The Indonesian Biomedical Journal

Editorial Team

Editor in Chief

Dr. Dewi Muliaty, Prodia Clinical Laboratory, Jakarta, Indonesia

Managing Editor

Dr. Anna Meiliana, Universitas Padjadjaran, Bandung, Indonesia

Board of Editors

Dr. Andi Wijaya, Prodia Clinical Laboratory, Bandung, Indonesia
Prof. Dinath Ratnayake, The University of Western Ontario, Ontario, Canada
Prof. Geraldine Budomo Dayrit, Trinity University of Asia, Quezon, Philippines
Prof. Joseph Bercmans Lopez, MAHSA University College, Jenjarom, Malaysia
Prof. Koichi Nakayama, Saga University, Saga, Japan
Prof. Rajiv Timothy Erasmus, Stellenbosch University, Cape Town, South Africa
Dr. Rizky Abdulah, Universitas Padjadjaran, Bandung, Indonesia
Prof. Dr. Roberto Volpe, Italian National Research Council, Rome, Italy
Prof. Tar Choon Aw, ICON Central Laboratory, Singapore, Singapore
Dr. Trilis Yulianti, Prodia Clinical Laboratory, Jakarta, Indonesia

Indexed by:

The Prodia Education and Research Institute

The Indonesian Biomedical Journal

Vol 12, No 3 (2020)

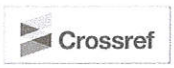
Table of Contents

Review Article

Research Article

Indexed by:

The Prodia Education and Research Institute



DOI: 10.18585/inabj.v12i3.1041

RESEARCH ARTICLE

Zinc Administration Affects Bronchial Mucosal NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β of Zinc-deficient Rats

Reza Gunadi Ranuh[1], Andy Darma[1], Alpha Fardah Athiyyah[1], Ergia Latifolia[1], Retno Asih Setyoningrum[1], Boerhan Hidajat[1], Siti Nurul Hidayati[1], Anang Endaryanto[1], Ferry Sandra[2,*], Subijanto Marto Sudarmo[1]

[1]Department of Child Health, Faculty of Medicine, Universitas Airlangga/Dr. Soetomo Regional General Hospital, Jl. Prof. Dr. Moestopo No 6-8, Surabaya, Indonesia
[2]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No.260, Jakarta, Indonesia

*Corresponding author. E-mail: ferry@trisakti.ac.id

Received date: Nov 14, 2019; Revised date: Jul 12, 2020; Accepted date: Jul 17, 2020

Abstract

BACKGROUND: Risk of acute respiratory infections in children less than 5 years of age is up to 95%. Zinc deficiency is one of the main risk factors. This study aimed to explore the effect of zinc on the bronchial mucosae inflammatory status expressed by nuclear factor (NF)-κB p105/p50, NF-κB p65, interleukin (IL)-8, and IL-1β.

METHODS: Twenty-four Wistar rats were divided into 4 groups: normal zinc diet group without zinc supplementation (Z1), normal zinc diet group with zinc supplementation (Z2), zinc deficient diet group without zinc supplementation (Z3), and zinc deficient diet group with zinc supplementation (Z4). NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β were measured by immunohistochemical staining.

RESULTS: The inflammatory status of bronchial mucosae between Z1 and Z2 groups showed no difference (p=0.055). However, the inflammatory status of bronchial mucosae between Z3 and Z4 groups showed significant difference (p<0.01). Multivariate factorial design showed that zinc supplementation was beneficial when given to zinc deficient diet group with regard to decrease p-NF-κB p65, IL-8 and IL-1β levels (p<0.001) and increase dendritic cell (p=0.022).

CONCLUSION: Zinc administration under conditions of zinc deficiency affects the inflammatory status, as shown by the decrease of p-NF-κB p65, IL-8 and IL-1β and the increase of NF-κB p105/p50.

KEYWORDS: zinc, NF-κB, p105/p50, p65, IL-8, IL-1β, rat

Indones Biomed J. 2020; 12(3): 245-50

Introduction

Child mortality due to acute respiratory infection (ARI) reached 1.9 million in 2000, with the highest levels seen in developing countries, particularly in children under 5 years old. In the developing countries, this age group has the highest risk of death from ARI, reaching as high as 95%.(1) The Basic Health Research conducted in Indonesia states that the national prevalence of overall ARI reached 25.50%. While in the group of infants and children younger than 5 years, as many as 35% suffered from ARI.(2) Zinc deficiency is one of the main risk factors for acute respiratory infections.(3)

Zinc is an essential component of several enzymes and cofactors in signaling pathways. Controlling inflammatory signaling pathway through correlated underlying factors such as interleukin (IL)-1β (4-6) and IL-8 (7), and also transcription factors, such as NF-κB (6-9), is required for normal development (10,11), apoptotic induction of tumor/cancer cell (12-14), and cellular function.(15)

Zinc plays a role in the inflammatory system by a variety of mechanisms such as protecting the mucociliary cytoplasmic apparatus (tubulin and basal bodies), and

CC BY NC

inhibiting Nuclear Factor (NF)-κB translocation into the nucleus, which prevents the subsequent expression of pro-inflammatory cytokines. Zinc deficiency will provoke pro-inflammatory cytokines, which could damage the mucous membrane of respiratory tract, leading to respiratory tract infections.(15-17) Zinc deficiency affects the function of cells belonging to innate and acquired immunity and can cause complications such as secondary infections and cell damage.(18) Zinc deficiency increased the levels of tumor necrosis factor (TNF)-α, interleukin (IL)-1β, and IL-8 cytokines.(19,20)

Unfortunately, effects of zinc supplementation on the inflammatory system of bronchial mucosa were not extensively studied. There was one study conducted in septic mice (21), but not in healthy rats. Therefore, current research was conducted to investigate the effect of zinc administration on the inflammatory bronchial mucosal status by analysing NF-κB p105/p50, phosphorylated-NF-κB (p-NF-κB) p65, IL-8, and IL-1β of normal and zinc deficiency healthy rats.

Methods

Animal Treatment and Sample Collection

Twenty-four male Wistar rats, aged 5 weeks, 60-100 g were divided into 4 groups: normal zinc diet group without zinc supplementation (Z1), normal zinc diet group with 60 ppm/day zinc supplementation (Z2), zinc-deficient diet group without zinc supplementation (Z3), and zinc-deficient diet group with 120 ppm/day zinc supplementation (Z4). All treatments were performed until 3 weeks. After that, rats were sacrificed and bronchial mucosae were collected. The mucosae were fixed and processed for making paraffin blocks. The study was conducted in the Biochemistry Laboratory, Faculty of Medicine, Universitas Airlangga/ Dr. Soetomo General Hospital, Surabaya. The research protocol was approved by the Research Ethics Committee for Animal Care and Use, Faculty of Veterinary Medicine, Universitas Airlangga (No. 055-KE).

