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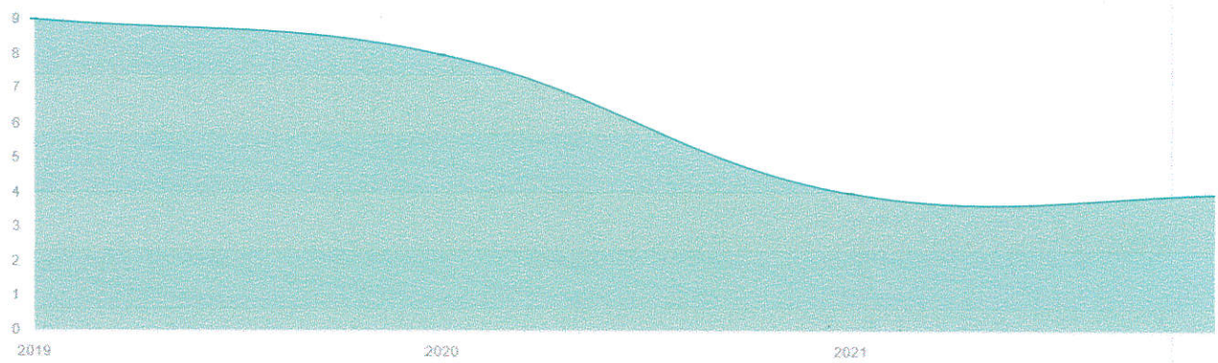

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J. Paek, B.C. Kang, H. Yang, Y. Lee

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International Journal of Probiotics and Prebiotics, Volume 16; https://doi.org/10.37290/ijpp2641-7197.16:16-21 (https://doi.org/10.37290 /ijpp2641-7197.16:16-21)

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Published: 4 January 2021 by New Century Health Publishers LLC (/publisher/13950) in International Journal of Probiotics and Prebiotics (/journal /382001)

International Journal of Probiotics and Prebiotics, Volume 16; https://doi.org/10.37290/ijpp2641-7197.16:22-30 (https://doi.org/10.37290 /ijpp2641-7197.16:22-30)

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Published: 1 December 2020 by New Century Health Publishers LLC (/publisher/13950) in International Journal of Probiotics and Prebiotics (/journal /382001)

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I.N.D. Roslan, N.A. Kamaruding, N. Ismail, S. Shaharuddin

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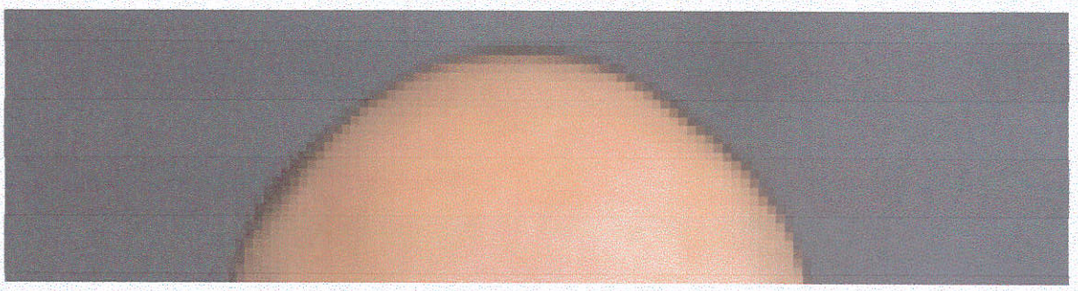

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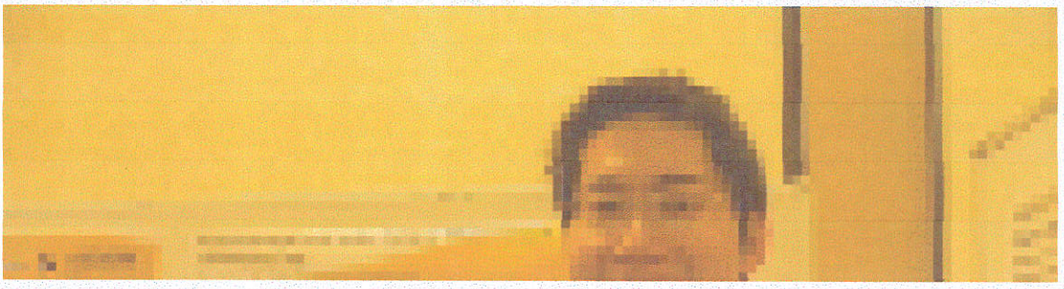
Research Interests
citation analysis; science of science; scientometrics; bibliometrics; mapping knowledge domains



