EXPERIMENTAL STUDY

Histological and electroencephalographic demonstration of probiotic effect for reduce of oxidative stress and apoptosis in experimental traumatic brain injury

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ABSTRACT

BACKGROUND: The gut microbiota modulates nervous system function. In the literature, it has been shown that this modulation is used in many nervous system injuries through oxidative stress (OS) and apoptosis mechanisms. In this study, it was aimed to investigate the neuroprotective effects of probiotic (PB) treatment in a rat traumatic brain injury (TBI) model with histological and electroencephalographic (EEG) data.

METHODS: Forty male Wistar albino rats were divided into four groups. Group 1 was the control group (CONTROL, n=10) and no trauma was applied. Group 2 was the trauma group with the weight-drop technique (TBH, n=10). Group 3 was the sham group (SHAM), (TBH+sterile saline [SS], n=10) rats were given 500 µL of SS per day by oral gavage. Group 4 was the PB treatment group, (TBH+PB, n=10) rats were treated daily for 7 days with 500 µL of PB oral gavage. Brain samples were collected 7 days after trauma. Histopathological evaluation of brain samples was done with HE. OS with Endothelial nitric oxide synthase, vascularization with Vascular Endothelial Growth Factor, gliosis with S100, and apoptosis with caspase 3 were evaluated immunohistochemically. Apoptotic index was determined with TUNEL. In addition, EEG and somatosensory evoked potential (SEP) recording findings were compared.

RESULTS: It was determined by HE staining that there was a significant (P<0.001) damage in the TBI and sham groups compared to the control group. It was found that PB treatment provided a significant (P<0.01) improvement in the damage created. While OS (P<0.01), gliosis (P<0.01), and apoptosis (P<0.05) decreased with PB treatment, angiogenesis (P<0.01) increased. In support of these findings, in the software-mediated EEG and SUP examination; Delta wave power and theta/alpha ratio increased with TBI and decreased with PB treatment.

CONCLUSION: The results showed that PB treatment provided a significant improvement in rats by reducing OS, apoptosis, and gliosis and increasing vascularity. To the best of our knowledge in the literature, it was shown for the 1st time that histological results for the treatment of PB were supported by software-mediated EEG and SEP analysis.

Keywords: Electroencephalography; histology; probiotic; traumatic brain injury.

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INTRODUCTION

In all parts of the world, traumatic brain injury (TBI) is known as the primary causes of disability and mortality. TBI causes morphologic and physiological damage to brain function due to an external force.[1] TBI has two distinct phases; primary (direct) and secondary (indirect). Excitotoxicity, neuroinflammation, cytokine activation, oxidative damage, and eventually cell death are common mechanisms of secondary injury that are frequently linked to molecular mechanisms that occur after TBI. These prominent mechanisms of injury contribute to the hypercatabolic state, which causes numerous complications both during and after hospitalization.[2] Consequently, TBI patients lose weight rapidly, have a negative nitrogen balance, and have increased whole-body protein breakdown.[3] Following TBI, several problems of intestinal dysfunction have been reported such as stomach ulcers, gastritis including Cushing's ulcer, protracted ileus, and other motility issues. TBI has therefore reportedly been shown to have a significant impact on intestinal function.[4] The systemic immune system is activated by the gut microbiota, which has recently been found to be a major cause of various diseases and may therefore be a new player in infectious complications.[3,5]

Live microorganisms known as probiotics (PB) provide the host with health benefits when given in sufficient doses. Foods, dietary supplements, and medications can all include PBs, which are primarily Bifidobacterium and Lactobacillus species.[6] Consuming PB has some benefits, including improved lipid metabolism, reduced gut inflammation, and increased insulin sensitivity.[7] The gut microbiota play a role for many diseases such as neurodegenerative pathologies and also CNS injury according to previous research.[8-11]

Oxidative stress (OS) plays an important role in many pathophysiological changes that occur after TBI. OS downregulates tight junction proteins and indirectly activates matrix metalloproteinases (MMPs) that contribute to blood-brain barrier (BBB) opening of the BBB. OS-induced activation of MMPs and fluid channel aquaporins leads to vascular and perivascular disruption, edema, increasing BBB leakage, and leading to progression of neuroinflammation. Similarly, OS activates inflammatory cytokines and growth factors such as IL-1β, tumor necrosis factor-α, and transforming growth factor-beta, either directly or indirectly by activating MMPs. In another pathway, OS-induced degradation by MMPs for the Vascular Endothelial Growth Factor (VEGF) receptor-2 (VEGFR-2) leads to subsequent elevation of serum VEGF level. A decrease in VEGFR-2 followed by an increase in VEGF-A leads to apoptosis (AP) and neuroinflammation through activation of caspase-1 and 3 and IL-1β release.[12-14]

This study aimed to investigate the mechanisms such as OS and AP for the therapeutic effects of PB on rat model of TBI. We did not find any existing study to show this effect using electroencephalographic (EEG) monitoring for evidence of histopathologic alterations.