Immunohistochemistry

Bronchial mucosal paraffin blocks were sliced in 4 μm, de-paraffinized and antigen retrieved. After washing with phosphate buffered saline (PBS), the tissue sections were incubated with 3% hydrogen peroxide and incubated with 2% bovine serum albumin. Then each of the following primary antibodies was applied. For NF-κB p105/p50 detection, a mouse monoclonal anti-NF-κB p105/p50 antibody (Cat# NB100-56583, Novus Biologicals, Centennial, CO, USA) was applied. For p-NF-κB p65 detection, a mouse monoclonal anti-phospho-NF-κB p65 (Ser536) antibody (Cat# sc-136548, Santa Cruz Biotechnology, Dallas, TX, USA) was applied. For IL-8 detection, a rabbit polyclonal anti-IL-8 antibody (Cat# orb229133, Biobyt, St. Louis, MO, USA) was applied. For IL-1β detection, a rabbit polyclonal anti-IL-1β antibody (Cat# AAR15G, Bio-Rad, Hercules, CA, USA) was applied. After the first antibody, N-Histofine High Stain HRP (MULTI) (Nichirei Biosciences, Tokyo Japan) kit was used. The peroxidase activity was visualized by immersing tissue sections in N-Histofine DAB-2V (Nichirei Biosciences), resulting in a brown reaction product. Tissue sections were finally counterstained with hematoxylin and mounted.

Immunohistochemical Evaluation

Cells with overexpressions of NF-κB p105/p50, p-NF-κB p65, IL-8 and IL-1β were examined, documented and counted. Five fields/slide/rat were selected and documented under a light microscope with 400x magnification, then counted by two trained examiners.

Statistical Analysis

Counted cells were statistically analyzed with SPSS Statistics, version 17.0 (SPSS Inc., Chicago, IL, USA). Student's t-test was used to analyse differences of number of cells expressing NF-κB p105/p50, p-NF-κB p65, IL-8 and IL-1β in each group. The multivariate analysis of variance (MANOVA) factorial design was used to determine the effect between zinc status and zinc supplementation on immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β. Data were analysed with 95% confidence level. The p-value<0.05 was considered significant.

Results

Immuno-expressions of NF-κB p105/p50 (Figure 1), p-NF-κB p65 (Figure 2), IL-8 (Figure 3) and IL-1β (Figure 4) were seen clearly in bronchial mucosae of all Z1-Z4 groups. Immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β in the bronchial mucosae of Z1 and Z2 groups were not significantly different (Table 1). Immuno-expression levels of NF-κB p105/p50, IL-8, and IL-1β in the bronchial mucosae of Z1 group were merely slightly higher than the ones in Z2 group.

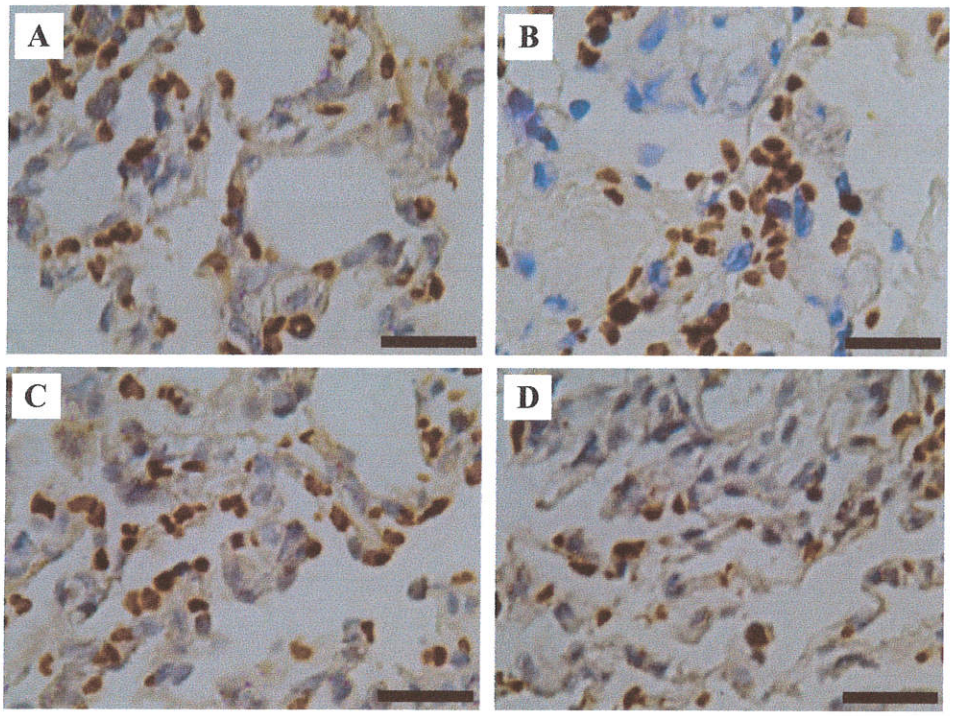


Figure 1. Immuno-expression of NF-κB p105/p50 in bronchial mucosae. A: Z1 group, B: Z2 group, C: Z3 group, D: Z4 group. Black bar: 10 μm.

Meanwhile immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β in the bronchial mucosae of Z3 and Z4 groups were significantly different (Table 2). Immuno-expression levels of, p-NF-κB p65, IL-8, and IL-1β in the bronchial mucosae of Z4 group were significantly lower than the ones in Z3 group. Interestingly, immuno-expression level of NF-κB p105/p50 in the bronchial mucosae of Z4 group was significantly higher than the one in Z3 group. The MANOVA factorial design showed significant results of zinc status with zinc supplementation on the immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β (Table 3). In addition, individual zinc status and individual zinc supplementation were shown to significantly affect immuno-expression levels of p-NF-κB p65, IL-8, and IL-1β but not the one of NF-κB p105/p50.

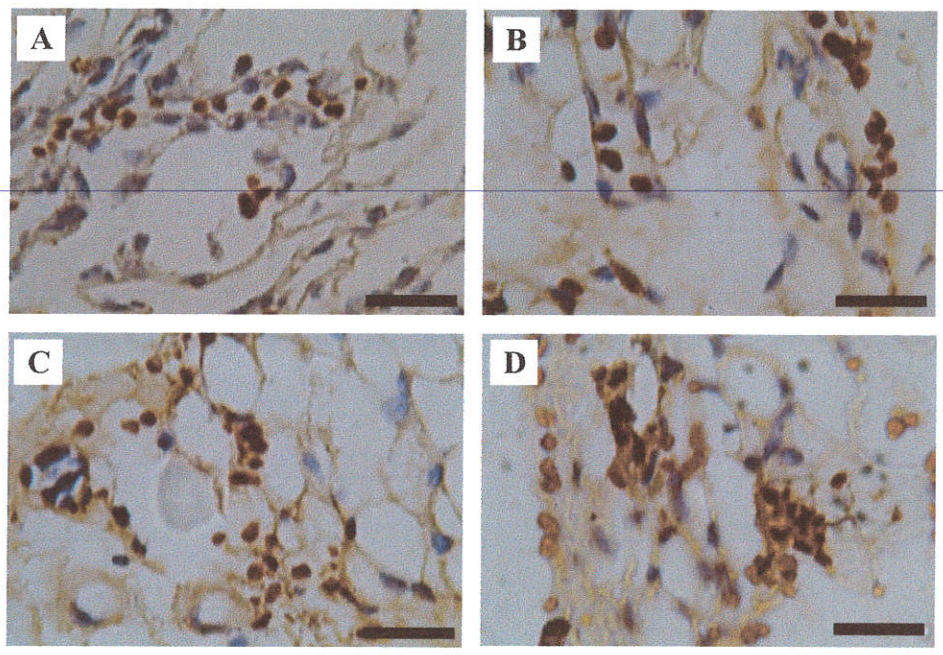


Figure 2. Immuno-expression of p-NF-κB p65 in bronchial mucosae. A: Z1 group, B: Z2 group, C: Z3 group, D: Z4 group. Black bar: 10 μm.