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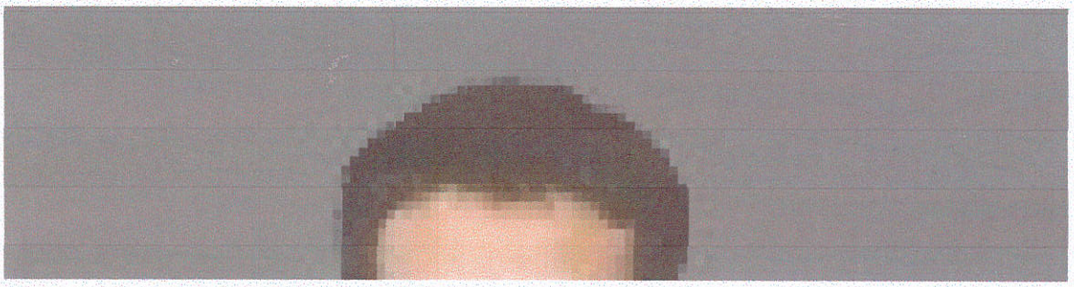
Research Interests

data management; data science; databases; data mining; scientometrics



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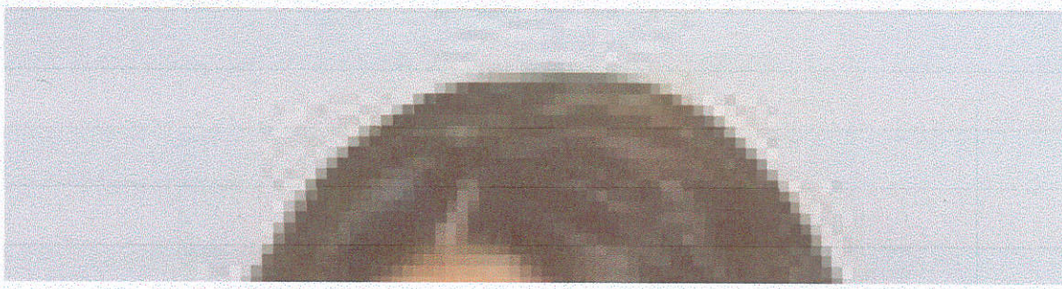

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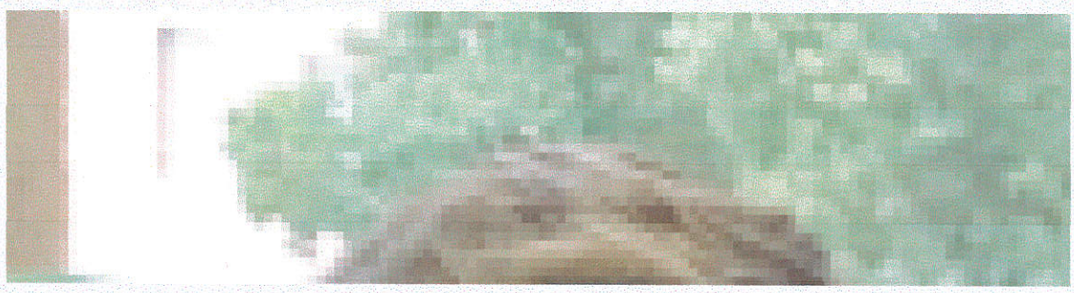
open access; open science; scientific communication, journalism, academic



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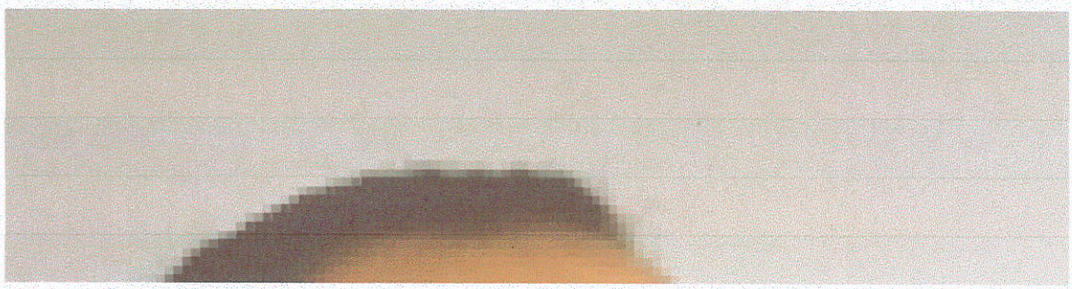
Research Interests

scientometrics; citation databases; bibliometric indicators; library and information science