MATERIALS AND METHODS

Experimental Protocol

Forty adult male Wistar albino rats (200±50 g) were kept under optimal laboratory conditions for experiments. This study was approved by the Animal Experiments Local Ethics Committee of Manisa Celal Bayar University (2021/77.637.435/189), Türkiye. Forty male Wistar albino rats were divided into four groups. Group 1, the control group (n=10) only skin incision was made and no trauma was applied. Group 2, the trauma group with the weight-drop technique (TBH, n=10). Group 3, was the sham group (TBH+SS, n=10) rats were given 500 µL of sterile saline (SS) per day by oral gavage for 7 days after trauma. Group 4, the PB treatment group (TBH+PB, n=10) rats were treated daily 500 µL of PB oral gavage for 7 days after trauma.[15-18]

TBI Procedure

Xylazine (10 mg/kg, Bayer, Istanbul, Türkiye) and ketamine hydrochloride (75 mg/kg, Parke Davis Istanbul, Türkiye) were used by intraperitoneal injection for anesthesia. TBI was provided by the drop weight technique which described and modified by the previous studies.[16] EEG monitoring was performed before and after PB administration at 7 days after TBI, the rats were sacrificed at the end of the experiment and brain samples were used for histopathology.[15,18-21]

PB Treatment

Non-effective NBL PB Gold drugs (Nobelpharma) including Lactobacillus acidophilus, Lactobacillus rhamnosus, Bifidobacterium bifidum, Bifidobacterium longum, Enterococcus faecium bacteria were used with a support of PB Probiatop®. L. rhamnosus HN001, and L. acidophilus NCFM® which were used approximately at a dose of 2.5 × 109 CFU through oral gavage once a day as 500 µL for 7 days.[17,18,22]

Immunohistochemistry

The samples were deparaffinized at 60°C overnight. Then, they were kept in xylene for 30 min. for dehydration in ethanol. Samples were then washed in phosphate-buffered saline (PBS). They were treated with 0.5% trypsin solution (800.729.8350, ScyTek Laboratories, Inc., Logan, UT, USA) for 15 min at 37°C. 3% hydrogen peroxide was used for inhibition of endogenous peroxidase activity. Samples were washed in PBS for 3 times. Blocking solution (TA-125-UB; Lab Vision, Fremont, CA, USA) was used for 1 h. The sections were incubated with the primary antibodies for 18 h at 4°C. Endothelial nitric oxide synthase (eNOS; sc-654) for OS, caspase-3 (sc-56053) for apoptosis, vascular endothelial growth factor (VEGF; ab1316) for vascularization, and S100 (sc58839) for reactive astrocytes were used as primary antibodies. All antibodies were from Santa Cruze biyoteknology. The samples were treated with PBS for negative control. After overnight incubation, samples washed in PBS for 3 times. Then, the anti-mouse biotin-streptavidin hydrogen peroxidase secondary antibody (85–9043 Zymed Histostain

kit; Zymed, San Francisco, CA, USA) was applied to the sections for 30 min. Sections were washed again in PBS for 3 times. The diaminobenzidine (DAB; ScyTek) staining was performed for the appearance of the immunohistochemical reaction., Mayer's hematoxylin (72804E; Microm, Walldorf, Germany) was used for counterstaining. The sections were mounted with entellan. İmages were produced under an Olympus BX40 (Tokyo, Japan) light microscope. H-score was scored by two histologist as blind manner. Scoring of samples were done as; weak (+), moderate (++), and strong (+++), respectively. The labeled cells were counted for each staining degree. The H-score calculation was used where H-Score = Pi (intensity of staining + 1). Pi means the percentage of stained cells for each intensity.[15,18-20]

TUNEL Assay

An ApopTag Plus Peroxidase in situ apoptosis detection kit (S7101; Millipore, Billerica, MA, USA) was used for the detection of apoptosis with the manufacturer's protocol. The brain tissue samples were treated with 20 µg/mL proteinase K treatment for 10 min at 37°C. They were washed in PBS for 3 times. Samples were incubated with terminal deoxynucleotidyl transferase enzyme for 1 h at 37°C after the treatment with an equilibration buffer,. An anti-digoxigenin peroxidase conjugate was applied to the samples for 30 min following the washing in Stop Wash Buffer for 10 min. Then, the apoptotic cells were visualized using the diaminobenzidine (DAB; ScyTek). Mayer's hematoxylin (72804E; Microm, Walldorf, Germany) was used for counterstaining. The percentage of apoptotic cells was evaluated as apoptotic index by.[18,20,21]

EEG Monitoring

EEG monitoring was performed before and after TBI. A rostrocaudal incision was made in the scalp using stereotaxy device. Three screw electrodes were placed on the scalp. Two screw electrodes were placed in the right somatocortex skull bone. The first screw electrode was placed 3 mm lateral and 4 mm rostral to bregma and the second screw electrode was placed 3 mm lateral and 4 mm caudal to bregma. Finally, reference electrode was placed 3 mm lateral and 4 mm caudal to bregma on left-somatocortex skull bone.[23-25] Then, dental acrylic was used to fix electrodes to the skull. Powerlab/SP8 ADInstruments (Australia) recording system as 10,000 samples per second was used for records. Fourier analysis was applied by quantitative EEG method for the digitalization of recorded EEG waves. Digitalization of observational data was provided. Delta: 0.5–4 Hz, Theta: 4–8 Hz, Alpha: 8–12 Hz, and Beta 12–30 Hz were determined as wave ranges.[15,26]

