levels in a dose-dependent manner in high-fat fed rats (21).

Apart from changes in cytokine levels, sepsis also activates neuropathophysiological mechanisms, including the initiation of oxidative stress (1). As a matter of fact, oxidative stress and inflammation are closely linked cellular mechanisms that can trigger each other. The inflammatory signaling stimulated by ROS can worsen the production of ROS at the site of inflammation, leading to a self-perpetuating cycle (22). Thus, along with the regulation of inflammatory cytokines, controlling oxidative stress is a crucial therapeutic goal in treating sepsis. According to Barichello et al.'s (2007) report, the mitigation of oxidative stress in septic rats led to a decrease in cognitive impairment that persisted over the long term (23). Oxidative stress is strongly linked with cellular damage, necrosis, and apoptosis. The process of apoptosis is complicated and involves a number of features such as cell shrinkage, chromatin condensation, internucleosomal DNA fragmentation, and the creation of apoptotic bodies (24). Many protease families are linked to apoptosis, with caspases being the most significant. The role of the intrinsic apoptotic pathway is crucial in the development of sepsis-induced neuroinflammation. The rise in ROS caused by sepsis enhances the permeability of the outer membrane of mitochondria, leading to the release of multiple proteins including cytochrome c. Cytochrome c consequently triggers the activation of caspase-3 (25).

Our present study demonstrated that h-BN nanoparticles dose dependently mitigate the elevated oxidative stress index resulting from decreased total antioxidant status and increased total oxidant status in the brain induced by LPS administration. We also showed that h-BN nanoparticles suppressed sepsis-induced intrinsic apoptotic pathway activation by reducing cytochrome c and caspase-3 levels. There are very few studies in the literature regarding the antioxidant features of h-BN nanoparticles, and the existing studies are limited to *in vitro* investigations. In parallel with our study, it has been shown that h-BN nanoparticles and boric acid increase the viability of embryonic mouse hippocampal cells by ameliorating oxidative stress caused by the anti-cancer drug doxorubicin. A recent research conducted by Aydin et al., (2022) presented evidence that it was demonstrated that h-BN nanoparticles

reduced oxidative stress by regulating both TAS and TOS levels in an *in vitro* Alzheimer's model created by the amyloid beta application in embryonic mouse hippocampal cells. They also indicated that this antioxidative effect was accompanied by inhibition of apoptotic/necrotic cell death characterized by nuclear fragmentation and chromatin condensation (12). Moreover, Kücükdogru and colleagues revealed that h-BN nanoparticles enhance the antioxidant capacity of pluripotent human embryonal carcinoma cells in the presence of the neurotoxin 1-methyl-4-phenylpyridinium, thereby they reduce oxidative stress and inhibit apoptosis (13).

Histopathologically, we observed indications of neurodegenerative changes in neuronal and neuroglial cells, such as nuclear condensation and shrinkage, as well as hemorrhage and capillary dilation in the cerebral cortex of rats following LPS administration. Similarly, pycnosis was present in neurons in both CA1 and CA3 regions, along with focal inflammatory cell infiltration in the hippocampus. h-BN nanoparticles were effective in alleviating these degenerative effects at 50 µg/kg and 100 µg/kg concentrations, with a greater protective impact seen at the higher dosage. It should also be noted that the nanoparticles in the dose used did not cause any damage to the brain tissue. As there is currently no *in vivo* research on the impacts of h-BN nanoparticles on neural tissues, our findings are particularly noteworthy since they are the first evidence on this matter. We suppose that the neuroprotective effect of the nanoparticles we observed is a result of the presence of boron in their structure, as well as boric acid which may potentially be a degradation product. Several studies have been conducted regarding the neuroprotection abilities of boron and boric acid in the brain tissue. As an instance, Acaroz et al. (2018) revealed that boron improved the tissue damage induced by acrylamide in multiple organs, such as the kidney, liver, heart, lung, testis, and brain. Additionally, the findings of the researchers emphasize the impact of the antioxidant and anti-inflammatory properties of boron (26). There is another study indicating that boric acid can provide protection against brain damage in rats with traumatic brain injury, through the reduction of oxidative stress by suppressing the increase in malondialdehyde levels and catalase activity (27). Additionally, the treatment of boric acid was found to reduce neuronal degeneration in rats with Parkinson's Disease induced by rotenone (28). Moreover, a recent investigation demonstrated that boron compounds, such as boric acid, borax, colemanite, and ulexite, can prevent the histopathological and ultrastructural alterations in the brain that arise from oxidative damage induced by AlCl3 (29).

In conclusion, the findings of this study indicate that the administration of h-BN nanoparticles can improve degenerations triggered by LPS-induced sepsis in rat brain. h-BN nanoparticles also diminish the concentrations of the pro-inflammatory cytokines TNF-α and IL-1β, alleviate oxidative stress by decreasing total oxidant status and increasing total antioxidant status, and hinder apoptosis by mitigating cytochrome c and caspase-3 levels in the septic rat brain. Although additional research is needed to comprehend the impact of h-BN nanoparticles, our findings provide novel insights into the mechanisms through which h-BN nanoparticles function in protection from brain injury related to sepsis induced by LPS.

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Ethics

Ethics Committee Approval: The study was approved by Kütahya University of Health Sciences Animal Experiments Local Ethics Committee (Decision no: 2023.01.03, Date: 05.01.2023

Informed Consent: This study is animal experiment

Authorship Contributions: Surgical and Medical Practices: AÇG, FK. Concept: AÇG, FK. Design: AÇG, FK. Data

Collection or Processing: AÇG, FK. Analysis or Interpretation: AÇG, FK. Literature Search: AÇG. Writing: AÇG, FK.

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