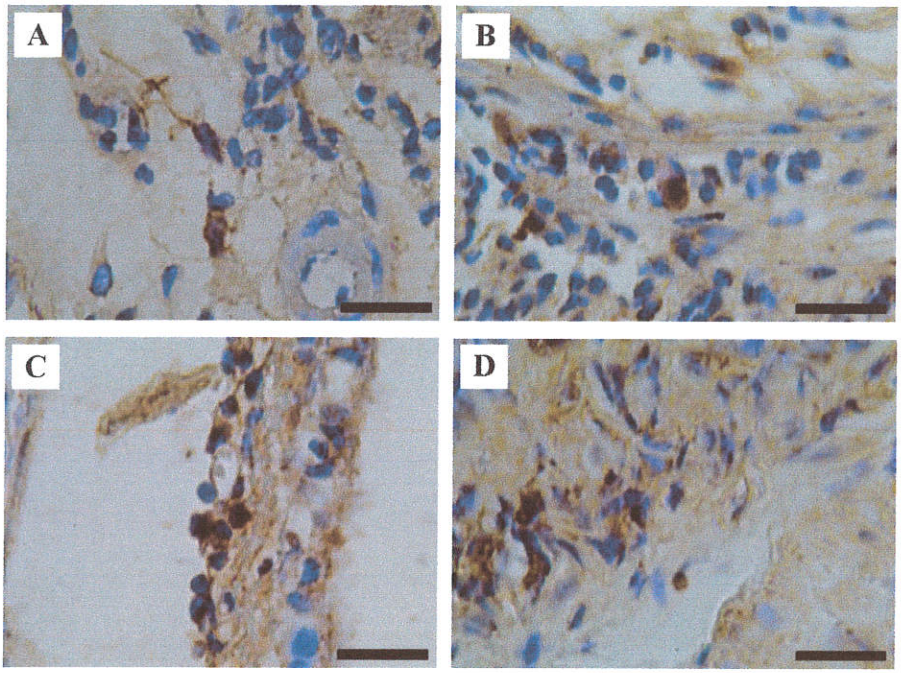


Figure 3. Immuno-expression of IL-8 in bronchial mucosae. A: Z1 group, B: Z2 group, C: Z3 group, D: Z4 group. Black bar: 10 μm.

Discussion

Present study showed that zinc supplementation under normal conditions had no effect on the inflammatory status of bronchial mucosae, as shown by immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β. Previous study reported that zinc supplementation on respiratory tract infections of children with cystic fibrosis showing a not significant difference of IL-8 and IL-1β serum levels compared to placebo.(22) Zinc supplementation would not be beneficial to individuals with normal zinc level, but zinc-deficient individuals responded to zinc supplementation. (23) Present results also showed that zinc administration had an effect on bronchial mucosal inflammatory status in deficient condition.

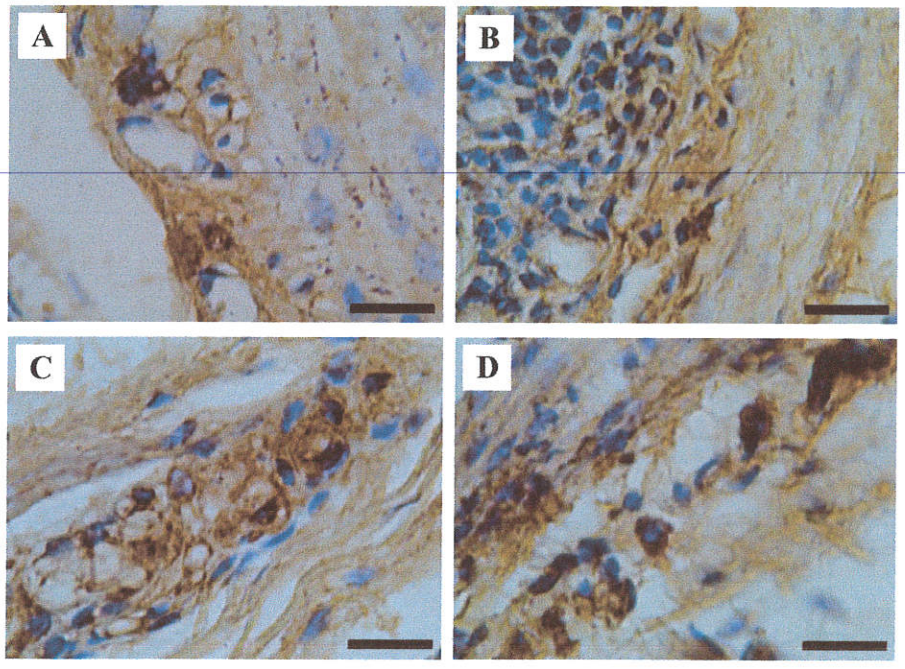


Figure 4. Immuno-expression of IL-1β in bronchial mucosae. A: Z1 group, B: Z2 group, C: Z3 group, D: Z4 group. Black bar: 10 μm.

Table 1. Immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β in the bronchial mucosae of normal zinc diet group. Z1 group: normal zinc diet group without zinc supplementation, Z2 group: normal zinc diet group with zinc supplementation.

Variable	Normal Zinc Diet Group		*p** (univariate)	*p*** (multivariate)
	Z1 Group (Mean±SD)	Z2 Group (Mean±SD)		
NF-κB p105/p50	12.40±3.21	20.20±0.84	0.001	0.055
p-NF-κB p65	2.40±1.14	2.00±0.71	0.524	
IL-8	3.00±1.23	3.80±1.48	0.380	
IL-1β	3.00±1.58	3.60±1.52	0.557	

*Independent t-test; **Hotelling's T2 test.

Table 2. Immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β in the bronchial mucosae of zinc deficient diet group. Z3 group: zinc deficient diet group without zinc supplementation, Z4 group: zinc deficient diet group with zinc supplementation.

Variable	Zinc Deficient Diet Group		*p** (univariate)	*p*** (multivariate)
	Z3 Group (Mean±SD)	Z4 Group (Mean±SD)		
NF-kB p105/p50	5.50±1.38	12.60±2.70	<0.001	<0.001
p-NF-kB p65	19.30±1.97	7.80±2.86	<0.001	
IL-8	20.30±1.63	11.20±1.30	<0.001	
IL-1b	22.17±3.97	10.40±1.14	<0.001	

*Independent t-test; **Hotelling's T2 test.

In present study, p-NF-κB p65, IL-8 and IL-1β were increased in zinc-deficient rats without zinc supplementation while NF-κB p105/p50 was not influenced by zinc supplementation. Theoretically zinc deficiency might cause epithelial damage, which stimulate dendritic cells to secrete IL-8 and IL-1β in the inflammatory process of the bronchial mucosal epithelium. Then the signal transduction will occur, leading to increment of NF-κB p65 phosphorylation, then p-NF-κB p65 translocate into the nucleus. As for the inactive form of NF-κB will decrease, including NF-κB p105/p50, which is remained in the cytoplasm.

Zinc administration will increase zinc levels in the intracellular system and inhibit the production of proinflammatory cytokines through several channels. In

Table 3. MANOVA factorial design of zinc status and zinc supplementation on immuno-expression levels of NF-κB p105/p50, p-NF-κB p65, IL-8, and IL-1β in the bronchial mucosae of Wistar rats.