Statistical Analysis

The data were presented as mean ± standard deviation. The results were analyzed for statistic by GraphPad software (San Diego, CA, USA) using one-way analysis of variance (ANOVA). P≤0.05 was accepted as significant.[15,20,21]

RESULTS

Histopathological Findings

Pathologic alterations due to TBI were evaluated by HE staining. While control samples showed normal histology, TBI samples caused the reduction of cell concentration and sheet thickness, disorganization of cell with pycnosis, increase of vacuolization, and dilation of capillaries. Wide anoxic neurons, nucleus, and cell shape anomalies with condensation were observed. There was significantly (P<0.001) increase for these alterations in TBI (3.88±0.59) or sham group (3.58±0.58) compared to that of control group (1.65±0.45). PB treatment (2.85±0.53) after TBI significantly (P<0.01) reduced these pathologic findings (Fig. 1).

The apparent neuroprotective effect of PB application was checked by immunocytochemistry of eNOS, VEGF, and S100 stainings.

For eNOS which is an OS marker due to inflammation increased in TBI and sham groups. The differences between the control group (83.88±11.77) and TBI group (185.88±25.55) was statistically significant (P<0.001). Similarly, difference between the control group (83.88±11.77) and sham group (179.84±28.98) was statistically significant (P<0.001). On the contrary, PB treatment (144.55±21.20) reduced OS and difference between control group and PB treatment was statistically (P<0.01) significant (Fig. 2).

For S100, which demonstrated gliosis, there were significant (P<0.001) increase in TBI (219.87±29.98) and sham group

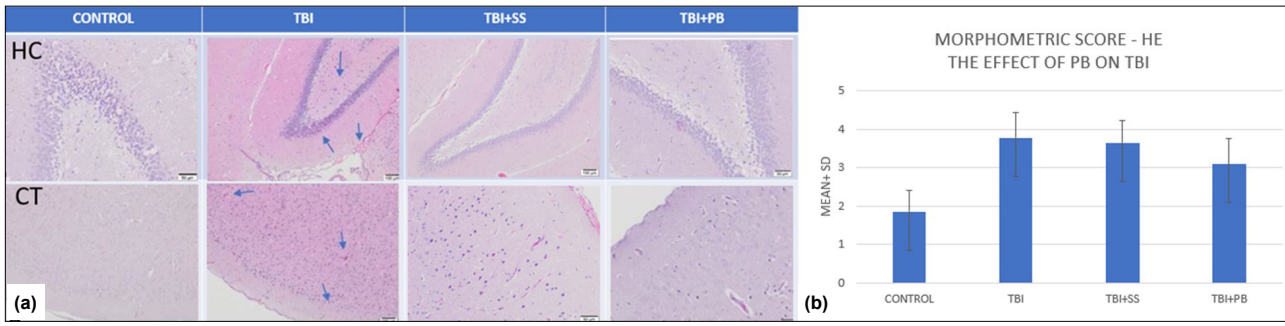

Figure 1. The effect of probiotic (PB) application after traumatic brain injury (TBI) with HE staining of HC (hippocampus) and CT (Cortex). It was determined that the pathological changes (blue arrows) due to TBI **(a)**. Morphological evaluation of PB application for TBI with morphometric score was regressed significantly after PB treatment **(b)**.

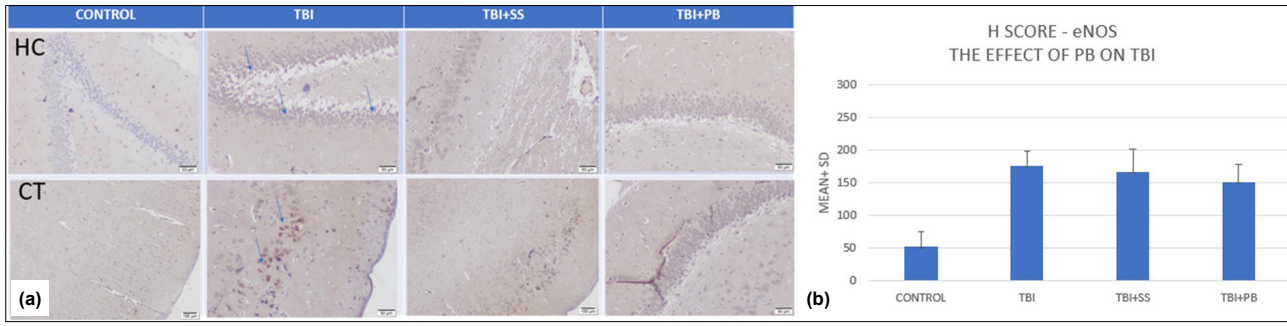

Figure 2. Immunocytochemical staining of Endothelial nitric oxide synthase (eNOS) for oxidative stress. eNOS staining was increased after traumatic brain injury (blue arrows) and decreased with probiotic (PB) treatment **(a)**. In the evaluation of eNOS staining with H-score, it was determined that decrease of oxidative stress with PB treatment was statistically significant **(b)**