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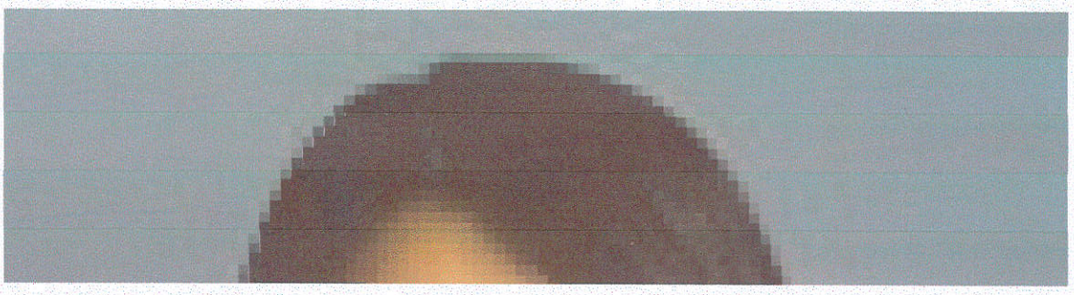
Research Interests

bibliometrics; big data; data mining; network analysis; scientometrics; text mining; visualization



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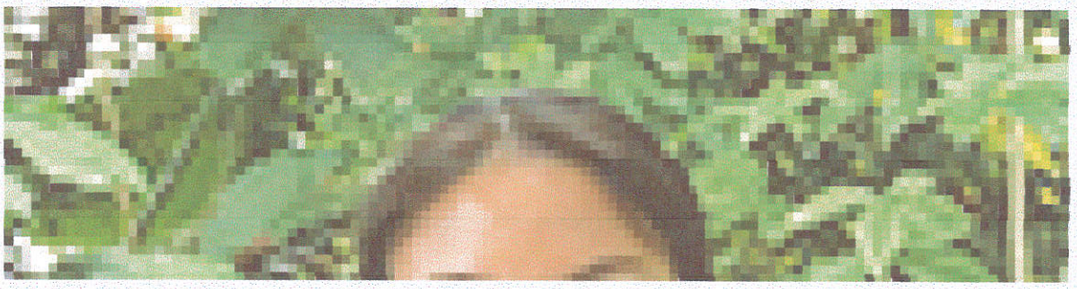

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Research Article

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Gut microbiota is a complex community that helps maintain the dynamic metabolic ecological balance of the brain through the gut–brain axis and keeps the blood–brain barrier structure intact. However, the knowledge of how the gut microbiota responds to exogenous influences on the blood–brain barrier structure remains limited. This study hypothesizes that probiotic *Lactobacillus plantarum* IS 10506 supplementations could ameliorate the disruption of the blood–brain barrier structure. To this end, we examined effect of the probiotic *L. plantarum* IS 10506 on the expression of glial fibrillary acidic protein and platelet endothelial cell adhesion molecule-1 in the control and *E. coli* serotype O55:B5 lipopolysaccharide treated blood–brain barrier disruption model of Wistar rats. The rats receiving *L. plantarum* IS 10506 alone or along with *E. coli* serotype O55:B5 lipopolysaccharide exhibited upregulation of the expression of glial fibrillary acidic protein and platelet endothelial cell adhesion molecule-1. In conclusion, the probiotic *L. plantarum* IS-10506 stimulates the restoration of blood–brain barrier disruption.

Keywords: Blood–brain barrier, Brain injury, Glial fibrillary acidic protein, Gut microbiota, *Lactobacillus plantarum* IS-10506, Platelet endothelial cell adhesion molecule-1, Probiotic

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INTRODUCTION

The blood–brain barrier (BBB) functions as a gatekeeper to control the passage and exchange of molecules and nutrients between the circulatory system and the brain parenchyma. It is essential as a physical barrier for maintaining a precisely regulated intracerebral microenvironment, ensuring homeostasis of the central nervous system (CNS) to brain development and function (Banks et al., 2015; Moretti et al., 2015; Wimmer et al., 2019). BBB characteristics limit paracellular diffusion while allowing larger molecules' tightly controlled receptor-mediated endocytosis and the transporter-mediated intake of smaller nutrients such as glucose, insulin, and iron. Endothelial cells interact closely with other CNS cells such as neurons, pericytes, and astrocytes, through adherent junctions, influx and efflux transporters, metabolic enzymes, and extracellular matrix (Abbott et al., 2006; Sharif et al., 2018; Sofroniew and Vinters, 2010). Breakdown of the BBB and increased immune cell trafficking into the CNS are hallmarks of the pathogenesis of many CNS diseases (Banks et al., 2015; Sharif et al., 2018).