Main Effects and Interaction Effects	Variable	*Mean square*	*p**	*p***
Effects of zinc status (normal/ zinc deficiency)	NF-κB p105/p50	322.73	<0.001	<0.001
	p-NF-κB p65	668.48	<0.001	
	IL-8	784.1	<0.001	
	IL-1β	850.85	<0.001	
Effects of zinc supplementation (yes/no)	NF-κB p105/p50	253.8	<0.001	<0.001
	p-NF-κB p 65	192.61	<0.001	
	IL-8	112.4	<0.001	
	IL-1β	154.93	<0.001	
Interaction effects of zinc status (normal/deficiency) with zinc supplementation (yes/no)	NF-κB p105/p50	0.013	0.721	<0.001
	p-NF-κB p65	148.44	<0.001	
	IL-8	100.74	<0.001	
	IL-1β	200	<0.001	

*Tests of Between-Subjects Effects; **Wilks' Lambda.

addition, zinc induces zinc finger protein and inhibits activation of the NF-κB pathway through Tumor Necrosis Factor Receptor (TNF-R)-associated Factor (TRAF).(20,23) Zinc administration in HL-60 cells was shown to decrease gene expression and the production of inflammatory cytokines, as well as decreasing markers of oxidative stress. (20) Therefore, in present study, by supplementation of zinc, the zinc deficiency-caused inflammatory signaling pathway was inhibited, resulting down-regulation/decrease of p-NF-κB p65 and upregulation/increase of NF-κB p105/p50.

Conclusion

Zinc administration has an effect on bronchial mucosal inflammatory status, which is expressed by the decrease of p-NF-κB p65, IL-8 and IL-1β levels and the increase of NF-κB p105/p50 level in rats with zinc deficiency.

References

1. Daili SF, Williams BG, Gouws E, Boschi-Pinto C, Bryce J, Dye C. Estimates of world-wide distribution of child deaths from acute respiratory infections. Lancet Infect Dis. 2002; 2: 25-32.
2. Badan Penelitian dan Pengembangan Kesehatan Kemenkes RI. Riset Kesehatan Dasar 2010. Jakarta: Kementrian Kesehatan; 2010.
3. Bhutta ZA, Black RE, Brown KH, Gardner MJ, Gore S, Hidayat A, *et al.* Prevention of diarrhea and pneumonia by zinc supplementation in children in developing countries: Pooled analysis of randomized controlled trials. J Pediatr. 1999; 135: 689-97.
4. Todingrante A, Arief M, Bahrun U, Sandra F. Study of low-grade chronic inflammatory markers in men with central obesity: cathepsin s was correlated with waist circumference. Indones Biomed J. 2013; 5: 115-20.
5. Afifah E, Mozef T, Sandra F, Arumwardana S, Rihibiha DD, Nufus H, *et al.* Induction of matrix metalloproteinases in chondrocytes by interleukin IL-1β as an osteoarthritis model. J Math Fund Sci. 2019; 51: 103-11.
6. Hamuaty RB, Sukmawati IR, Sandra F. Relationship between sRAGE and hsCRP as markers of cardiovascular disease risk factors in diabetic and non-diabetic men with central obesity. Mol Cell Biomed Sci. 2017; 1: 70-4.
7. Ketherin, Sandra F. Osteoclastogenesis in periodontitis: signaling pathway, synthetic and natural inhibitors. Mol Cell Biomed Sci. 2018; 2: 11-8.
8. Sandra F, Ketherin. Caffeic acid inhibits RANKL and TNF-α-induced phosphorylation of p38 mitogen-activated protein kinase in RAW-D cells. Indones Biomed J. 2018; 10: 140-3.
9. Sandra F, Kukita T, Tang QY, Iijima T. Caffeic acid inhibits NFkB activation of osteoclastogenesis signaling pathway. Indones Biomed J. 2011; 3: 216-22.
10. Sandra F. Role of herbal extract in stem cell development. Mol Cell Biomed Sci. 2018; 2: 19-22.
11. Sandra F, Kukita T, Muta T, Iijima T. Caffeic acid inhibited receptor activator of nuclear factor κB ligand (RANKL)-tumor necrosis factor (TNF) α-TNF receptor associated factor (TRAF) 6 induced osteoclastogenesis pathway. Indones Biomed J. 2013; 5: 173-8.
12. Sandra F. Targeting ameloblatoma into apoptosis. Indones Biomed J. 2018; 10: 35-9.
13. Sandra F, Hudono KF, Putri AA, Putri CAP. Caspase inhibitor diminishes caffeic acid-induced apoptosis in osteosarcoma cells. Indones Biomed J. 2017; 9: 160-4.
14. Hendarmin L, Kawano S, Yoshiga D, Sandra F, Mitsuyasu T, Nakao Y, *et al.* An anti-apoptotic role of NF-κB in TNFα-induced apoptosis in an ameloblastoma cell line. Oral Sci Int. 2008; 5: 96-103.
15. Truong-Tran AQ, Carter J, Ruffin R, Zalewski PD. New insights into the role of zinc in the respiratory epithelium. Immunol Cell Biol. 2001; 79: 170-7.
16. Prasad AS. Clinical, immunological, anti-inflammatory and antioxidant roles of zinc. Exp Gerontol. 2008; 43: 370-7.
17. Ngom PT, Howie S, Ota MO, Prentice AM. The potential role and possible immunological mechanisms of zinc adjunctive therapy for severe pneumonia in children. Open Immunol J. 2011; 4: 1-10.
18. Overbeck S, Rink L, Haase H. Modulating the immune response by oral zinc supplementation: A single approach for multiple diseases. Arch Immunol Ther Exp (Warsz). 2008; 56: 15-30.
19. Prasad AS. Effects of zinc deficiency on Th1 and Th2 cytokine shifts. J Infect Dis. 2000; 182 (Suppl1): S62-8.
20. Prasad AS. Zinc: mechanisms of host defense. J Nutr. 2007; 137: 1345-9.
21. Bao S, Liu MJ, Lee B, Besecker B, Lai JP, Guttridge DC, *et al.* Zinc modulates the innate immune response in vivo to polymicrobial sepsis through regulation of NF-kappaB. Am J Physiol Lung Cell Mol Physiol. 2010; 298: L744-54.
22. Abdulhamid I, Beck FW, Millard S, Chen X, Prasad A. Effect of zinc supplementation on respiratory tract infections in children with cystic fibrosis. Pediatr Pulmonol. 2008; 43: 281-7.
23. Prasad AS. Zinc: role in immunity, oxidative stress and chronic inflammation. Curr Opin Clin Nutr Metab Care. 2009; 12: 646-52.

RESEARCH ARTICLE

Lactobacillus plantarum IS-20506 Probiotic Restores Galectin-4 and Myosin-1a Expressions in Duodenum, Jejunum and Ileum of Lipopolysaccharide-induced Rats

Reza Gunadi Ranuh[1], Alpha Fardah Athiyyah[1], Andy Darma[1], Wibi Riawan[2], Ingrid Suryanti Surono[3], Ferry Sandra[4,*], Subijanto Marto Sudarmo[1]

[1]Department of Child Health, Dr. Soetomo Hospital, Faculty of Medicine, Universitas Airlangga, Jl. Prof. Dr. Moestopo No. 6-8, Surabaya, Indonesia
[2]Laboratory of Biochemistry and Biomolecular, Universitas Brawijaya, Jl. Veteran, Malang, Indonesia
[3]Food Technology Department, Faculty of Engineering, Bina Nusantara University, Jakarta 11480, Indonesia
[4]Department of Biochemistry and Molecular Biology, Division of Oral Biology, Faculty of Dentistry, Universitas Trisakti, Jl. Kyai Tapa No.260, Jakarta, Indonesia

*Corresponding author. E-mail: ferry@trisakti.ac.id

Received date: Dec 16, 2019; Revised date: Aug 15, 2020; Accepted date: Aug 18, 2020

Abstract

BACKGROUND: Galectin-4 and Myosin-1a are important proteins for normal intestinal brush border structure and composition. Damage of these proteins by inflammation may alter digestion, absorption and barrier function. Probiotic has been widely known in maintaining gut health. However, the molecular mechanism of *Lactobacillus plantarum* IS-2056 probiotic in repairing intestinal brush border is not well defined. Therefore, current study was conducted by investigating the Galectin-4 and Myosin-1a expressions in a rodent model.