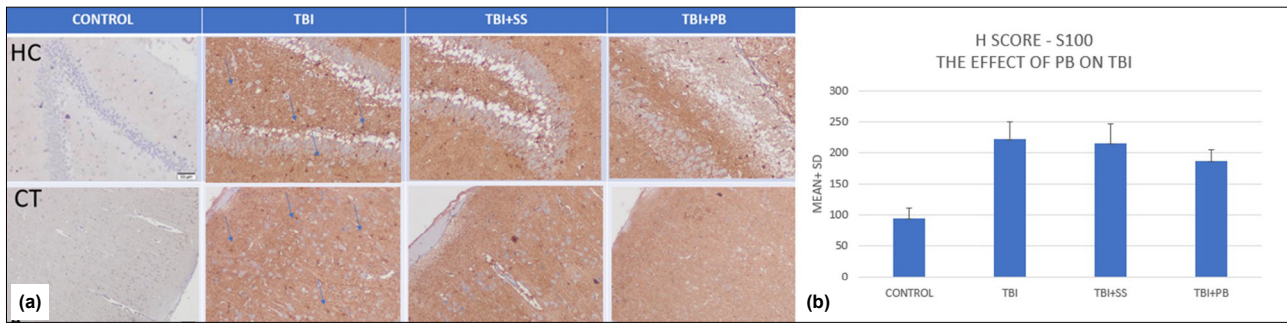

Figure 3. Immunocytochemical staining of S100 for gliosis. S100 staining was increased after traumatic brain injury (blue arrows) and decreased after probiotic (PB) treatment **(a)**. The evaluation of S100 staining with H-score showed that PB treatment significantly reduced gliosis.

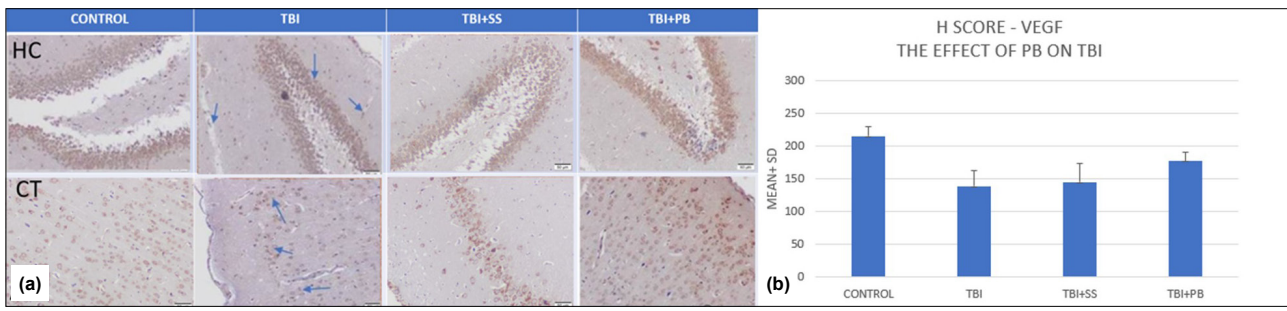

Figure 4. Immunocytochemical staining of VEGF for angiogenesis. The staining was decreased after traumatic brain injury and increased after probiotic (PB) treatment **(a)**. In the evaluation of VEGF staining with H-score, it was found that PB treatment significantly increased vascularization **(b)**

(212.98±28.08) compared to control group (88.68±19.45). PB treatment (177.41±21.12) significantly (P<0.01) reduced gliosis compared to control group (Fig. 3).

Angiogenesis, vasculogenesis and endothelial cells were shown by VEGF staining. Decrease in VEGF staining was statistically significant (P<0.001) in TBI (138.43±28.18) and sham (146.22±34.88) group compared to control group (205.57±19.92). PB treatment (188.71±22.44) after TBI increased VEGF staining and this was statistically significant (P<0.01) compared to control group (Fig. 4).

We performed Caspase-3 immunostaining for apoptotic pathway and TUNEL labeling for apoptotic index. Caspase-3 staining was increased in TBI (198.23±29.12) and sham groups (184.55±22.24) compared to control group (68.77±24.45) and differences were statistically significant (P<0.001). Caspase-3 staining was decreased after PB treatment (148.08±33.66) and differences between PB treatment group and TBI or sham group were statistically (P<0.01) significant (Fig. 5).

Results for h-score of Caspase-3 were similar to TUNEL labeling. The apoptotic index of control group (14.22±3.87) was significantly (P<0.001) increased in TBI (59.33±7.32) and sham group (56.87±8.23). PB treatment (45.99±9.98) reduced apoptosis and difference was statistically (P<0.01) significant (Fig. 6).Moreover, these findings might indicate that PB therapy has a neuroprotective effect on neural tissue cell death following TBI as shown in Figures 5 and 6.