Gut microbiota is a complex community that helps maintain dynamic metabolic ecological balance and keep the BBB

structure intact. The normal structure of the BBB is essential as defender brain functions from external intruders to the CNS (Braniste et al., 2014; Logsdon et al., 2018; Varatharaj and Galea, 2017). Glial fibrillary acidic protein (GFAP) of astrocytes is a vital player in the complex cascade of cellular adaptations taking place in the CNS in response to injury and disease (Mandyam et al., 2017; Parker et al., 2020; Winger et al., 2014). Platelet endothelial cell adhesion molecule-1 (PECAM-1; CD31) is an essential factor for supporting the BBB, expressed on vascular compartment cells, and regulated vascular integrity and immune cell trafficking (Wimmer et al., 2019).

The knowledge of how gut microbiota affects the GFAP of astrocyte and PECAM-1 of the BBB regulation as a response to exogenous influence remains limited. The present study investigated whether the probiotic *Lactobacillus plantarum* IS strain 10506, prevalent in Indonesia and a typical intestinal resident, can influence the BBB.

MATERIAL AND METHODS

Animals

Thirty-six male, 12-weeks old, Wistar rats weighing 100–120 g were procured from the central animal facility of the Cellular and Molecular Biology Laboratory, Faculty of Science, Brawijaya University, Malang, Indonesia. All the rats were given water and a standard pellet diet containing 20–25% protein, 5–12% fat, 2.5% fiber, and 45–60% carbohydrate ad libitum. After 14 days of acclimatization, the rats were divided into four groups of nine rats per group as follows:

Group K1: Treated with distilled water daily through gavage
Group K2: Treated with 2.5 mg/kg lipopolysaccharide (LPS) derived from the *E. coli* serotype O55:B5 through gavage on the first day, then treated with distilled water daily for 13 additional days
Group K3: Treated with 2.5 mg/kg LPS derived from the *E. coli* serotype O55:B5 through gavage on the first day, then treated with 2.5 mL of 2.67×10^9 CFU/mL *L. plantarum* IS-1056 daily for 7 following days
Group K4: Treated with 2.5 mg/kg LPS derived from the *E. coli* serotype O55:B5 through gavage on the first day, then treated with 2.5 mL of 2.67×10^9 CFU/mL *L. plantarum* IS-1056 daily for 13 following days

The probiotic used was from freeze-dried powder of *L. plantarum* IS-10506 (Gen Bank accession No. DC860149). The rats were examined and weighed daily. At the end of the experiment, day 14, the brain tissue was dissected. The study reported herein received ethical approval from the Animal Care and Use Committee at the Faculty of Veterinary Medicine, Brawijaya University, Malang, Indonesia (KEP:100-KEP-UB-2000).

Probiotic Supplementation

Microencapsulated *L. plantarum* strain IS-10506 (GenBank accession No. DQ860148) was packed in an aluminum foil sachet at the Pharmacy Installation of Dr. Soetomo Hospital (Surabaya, Indonesia) and dissolved in 1.5 mL sterile water and administered to the rats via a gastric tube once daily for 7 days at a dose of 2.67×10^9 CFU/day. Probiotic viability was assessed 1 week prior to the treatment.

LPS

For LPS dose-response and time studies, the male Wistar rats were weighed and given an intraperitoneal injection of 3 mg/kg LPS. The LPS was derived from the *E. coli* serotype O55:B5 (Cat. No. L5418, Sigma-Aldrich, St. Louis, MO, USA) dissolved in sterile normal saline.

Immunohistochemistry

The brain tissues were fixed in 10% formalin solution, followed by dehydration and paraffin embedding. Serial sections of the tissues were cleaned and fixed in 10% formalin buffer solution. Then, this procedure is followed by dehydration, clearing, and embedding. The tissue sections were probed with antibodies against the GFAP (Cat. No. sc-36673, Sigma-Aldrich, St. Louis, MO, USA) of astrocyte expression and PECAM-CD31 (Cat. No. sc-376764, Sigma-Aldrich, St. Louis, MO, USA). The sections were observed under a light microscope (CX21; Olympus, Tokyo, Japan) and photographed with an ILCE6000 camera (Sony, Tokyo, Japan). The number of immunopositive cells in 20 random fields at 100X and 400X magnification was counted.