METHODS: Male Wistar rats were induced with/without lipopolysaccharide (LPS) and treated with/without *L. plantarum* IS-2056 probiotic. On the seventh day, duodenum, jejunum, and ileum were collected and analyzed with western blot and immunohistochemistry for Galectin-4 and Myosin-1a expressions.

RESULTS: Rats administrated with *L. plantarum* IS-2056 probiotic showed significant increase of Galectin-4 and Myosin-1a expressions in duodenum, jejunum, and ileum compared to the control group ($p<0.05$). While in control group, Galectin-4 level tended to increase in more distal of intestinal segment and Myosin-1a level tended to decrease in more distal intestinal segment.

CONCLUSION: *L. plantarum* IS-20506 probiotic may facilitate the repairment of damaged intestinal brush border as demonstrated by significant restoration of Galectin-4 and Myosin-1a expressions in duodenum, jejunum, and ileum of LPS-induced rats.

KEYWORDS: *Lactobacillus plantarum,* IS-20506, probiotic, galectin-4, myosin-1a, duodenum, jejunum, ileum

Indones Biomed J. 2020; 12(3): 283-7

Introduction

Intestinal mucosal defense as a cellular unit is comprised by brush border membrane, microvilli core, and epithelial tight junction. There are several proteins play important roles in the defense. Galectin-4 serves as membrane structure of brush border, molecule transport, cell adhesion, and wound healing.(1) Villin, Fimbrin, Myosin-1a, Myosin-1c, Myosin-1e, and Calmodulin maintain the integrity of microvilli.(2) Occludin and ZO-1 provide important part in maintaining integrity of impermeable tight junction as a complex protein.(3) Together, these provide the first line defense unit called intestinal mucosal barrier.

Intestinal mucosal barrier dysfunction could be caused by immune aberrancy and infection, which are the main pathogenesis of many gastrointestinal diseases. Inflammation induced by pathogenic lipopolysaccharide

CC BY NC

(LPS) will down regulate mucosal barrier proteins, resulting in increasing permeability and susceptibility of bacterial invasion.(4) Tight junction is the main structure connecting intestinal mucosal epithelial cells needed to maintain normal anatomy and physiology of intestinal mucosal barrier.(4)

Probiotics are living bacteria or yeast that could give positive benefits to the host.(5) *Lactobacillus plantarum* IS-20506 is a one of two *L. plantarum* strains isolated from *dadih*, a fermented buffalo milk from Sumatra Island.(6) *L. plantarum* IS-10506 showed significant role in intestinal stem cells and intestinal mucosal barrier integrity (7), while *L. plantarum* IS-20506 activity towards mucosal barrier integrity is still unknown. In this study, we investigated the probiotic effect of *L. plantarum* IS-20506 on intestinal mucosal tight junction protein during intestinal inflammation.

Methods

Animal Preparation and Treatment

Fifteen male, weighted 100-120 gram, aged 12 weeks, Wistar rats were procured from central animal facility of Cellular and Molecular Biology Laboratory, Faculty of Science, Universitas Brawijaya, Malang, Indonesia. All rats were given water ad libitum and normal pellet diet containing 20-25% protein, 5-12% fat, 2.5% fiber, and 45-60% carbohydrate. After 14 days of acclimatization, fifteen rats were equally assigned into 3 groups. The first group was treated with distilled water daily through gavage as control group. The second group was induced with 2.5 mg/kg LPS derived from *Eschericia coli* serotype O55:B5 (Cat. No. L5418, Sigma-Aldrich, St. Louis, MO, USA) through gavage on the first day, then treated with distilled water daily on the following days. The third group was also induced with 2.5 mg/kg LPS through gavage on the first day, but then treated with 2.5 mL of 2.67x109 CFU/ mL *L. plantarum* IS-2056 daily on the following days. Freeze-dried powder of *L. plantarum* IS-20506 (GenBank accession no. DC860149) was obtained from the Research and Development Laboratory of PT Ultrajaya Milk Industry, Jakarta, Indonesia. Rats were examined and weighted daily. On day 7, rats were sacrificed, then duodenum, jejunum, and ileum were dissected and processed for western blot and immunohistochemistry. This study protocol was approved by Ethical Committee of Faculty of Veterinary Medicine, Universitas Airlangga (No. 028-KE, March 2018).

Western Blot

Dissected duodenum, jejunum, and ileum were minced and homogenized in a cold lysis buffer. After centrifugation, supernatants were collected as samples. The samples were separated by SDS-polyacrylamide gel electrophoresis and transferred to a polyvinylidene difluoride sheet. After blocking with 5% skim milk in phosphate-buffered saline, the sheet was incubated with 1:200 goat polyclonal anti-Galectin-4 (Q-20) (sc-19289, Santa Cruz Biotechnology, Dallas, TX, USA) or anti-Myosin-1a (C-12) (sc-32698, Santa Cruz Biotechnology) antibody. Secondary antibody was alkaline phosphatase-conjugated donkey anti-goat IgG (H+L) antibody (SAB3700286, Sigma-Aldrich). The bound antibodies were visualized using 5-bromo-4-chloro-3-indolyl phosphate (BCIP)/nitro blue tetrazolium (NBT) substrate system (B1911, Sigma-Aldrich).

Immunohistochemistry

Dissected duodenum, jejunum, and ileum were fixed and processed for making paraffin blocks. The paraffin blocks were sliced in 4 μm, de-paraffinized and antigen retrieved. After washing with phosphate buffered saline (PBS), the tissue sections were incubated with 3% hydrogen peroxide and incubated with 2% bovine serum albumin. Then 1:50 goat polyclonal anti-Galectin-4 (Q-20) (sc-19289, Santa Cruz Biotechnology) or anti-Myosin-1a (C-12) (sc-32698, Santa Cruz Biotechnology) antibody was applied. After the antibody, N-Histofine High Stain HRP (MULTI) (Nichirei Biosciences, Tokyo, Japan) kit was used. The peroxidase activity was visualized by immersing tissue sections in N-Histofine DAB-2V (Nichirei Biosciences), resulting in a brown reaction product. Tissue sections were finally counterstained with hematoxylin and mounted.

Immunohistochemical Evaluation and Data Analysis

Cells with overexpressions of Galectin-4 and Myosin-1a were examined and counted. Five fields/slide/rat were selected and documented under a light microscope with 400x magnification, then counted by two trained examiners. Counted cells were then statistically analyzed with SPSS Statistics, version 17.0 (SPSS Inc., Chicago, IL, USA). The p-value <0.05 was considered significant.