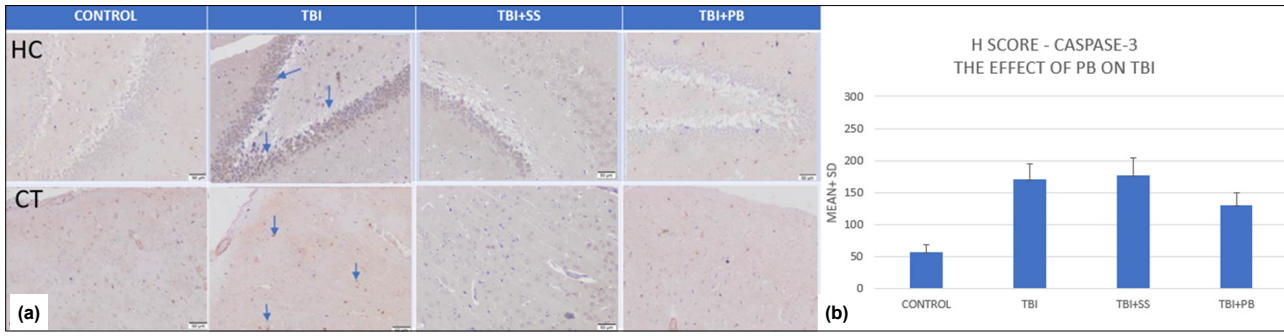

Figure 5. Immunohistochemistry staining with Caspase 3 apoptotic pathway. Apoptosis was increased with traumatic brain injury (blue arrows) and decreased with PB treatment **(a)**. The evaluation of Caspase 3 staining with H-scoring showed that apoptosis was decreased significantly (P<0.01) after PB treatment

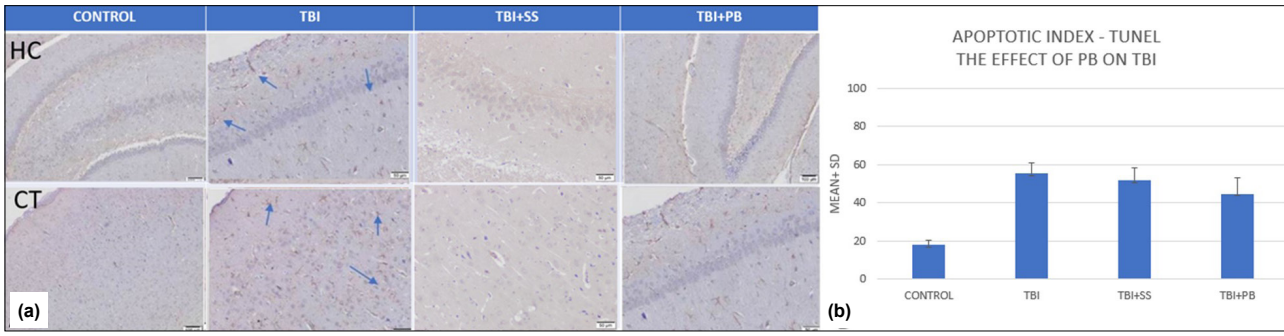

Figure 6. TUNEL labeling for apoptotic index. Labeling was increased with traumatic brain injury and was regressed with probiotic (PB) application **(a)**. Determination by apoptotic index showed that PB treatment significantly (P<0.01) reduced neuronal apoptosis after PB treatment **(b)**

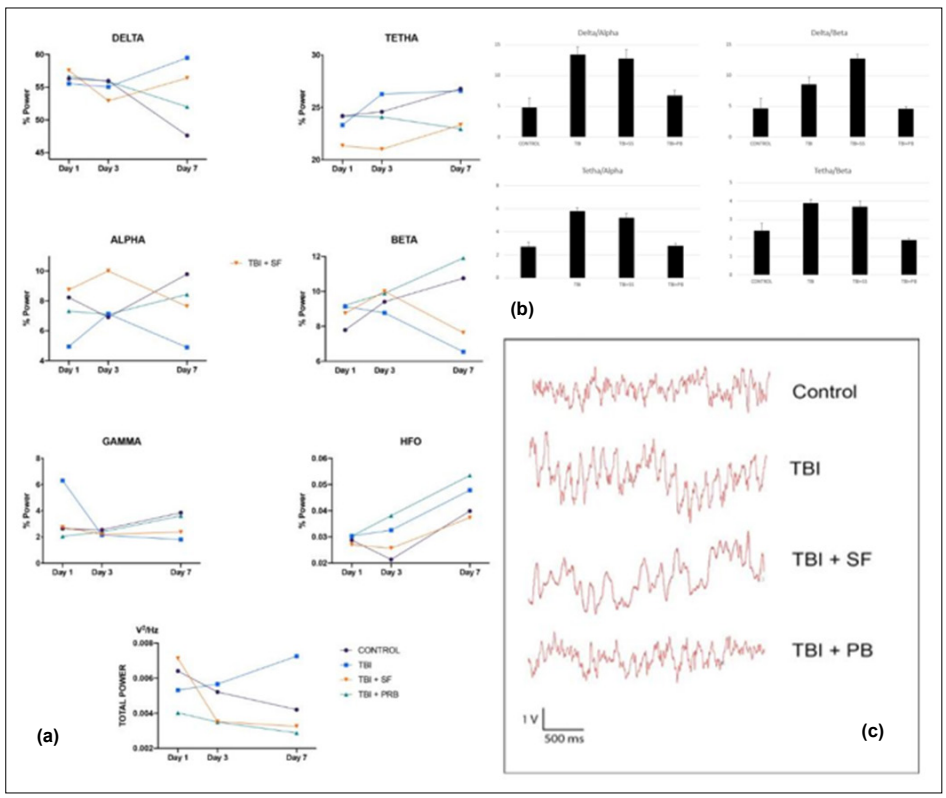

Figure 7. Waves for all groups **(a)**. Comparison of Delta/Alpha and Tetha/Alpha wave ratios at the 7th day for all groups **(b)**. Delta waves for all groups. Similarity of Delta waves for control and probiotic treatment group is remarkable **(c)**