RESULTS

The microscope visualization of the BBB structure and the results of the normal structure can be seen in Figure 1 for the control group of brain GFAP of astrocyte expression (brown color). The GFAP manifestation of the astrocyte expression group after being exposed by the LPS was shown in Figure 2. There was an improvement of the brain GFAP in the astrocyte expression group (brown color) after being treated by *L. plantarum* IS-10506 for 7 days (Fig. 3). However, *L. plantarum* IS-10506 treatment for 14 days showed a better result than 7 days, proven by the increased expression of GFAP of the astrocyte. (Fig. 4). The normal structure of the PECAM-1 in rats (brown color) as the control group was provided in Figure 5. The manifestation of the PECAM-1 expression after the LPS treatment was shown in Figure 6 and the black arrow showed the downregulation of the PECAM-1 expression. The result of this study confirmed that the probiotic *L. plantarum* IS-10506, treated for 7 days as a model of gut microbiota, improved (black arrow) disruption of PECAM-1 expression (Fig. 7). The long treatment period of 14 days also showed upregulation of the PECAM-1 expression (Fig. 8).

DISCUSSION

Gut microbiota is a complex community composed of trillions of microbes that perform several tasks which are essential to our healthy physiology and help to maintain dynamic metabolic and ecological balance and keep the structure of the BBB intact (Caspani et al., 2019; Gomes et al., 1999; Hol and Pekny, 2015; Varatharaj and Galea, 2017). Many studies using the probiotic *L. plantarum*

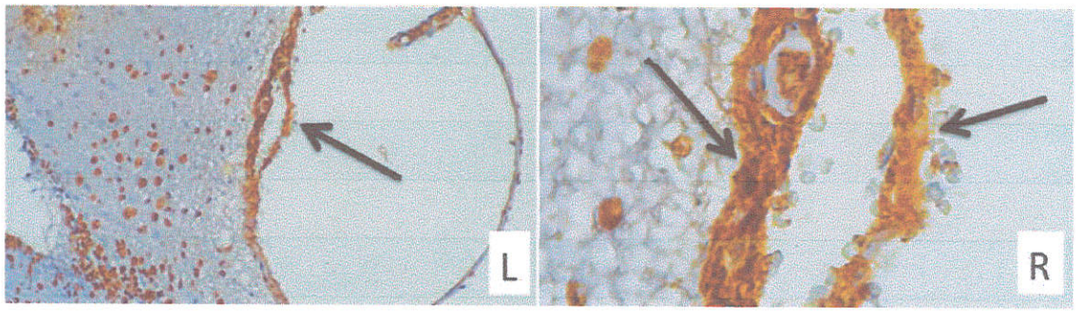

FIGURE 1 | Representative image of control group of brain glial fibrillary acidic protein (GFAP) of astrocyte expression (brown color in black arrow) in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

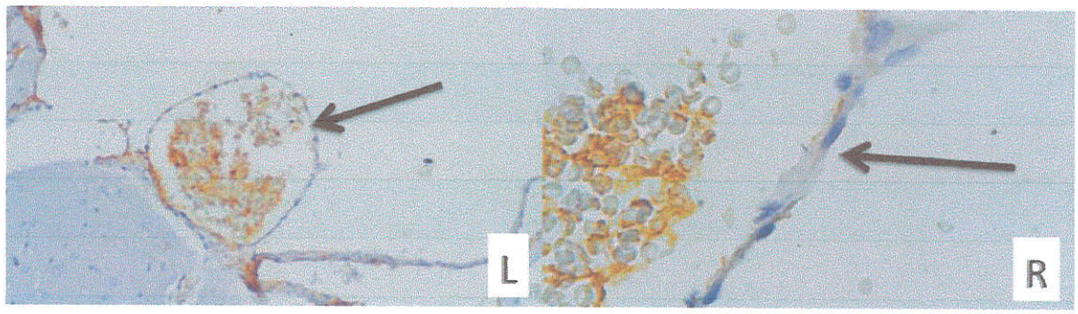

FIGURE 2 | Representative image of disruption brain GFAP of astrocyte expression (disappeared brown color in black arrow) on lipopolysaccharide group in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