Results

There was no significant different for age and body weight among the three groups prior to intervention ($p>0.05$). The rats induced by LPS showed slight inflammation

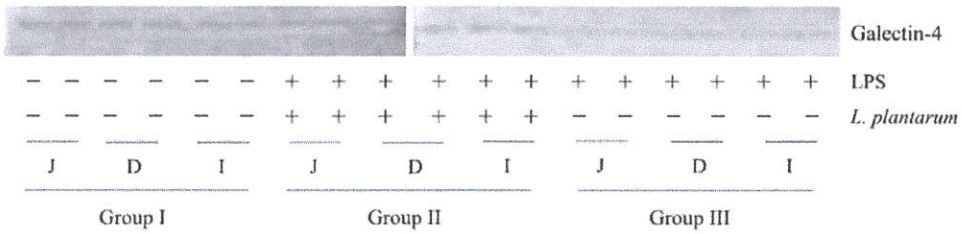


Figure 1. *L. plantarum* restored amount of Galectin-4 protein in duodenum, jejunum, and ileum of LPS-induced rats. Duodenum, jejunum, and ileum lysates of LPS-induced and/or *L. plantarum*-treated rats were subjected to western blot analysis to detect Galectin-4. J: jejunum, D: duodenum, I: ileum.

for 1-2 days. No adverse event was observed for administration of *L. plantarum.*

***L. plantarum* Restored Amount of Galectin-4 Protein in Duodenum, Jejunum, and Ileum of LPS-treated Rats**

Western blot results showed that Galection-4 was observed in all duodenum, jejunum, and ileum of Group I (Figure 1). However, Galection-4 protein was not clearly detected in Group II. LPS diminished Galection-4 protein in all duodenum, jejunum, and ileum. With the treatment of *L. plantarum*, Galection-4 protein was then clearly redetected as shown in Group III.

***L. plantarum* Restored Number of Galectin-4$^+$ Cells in Duodenum, Jejunum, and Ileum of LPS-treated Rats**

The highest number of Galectin-4$^+$ cells was found in ileum, followed by jejunum, then duodenum (Figure 2). Numbers of Galectin-4$^+$ cells were decreased significantly in the LPS-induced jejunum (p=0.007), duodenum (p=0.005) and ileum (p=0.004) (Table 1). Under the treatment of *L. plantarum*, numbers of Galectin-4$^+$ cells were then significantly restored.

L. plantarum Restored Amount of Myosin-1a Protein in Duodenum, Jejunum, and Ileum of LPS-treated Rats

Myosin-1a was observed in all duodenum, jejunum, and ileum of Group I (Figure 3). However, Myosin-1a protein was not detected in Group II. LPS totally abolished Myosin-1a protein in all duodenum, jejunum, and ileum. With the treatment of *L. plantarum*, Myosin-1a protein was then clearly redetected as shown in Group III.

***L. plantarum* Restored Number of Myosin-1a$^+$ Cells in Duodenum, Jejunum, and Ileum of LPS-treated Rats**

The highest number of Myosin-1a$^+$ cells was found in duodenum (Figure 4). Numbers of Myosin-1a$^+$ cells were decreased significantly in the LPS-induced jejunum (p=0.007), duodenum (p=0.001) and ileum (p=0.008) (Table 1). Under the treatment of L. plantarum, numbers of Myosin-1a$^+$ cells were then significantly restored.

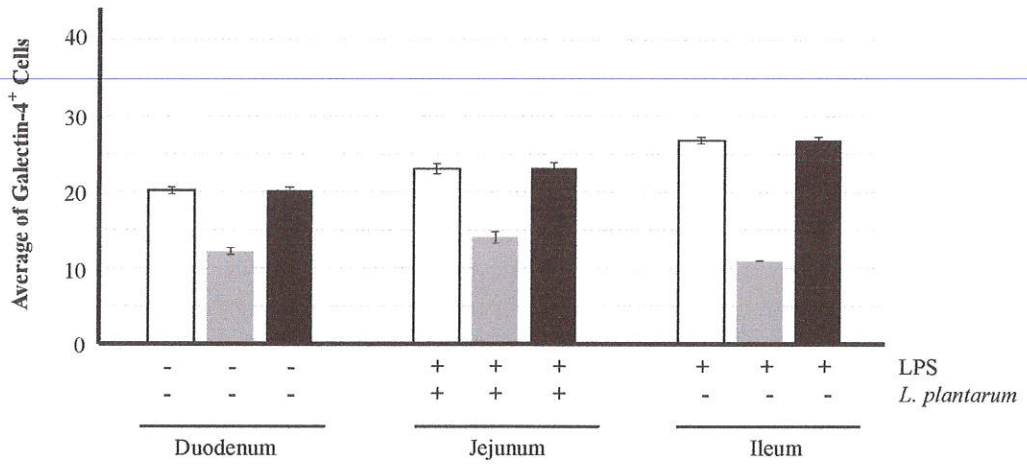


Figure 2. *L. plantarum* restored number of Galectin-4$^+$ cells in duodenum, jejunum, and ileum of LPS-induced rats. Galectin-4-immunohistochemically-overexpressed cells in duodenum, jejunum, and ileum of LPS-induced and/or *L. plantarum*-treated rats were examined and counted.

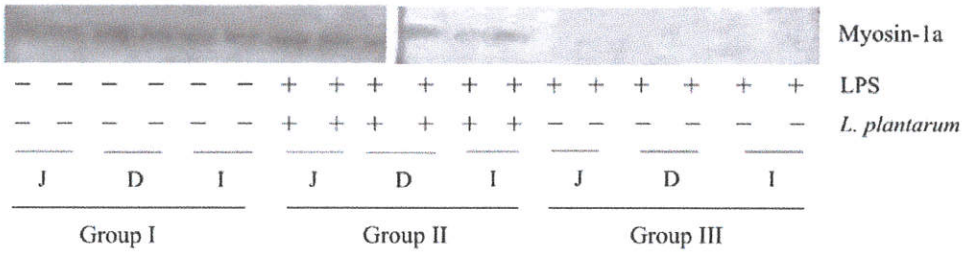


Figure 3. *L. plantarum* restored amount of Myosin-1a protein in duodenum, jejunum, and ileum of LPS-treated rats. Duodenum, jejunum, and ileum lysates of LPS-induced and/or *L. plantarum*-treated rats were subjected to western blot analysis to detect Myosin-1a. J: jejunum, D: duodenum, I: ileum.

Discussion

Galectin-4 is a protein that maintain integrity of intestinal membrane through stabilization of lipid raft and retain functionality of intestinal immune-regulation through selective T-cell apoptosis and cell cycle control.(1,8) Meanwhile, Myosin-1a is an important protein with its multi-functionality, and mainly maintain structure of villi core. (2,9,10) In human, Myosin-1a is found mainly in intestine and smooth muscle, with considerable amount found in duodenum.(11) Healing mechanism of mucosal barrier will involve the process of regaining impermeability of tight junction with Galectin-4 and Myosin-1a as biomarkers of tight junction repair.