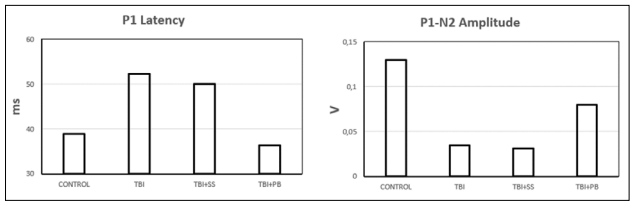

Figure 8. A comparison of SEP parameters between experimental groups.

EEG Recording Findings

In the TBI group, the relative power of the delta wave increased up to the 7th day. In the PB group, delta wave activity decreased. The relative powers of alpha and beta waves were found to considerably decrease up to the 7th day in the TBI and TBI+SS group, while PB was found to increase the activities of alpha and beta waves (Fig. 7a). Figure 7b shows changes in the ratios of the relative powers between groups. The ratio of the delta/alpha and the delta/beta increased after TBI. However, delta/alpha and delta/beta rates decreased on the 7th day after PB treatment. Theta/alpha and theta/beta rates decreased with PB treatment compared to TBI and TBI+SS. In the evaluation of EEG waveforms, the control and PB treatment group waveforms were more complex than the TBI group that is dominated by the slow waves (Fig. 7c).

Somatosensory Evoked Potentials (SEP) Findings

The P1 latencies and the P1-N2 amplitudes were compared between the groups. P1 latency was increased and P1-N2 amplitude was decreased in TBI and TBI+SS group compared to control group. In TBI+PB group , there was no changes in P1 latencies, whereas P1-N2 amplitude was slightly reduced compared to control group (Fig. 8).

DISCUSSION

In this study, we used histopathologic, immunohistochemical, and EEG monitoring assays to examine the neuroprotective effect of PB. Variables such as OS, vascularization, gliosis, neuronal loss, and apoptosis were evaluated in TBI rat model. We demonstrated that PB has a recovery effect on these parameters. It was discovered that PB reduced OS and gliosis, which were elevated with TBI, and also prevented neuronal cell death and support vascularization. In addition, EEG monitoring assay showed that; delta wave strength and theta/alpha ratio, which increase in TBI, decrease with PB treatment. Therefore, we think related research on PB will provide new insights on the rehabilitation treatment of brain function after TBI.

Simultaneous mechanical disruption of brain tissue is the primary injury brought on by exposure to external stress. It causes contusion, blood vessels damage, hemorrhage, and neuronal damage where axons are stretched and wavering.[27,28] Secondary injury arises from a series of metabolic, physiological, and molecular reactions. It starts minutes to months after the primary injury. This causes atrophy, morphological damage to brain cells, and the neural death.[6] Other possible explanation for OS, gliozis, angiogenesis, and neural death might be the effect of brain on the gut by the enteric nervous system and neuroendocrine signals. For example, these effects are vagal motor activity which has been shown to be impaired after TBI. Such results show promise in elucidating the CNS-gut axis and its role in secondary processes after brain injury. Alterations of microbiome can be seen after CNS injury to alleviate secondary injury through the brain-gut axis and alter the neuroinflammatory feedback. Acute phase changes, including bacterial metabolites, intestinal permeability, and GALT-associated immunity could be important mechanisms in TBI.[29] The treatment of TBI has analyzed by a number of experimental research showing encouraging outcomes in the last decades. Nevertheless, a pharmaceutical treatment for TBI that exhibited clinical efficacy has not been found. The aim of this study was to investigate the effects of PB treatment on post-TBI brain samples for morphological damage with EEG monitoring.

Too many of bacteria that make up the human gastrointestinal tract are collectively referred to as the gut microbiota. Intestinal microbes can control the maturation and operation of immune system under physiological settings. The most well-known bacteria that are good for the gut and can be transmitted from mother to child are Lactobacillus and Bifidobacterium.[30] Recent research reveals that cross-talk along the gut-brain axis regulates inflammatory nociception, inflammatory responses, and immune homeostasis. TBI induces a systemic immune response, which can progress to systemic immune response syndrome, releasing immune mediators of inflammation (cytokines and chemokines) into the circulation.[31] According to Hayakawa et al.[32] a rapid physiologic insult such as TBI causes the gut microbiota to fundamentally change within hours of injury. The "microbiota-gut-brain" axis is a line between the CNS and gut microbiota where they affect each other.[5] Afferent and efferent fibers make up the, which is crucial in the bidirectional transmission of important information between the gut and brain. On the one hand, gut homeostasis, gut motility, and gut microbiota are regulated by the autonomic and enteric nervous system, hypothalamic-pituitary-adrenal axis, and immunological pathway. On the other hand, hypothalamic neurons are activated and stimulated by microbial metabolites and enteroendocrine neuropeptides, which encourage pituitary production.[23,33] The most important mechanism responsible for the pathogenesis of TBI is OS. In particular, mitochondrial dysfunction leads to the aberrant production of reactive oxygen species (ROS), which are capable of oxidizing lipids and proteins, ultimately causing cell death.[14,34] Complex gut microbiota microbe-microbe and microbiota host interactions may also influence the oxidative state of the central nervous system (CNS), directly and indirectly, by interfering both with the level of endogenous and exogenous ROS and with antioxidant system.[8] These mechanisms are mostly speculative but are pertinent to the hypothesize that the oxidative state of the CNS could be regulated by the microbiota through the