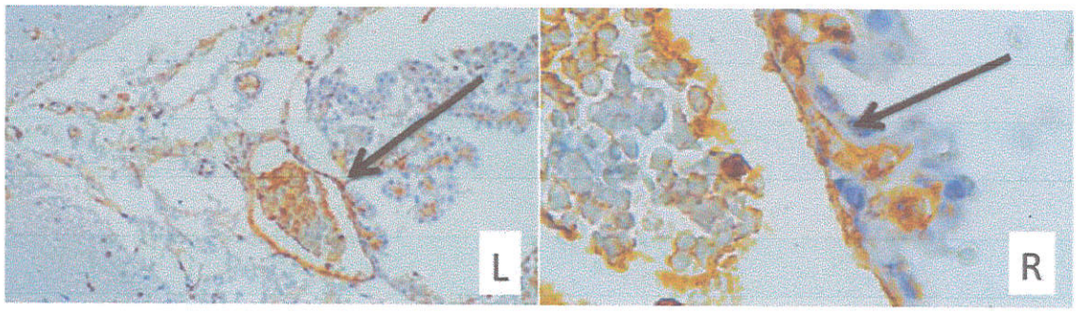

FIGURE 3 | Representative image of recovery brain GFAP of astrocyte expression (appearance of brown color in black arrow) in rats treated with *Lactobacillus plantarum* IS 10506 (7 days) in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

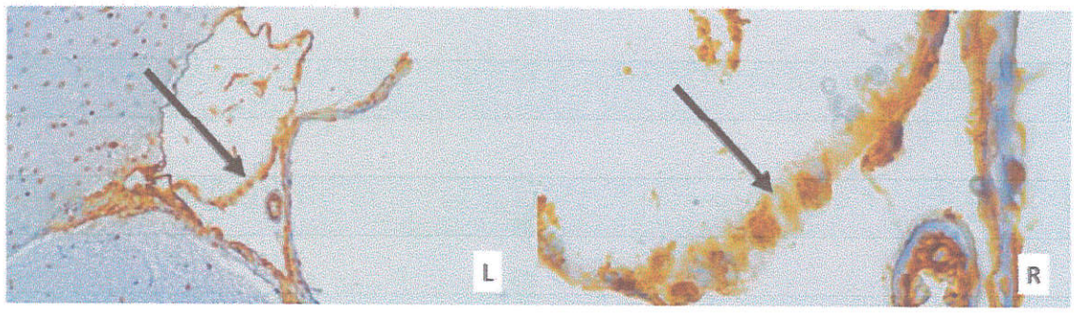

FIGURE 4 | Representative image of recovery brain GFAP of astrocyte expression (appearance clearer brown color in black arrow) treated with *L. plantarum* IS 10506 (14 days) in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

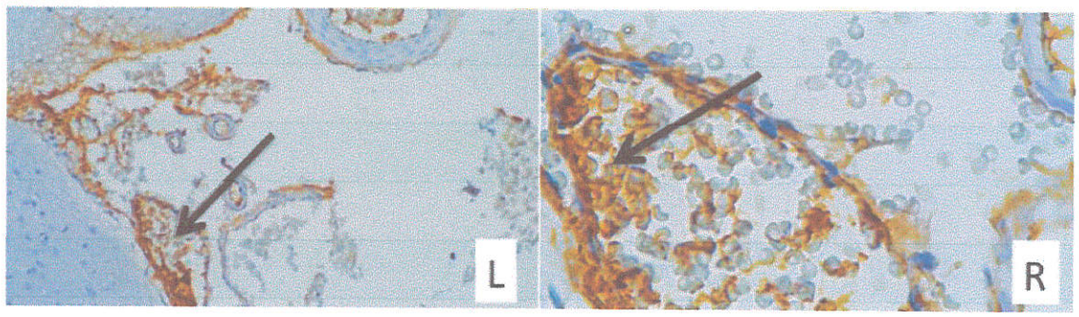

FIGURE 5 | Representative image of control group of brain CD31 (platelet endothelial cell adhesion molecule-1 (PECAM-1) expression (brown color in black arrow) in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