In this study, inflammation was induced by administrating LPS. *L. plantarum* IS-20506 probiotic significantly increased both Galectin-4 and Myosin-1a expressions in duodenum, jejunum, and ileum. It has been reported that probiotics may induce brush border repair through Mitogen-activated Protein Kinase (MAPK)/ Extracellular Signal-regulated Kinase (ERK) activation. MAPK/ERK has been well known to play important role in cell proliferation.(12) Peptidoglycan and teichoic acid exposure provided by probiotic may increase cells proliferation and promote the repair.(13)

Based on our findings, Galectin-4 is expressed more in ileum compared to duodenum and jejunum. *L. plantarum* IS-20506 probiotic relatively increased activity in more distal parts of LPS-induced intestine, as demonstrated by total Galectin-4 difference in each segment. This is in in line with the study of probiotic survivability using *L. plantarum* ST16Pa.(14) The study showed that, more activity of *L. plantarum* ST16Pa in the more distal part of intestine.(14) Hence, Galectin-4 may precede partial recovery rather than full recovery after administration of *L. plantarum* IS-20506. Myosin-1a exhibited contrasting behavior compared to Galectin-4. Although LPS-suppressed Myosin-1a was significantly restored by *L. plantarum* IS-20506 probiotic, the Myosin-1a expression was lower in distal part of

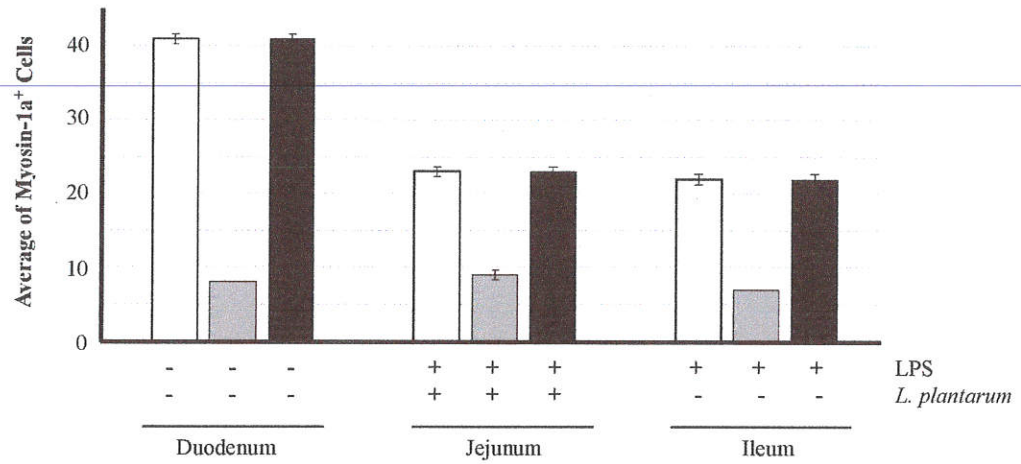


Figure 4. *L. plantarum* restored number of Myosin-1a$^+$ cells in duodenum, jejunum, and ileum of LPS-treated rats. Myosin-1a-immunohistochemically-overexpressed cells in duodenum, jejunum, and ileum of LPS-induced and/or *L. plantarum*-treated rats were examined and counted.

Table 1. Statistical Differences of Galectin-4 and Myosin-1a between groups.

Group	Galectin-4*			Myosin-1a*		
	Duodenum	Jejunum	Ileum	Duodenum	Jejunum	Ileum
I *vs.* II	0.005	0.007	0.004	0.001	0.007	0.008
I *vs.* III	0.510	0.701	0.471	0.531	0.571	0.570
II *vs.* III	0.004	0.007	0.003	0.001	0.005	0.005

*Mann-Whitney test, significant if $p<0.05$. I: first group, treated with distilled water daily; II: second group, treated with LPS on the first day, then treated with distilled water daily; III: third group, treated with LPS on the first day, then treated with *L. plantarum* daily on the following days.

intestine. This pattern is still unknown or whether exclusive to Wistar rat. However, a protein mapping study showed that Myosin-1a is expressed slightly higher in duodenum compared to the rest of intestine.(11)

Administration of *L. plantarum* IS-20506 probiotic after LPS induction in rats tended to increase Myosin-1a expression compared to the LPS-induced group. Our finding is line with the study of *Lactobacillus casei* probiotic in Giardia lamblia-infected Balb/c mice.(15) *L. casei* probiotic administration showed a significant increase of overall mass of intestine compared to control on both pre- and post-infection in brush border damaged due to giardiasis, malnutrition, and the combination of both; a finding that is supposed to be linear to the mass-creating nature of Myosin-1a.

Conclusion

L. plantarum IS-20506 probiotic may facilitate the repairment of damaged intestinal brush border as demonstrated by significant restoration of Galectin-4 and Myosin-1a expressions in duodenum, jejunum, and ileum of LPS-induced rats. Further studies are still needed to investigate the extra-intestinal effects, safety, long-term effect, and overtime time dependent efficacy of *L. plantarum* IS-20506 probiotic.

References

1. Cao ZQ, Guo XL. The role of galectin-4 in physiology and diseases. Protein Cell. 2016; 7: 314-24.
2. Tyska MJ, Mackey AT, Huang JD, Copeland NG, Jenkins NA, Mooseker MS. Myosin-1a is critical for normal brush border structure and composition. Mol Biol Cell. 2005; 16: 2443-57.
3. Tash BR, Bewley MC, Russo M, Keil JM, Griffin KA, Sundstrom JM, *et al.* The occludin and ZO-1 complex, defined by small angle X-ray scattering and NMR, has implications for modulating tight junction permeability. Proc Natl Acad Sci USA. 2012; 109: 10855-60.
4. Oshima T, Miwa H. Gastrointestinal mucosal barrier function and diseases. J Gastroenterol. 2016; 51: 768-78.
5. FAO/WHO. Probiotics in Food: Health and Nutritional Properties and Guidelines for Evaluation. Corboda: FAO/WHO; 2006.
6. Akuzawa R, Surono I. Fermented Milks. In: Roginski H, Fuquay J, Fox P, editors. Encyclopedia of Dairy Sciences. London: Elsevier; 2002. p.1045-9.
7. Athiyyah AF, Darma A, Ranuh R, Riawan W, Endaryanto A, Rantam FA, *et al.* Lactobacillus plantarum IS-10506 activates intestinal stem cells in a rodent model. Benef Microbes. 2018; 9: 755-60.
8. Paclik D, Danese S, Berndt U, Wiedenmann B, Dignass A, Sturm A. Galectin-4 controls intestinal inflammation by selective regulation of peripheral and mucosal T cell apoptosis and cell cycle. PLoS One. 2008; 3: e2629. doi: 10.1371/journal.pone.0002629.
9. Benesh AE, Nambiar R, McConnell RE, Mao S, Tabb DL, Tyska MJ. Differential localization and dynamics of class I myosins in the enterocyte microvillus. Mol Biol Cell. 2010; 21: 970-8.
10. McConnell RE, Tyska MJ. Leveraging the membrane - cytoskeleton interface with myosin-1. Trends Cell Biol. 2010; 20: 418-26.
11. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, *et al.* Proteomics. Tissue-based map of the human proteome. Science. 2015; 347: 1260419. doi: 10.1126/science.1260419.
12. Sandra F, Oktaviono YH, Widodo MA, Dirgantara Y, Chouw A, Sargowo D. Endothelial progenitor cells proliferated via MEK-dependent p42 MAPK signaling pathway. Mol Cell Biochem. 2015; 400: 201-6.
13. Sashihara T, Sueki N, Ikegami S. An analysis of the effectiveness of heat-killed lactic acid bacteria in alleviating allergic diseases. J Dairy Sci. 2006; 89: 2846-55.
14. Todorov SD, Leblanc JG, Franco BD. Evaluation of the probiotic potential and effect of encapsulation on survival for Lactobacillus plantarum ST16Pa isolated from papaya. World J Microbiol Biotechnol. 2012; 28: 973-84.
15. Shukla G, Sidhu RK. Lactobacillus casei as a probiotic in malnourished Giardia lamblia-infected mice: a biochemical and histopathological study. Can J Microbiol. 2011; 57:127-35.