production of various metabolites such as absorbable vitamins, short-chain fatty acids (SCFA), polyphenols, and highly diffusible antioxidant and oxidant gases.[8,14]

To confirm this hypothesis, we evaluated OS, vascularization, gliosis, and neuronal death through eNOS, VEGF, S100, and TUNEL/Caspase 3 stainings, respectively. We found that eNOS and S100 staining intensity were decreased after PB treatment. Moreover, VEGF staining intensity was increased after PB treatment. Similar to eNOS and S100 stainings, TUNEL and Caspase 3 stainings were decreased after PB treatment. These results can be interpreted as; PB treatment decreased neuronal cell loss by increasing vascularization and reducing OS and gliosis which have been increased with TBI. Similar to our results, some of these observations showed in the previous literature.[12,35-37]

It is known that TBI causes disruption of the BBB. Another mechanism of action may be SCFAs produced by bacteria. Namely; dietary carbohydrates are substrates for fermentation by certain gut bacteria, which produce short chain fatty acids have been shown to regulate intestinal motility and to be involved in central appetite regulation as well as being taken up directly into the bloodstream and transported to various organs, including the brain, where they modulate tissue development and function by decreasing BBB permeability.[38] Upregulation of the expression of tight junction proteins, like Claudin-5 and occludin, by the gut microbiota decreases BBB permeability.[39] Braniste et al. showed that monocolonization of the intestine of germ-free adult mice with either Clostridium tyrobutyricum, a bacterial strain producing butyrate, or Bacteroides thetaiotaomicron, which produces mainly acetate and propionate decreased BBB permeability by increasing the transepithelial electrical resistance and decreasing paracellular permeability.[38] Similarly, Ma et al. showed that treated mice with gut microbiota caused remodeling by L. acidophilus after TBI. According to the authors, administering LA after injury prevented brain edema and kept BBB permeability. In addition, L. acidophilus improved neuronal survival and reduced the amount of neurons that were degenerating in the perilesional cortex after TBI.[40] Du et al. reported that fecal microbiota transplantation can restore gut microbiota dysbiosis and alleviate neurological impairments, through the action of the enzyme trimethylamine-trimethylamine N-oxide-methionine sulfoxide reductase a signaling pathway following TBI in rats.[41] Another study found that antibiotic-induced gut microbial dysbiosis had an impact on neuroinflammation, neurogenesis, and fear memory. They suggest that gut microbial modulation may be a possible therapeutic intervention for TBI in murines. According to the authors, antibiotic exposure to gut microbiota for 1 week after TBI reduced cortical monocyte infiltration, raised microglial pro-inflammatory markers, and reduced T lymphocyte infiltration.[42] In addition to experimental studies, there are only a few clinical studies on this subject. In a prospective randomized pilot study, Tan et al.[42] treated 52 patients including 26 control patients and 26 PB patients. They had closed head injury. Patients received enteral nutrition within 48 h following hospital admission. Patients randomized to the PB group. They received seven sachets of viable PBs 3 times a day for 21 days. The authors reported that patients in control group were treated with significantly more types of antibiotics and length of intensive care unit was significantly longer in control group. The fact that we did not determine whether PBs affect the blood-brain barrier in our study is a limitation. One another limitation is; we could not evaluated antioxidants levels in brain tissue and blood.

In animal studies, the EEG demonstrated epileptiform activity with sharp waves or high-frequency which discharges right away after moderate TBI. Then, these activities were converted to widespread slowing waves in EEG. In people who suffered minor head trauma, the presence of widespread theta and delta slowing was detected for 15–20 min immediately after TBI. The most common alterations in qEEG following mild TBI were increased activities of theta, delta, and theta/alpha ratio, and decreased alpha. Posterior alpha activity is diminished with the generalized and/or focal slowing in the hours following the mTBI.[25,43]

The prolonged latency and the decreased amplitude in early potentials of the cortical response are important indicators in animal models of cortical impact and blast injury. Alteration in evoked potentials was found to be negatively correlated with survival prognosis. Furthermore, Carter and Butt compared the prognostic value of Glasgow Outcome Scale-photomotor reflex, SEPs, CT, and EEG in TBI and found that SEPs were the best predictor of outcome following brain injury.[25,43]