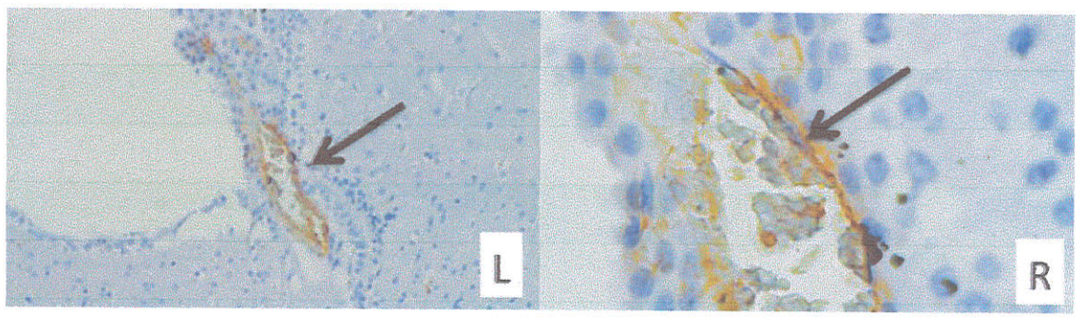

FIGURE 6 | Representative image of brain CD31 PECAM-1 expression (disappeared brown color in black arrow) on LPS group in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

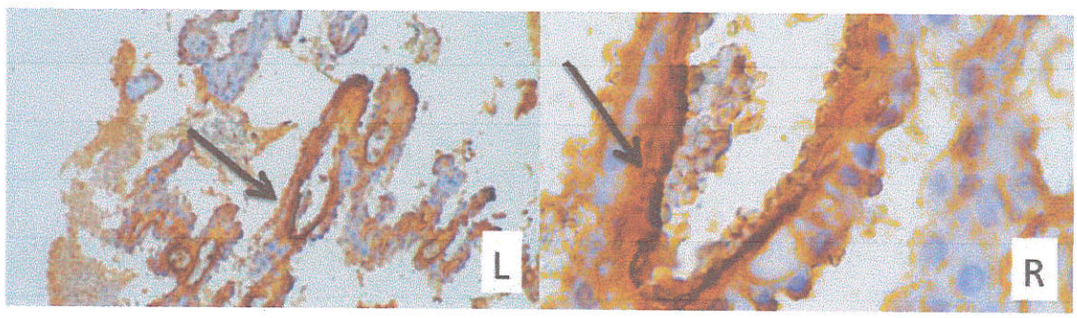

FIGURE 7 | Representative image of brain CD31 PECAM-1 expression (appearance of brown color in black arrow) treated with *L. plantarum* IS 10506 (7 days) in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

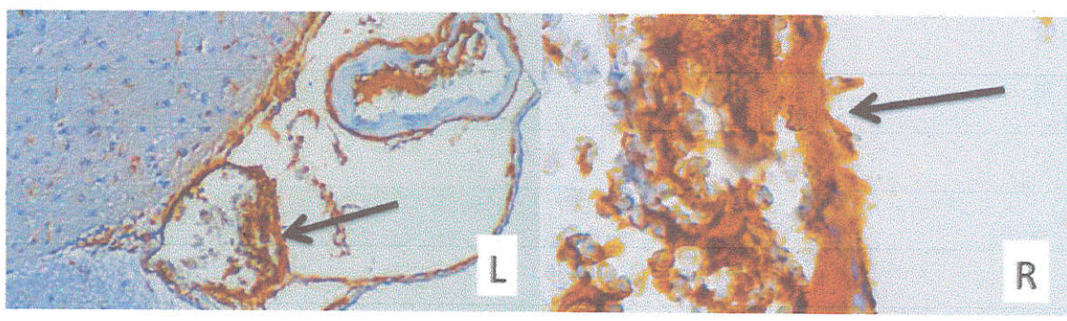

FIGURE 8 | Representative image of brain CD31 PECAM-1 expression (appearance clearer brown color in black arrow) on *L. plantarum* IS 10506 (14 days) in rats (100X magnification – L and 400X magnification – R); 1 bar = 0.01 mm.

IS 10506 showed a beneficial effect on many aspects, such as repairing damaged intestinal brush border (Ranuh et al., 2020), accelerating intestinal mucosa regeneration (Athiyyah et al., 2019), stimulating the gut–brain axis, promoting brain development and function (Ranuh et al., 2019), regulating innate immune response (Lubis et al., 2012). These studies proved the importance of gut microbiota, such as *L. plantarum* IS 10506, in animal models (Ranuh et al., 2020). The normal structure of the BBB is vital as the brain defender function from external intruders to the CNS. The BBB consists of highly specialized endothelial cells interconnected by complex and continuous tight junctions. These promote normal brain physiological function by restricting the entry of ions, macromolecules, and noxious blood-borne agents (Braniste et al., 2014; Logsdon et al., 2018).

Astrocytes, which are in close apposition to the cerebral vasculature, are crucial inducers of the BBB phenotype and help facilitate tight junction protein expression and maintenance through contact-dependent mechanisms and release soluble factors (Caspani et al., 2019; Gomes et al., 1999). GFAP is the hallmark intermediate filament (also known as nanofilament) protein in astrocytes, the primary type of glial cells in the CNS. Astrocytes have a range of control and homeostatic functions in health and disease. Astrocytes assume a reactive phenotype in acute CNS trauma, ischemia, and neurodegenerative diseases (Mandyam et al., 2017). The disruption of the brain GFAP of astrocyte at 100X magnification (left) and 400X magnification (right) after LPS treatment (a blur of brown color) is shown in Figure 2, which is a model disruption of BBB. Over the last few decades, considerable efforts have been made to elucidate the complex functions of astrocytes in healthy and CNS diseases. It is now established that astrocytes play essential roles beyond the simplistic view of supporting elements to neurons. Astrocytes are recognized to participate in functions that seem to be the exclusive prerogative of neurons, such as synaptic transmission and processing (Sofroniew and Vinters, 2010).

Studies regarding the CNS-related mechanism underlying the BBB development have not yet been fully explored, especially about the impact of the gut microbiota as a potential role on BBB integrity. Braniste et al. (2014) identified the gut microbiota as a probable regulator of the BBB integrity in both the fetal and adult mouse brain. This study confirmed that the probiotic *L. plantarum* IS-10506 administration in 14 days also has a better effect on the BBB restoration of disruption structure by increasing the GFAP of astrocyte expression and upregulation of GFAP of astrocyte expression. PECAM-1 is required for efficient monocyte and neutrophil diapedesis in the BBB (Dan et al., 2013). The PECAM-1 is a crucial component of endothelial cells. It has been implicated in several other neuropathologies that involve BBB damage and is essential in regulating endothelial cell integrity, especially during an inflammatory challenge (Wimmer et al., 2019). Breakdown of the BBB and increased immune cell trafficking into the CNS are hallmarks of the pathogenesis of many CNS diseases. The PECAM-1 (PECAM-1; CD31) is expressed on vascular compartment cells and regulates vascular integrity and immune cell trafficking (Wimmer et al., 2019). The disruption of the PECAM-1 increases the possibility of intruders such as an antigen moving on the brain (Dan et al., 2013). However, knowledge regarding how the PECAM-1 of the BBB regulation responds to gut microbiota's influence remains limited.

Other studies report that other gut microbiota such as *Clostridium butyricum*, *C. tyrobutyricum*, and *Bacteroides thetaiotaomicron* impact the BBB integrity. These microbial-derived metabolites have essential metabolic and signaling functions, which can modulate host homeostasis, including the BBB integrity and brain function (Parker et al., 2020). These results proved the potential of gut microbes as modulators of the BBB integrity for brain health.

CONCLUSION

This study indicates that *L. plantarum* IS-10506 shore up the BBB to improve the GFAP and PECAM-1 expression as a stimulator for restoring the BBB disruption. These findings suggest that probiotics potentially promote brain defense and offer the model for investigating the effects of gut microbiota on the BBB to prevent exogenous pathogens on the CNS infections.

CONFLICT OF INTEREST DECLARATION

The authors state that there are no conflicts of interest to disclose.

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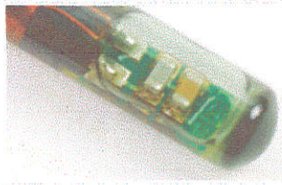

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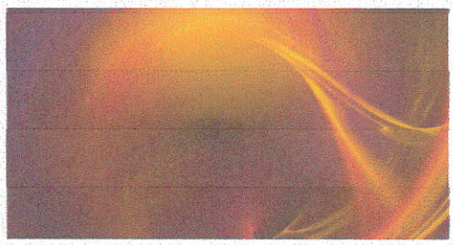
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Metrics based on Scopus® data as of April 2021

Astghik Pepoyan 2 years ago

Is this journal "active" now?

reply

SCImago Team

Melanie Ortiz 2 years ago

Dear Astghik,
Thank you for contacting us. Could you please expand a little bit your comment?
Best Regards, SCImago Team

Weerasak Taengphan 2 years ago

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reply

Madhu 2 years ago

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SCImago Team

Melanie Ortiz 2 years ago

Dear Madhu, thank you very much for your comment, we suggest you look for author's instructions/submission guidelines in the journal's website. Best Regards, SCImago Team

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