In our EEG evaluation, we found that the slow-wave relative power of EEG increased in the TBI group compared to the control group. This change did not occur when treated with PB and even decreased slow wave activity. In the TBI group, there were significant increases in the ratios of delta/alpha, delta/beta, theta/alpha, and theta/beta, which are known as TBI markers. There were no noticeable changes in the ratios with the PB treatment. In SEP evaluations, TBI prolonged P1 latency and decreased P1-N2 amplitude. On day 7, there was no significant difference in values of latency and amplitude between the PB group and the control group. In terms of changes after TBI, our EEG and SEP findings are consistent with those from other investigations.[16,44,45]

The neuroprotective potential of PB administration for the pathophysiological environment following TBI was highlighted by our SEP and qEEG data. Standard EEG analyses in acute conditions have been shown to foresee subsequent subcortical secondary damage as well as 6-month functional outcome, demonstrating that the level of secondary damage declared over the first 6-month post-injury may be determined very early after the initial insult and may be detectable using standard electrophysiological techniques.[46] Diffusion Tensor Imaging/Magnetic Resonance Spectroscopy has been shown molecular changes to caused by TBI effect of the generation, transmis-

sion, and processing of neural signals within and between brain regions. In addition, studies have found a strong relationship between DTI/MRS changes and anomalies in brain electrical activity, implying that EEG can be used to diagnose functional cerebral impairment. A consistent QEEG assessment has been established in studies to detect and characterize the level of TBI and significant axonal damage. QEEG can detect trauma-related alterations early and for an extended period of time. The TBI severity index can assess these alterations with 96% accuracy, 95% sensitivity, and 97% specificity.[46,47]

CONCLUSION

The CNS was previously shown to affect the microbiota and function of the intestine through the neuro-endocrino-immunological network although research on this area is still scarce. There are not very much treatment models that are used in intensive care units on feeding type of TBI patients, so we think that this kind of investigations may help to find a way to deal with TBI patients. The present study showed that PB has therapeutic effects against TBI by antioxidant, anti-inflammatory, anti-apoptotic, and vascularization effects; however, further clinical and experimental studies are needed.

Ethics Committee Approval: This study was approved by the Manisa Celal Bayar University Faculty of Medicine Ethics Committee (Date: 27.04.2021, Decision No: 77.637.435/189).

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DENEYSEL ÇALIŞMA - *ÖZ*

Deneysel travmatik beyin hasarında oluşan oksidatif stres ve apoptozun azalmasında probiotik etkisinin histolojik ve elektroensefalografik gösterilmesi

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AMAÇ: Bağırsak mikrobiyotası sinir sistemi fonksiyonunu modüle etmektedir. Literatürde bu modülasyonda oksidatif stres ve apoptoz mekanizmaları üzerinden birçok sinir sistemi hasarında kullanıldığı gösterilmektedir. Bu çalışmada sıçan travmatik beyin hasarı (TBH) modelinde probiyotik (PB) tedavisine bağlı nöroprotektif etkilerinin histolojik ve elektroensefalografik (EEG) veriler ile araştırılması amaçlandı.

GEREÇ VE YÖNTEM: 40 erkek Wistar albino sıçan 4 gruba ayrıldı. Grup 1, Kontrol grubu (KONTROL, n=10) olup travma uygulanmadı. Grup 2, ağırlık düşürme tekniği ile yapılan Travma grubuydu (TBH, n=10). Grup 3, taklit grubu (SHAM) olup (TBH+SS, n=10) sıçanlara günde 500 µl steril salin (SS) oral gavaj ile verildi. Grup 4, PB tedavi grubu olup (TBH+PB, n=10) sıçanlar günlük olarak 500 µl PB oral gavaj ile 7 gün tedavi edildi. Beyin örnekleri travmadan 7 gün sonra toplandı. Beyin örneklerinde histopatolojik değerlendirme HE ile yapıldı. eNOS ile oksidatif stres, VEGF ile damarlanma, S100 ile gliosis ve kaspaz 3 ile apoptoz ile immünohistokimyasal olarak değerlendirildi. TUNEL ile apoptotik indeks saptandı. Ek olarak, EEG ve Somatosensoriyal uyarılmış potansiyel (SUP) kayıt bulguları karşılaştırıldı.

BULGULAR: TBH ve sham gruplarında kontrol göre oldukça anlamlı ($p<0.001$) hasar oluşturulduğu HE boyamaları ile saptandı. Oluşturulan hasarda, PB tedavisinin anlamlı ($p<0.01$) bir iyileşmeyi sağladığı bulundu. PB tedavisi ile oksidatif stres ($p<0.01$), gliosis ($p<0.01$) ve apoptoz ($p<0.05$) azalırken, anjiyogenez ($p<0.01$) arttı. Bu bulguları destekler biçimde yazılım aracılı yapılan EEG ve SUP incelemesinde; Delta dalga gücü ve teta / alfa oranı TBH ile artarken PB tedavisi ile azaldı.

SONUÇ: Sonuçlar PB tedavisinin sıçanlarda oksidatif stres, apoptoz ve gliosizi azaltıp damarlanmayı artırarak anlamlı bir iyileşme sağladığını gösterdi. Literatürde bildiğimiz kadarı ile ilk defa yazılım aracılı EEG ive SUP analizi ile PB tedavisi için histolojik sonuçların desteklendiği gösterildi.

Anahtar sözcükler: Elektroensefalografi; histoloji; probiyotik; travmatik beyin hasarı.

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