Source details

Indonesian Biomedical Journal

Open Access

Scopus coverage years: from 2017 to Present

Publisher: Prodia Education and Research Institute

ISSN: 2085-3297 E-ISSN: 2355-9179

Subject area: Medicine: Medicine (miscellaneous)

Biochemistry, Genetics and Molecular Biology: General Biochemistry, Genetics and Molecular Biology

CiteScore 2019
0.3
Add CiteScore to your site

SJR 2019
0.141

SNIP 2019
0.536

View all documents > Set document alert Save to source list Journal Homepage

CiteScore CiteScore rank & trend Scopus content coverage

Improved CiteScore methodology

CiteScore 2019 counts the citations received in 2016-2019 to articles, reviews, conference papers, book chapters and data papers published in 2016-2019, and divides this by the number of publications published in 2016-2019. Learn more >

CiteScore 2019

$$0.3 = \frac{33 \text{ Citations 2016 - 2019}}{105 \text{ Documents 2016 - 2019}}$$

Calculated on 06 May, 2020

CiteScoreTracker 2020

$$0.5 = \frac{73 \text{ Citations to date}}{135 \text{ Documents to date}}$$

Last updated on 07 December, 2020 • Updated monthly

CiteScore rank 2019

Category	Rank	Percentile
Medicine — Medicine (miscellaneous)	#195/219	11th
Biochemistry, Genetics and Molecular Biology — General Biochemistry, Genetics and	#179/197	9th

View CiteScore methodology > CiteScore FAQ >

also developed by scimago: SCIMAGO INSTITUTIONS RANKINGS

SJR Scimago Journal & Country Rank Enter Journal Title, ISSN or Publisher Name

Home Journal Rankings Country Rankings Viz Tools Help About Us

Indonesian Biomedical Journal

Country Indonesia - SCIMAGO INSTITUTIONS RANKINGS

Subject Area and Category Biochemistry, Genetics and Molecular Biology
Biochemistry, Genetics and Molecular Biology (miscellaneous)
Medicine
Medicine (miscellaneous)

Publisher Prodia Education and Research Institute

Publication type Journals

ISSN 23559179, 20853297

Coverage 2017-2019

3
H Index

Scope The Indonesian Biomedical Journal is an open access, peer-reviewed journal that encompasses all fundamental and molecular aspects of basic medical sciences, emphasizing on providing the molecular studies of biomedical problems and molecular mechanisms. The Indonesian Biomedical Journal is dedicated to publish original research and review articles covering all aspects in biomedical sciences. The editors will carefully select manuscript to present only the most recent findings in basic and clinical sciences. All professionals concerned with biomedical issues will find this journal a most valuable update to keep them abreast of the latest scientific development.

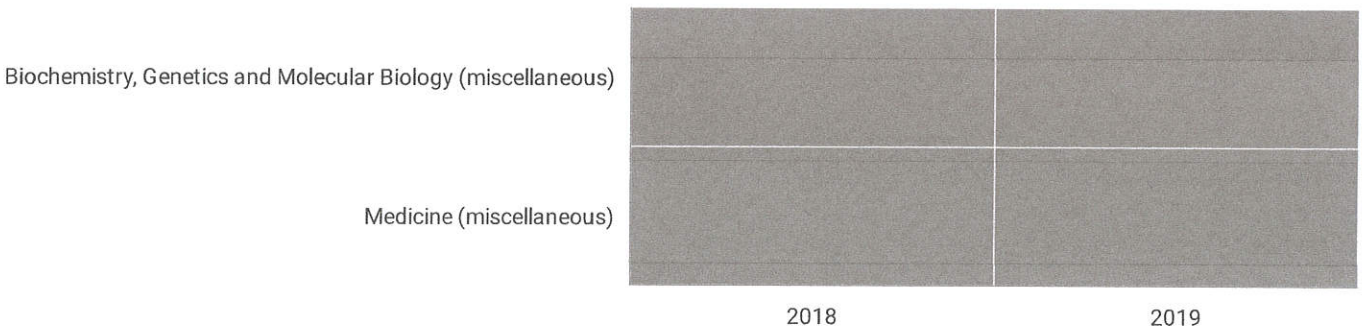
Homepage

How to publish in this journal

Contact

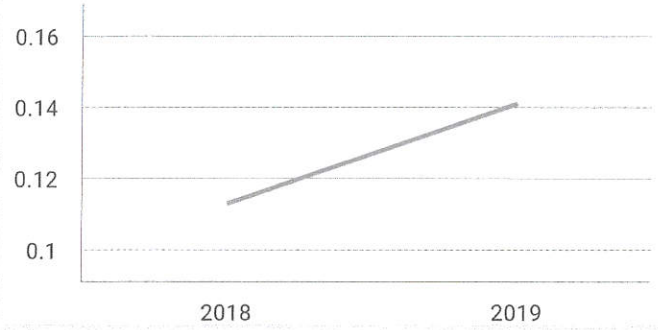
Join the conversation about this journal

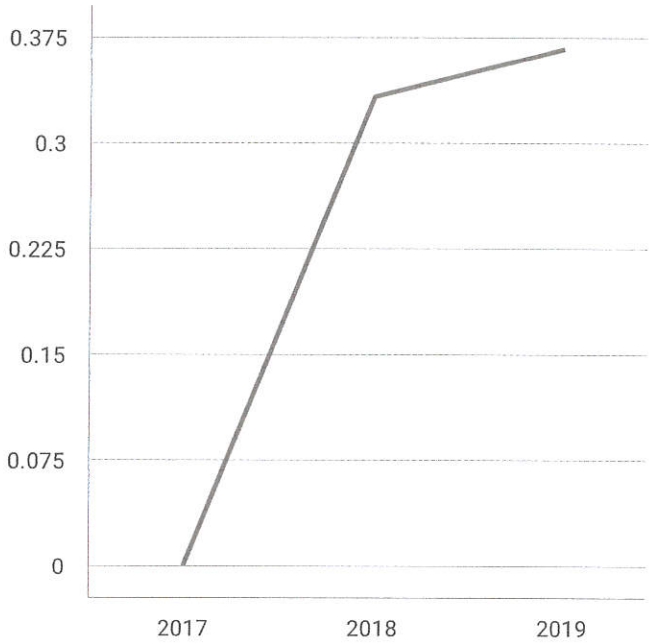
Quartiles



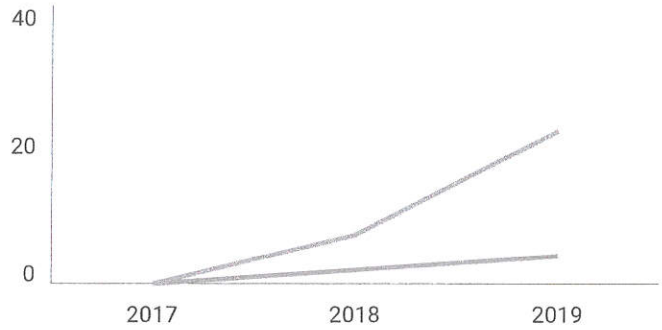
SJR

Citations per document

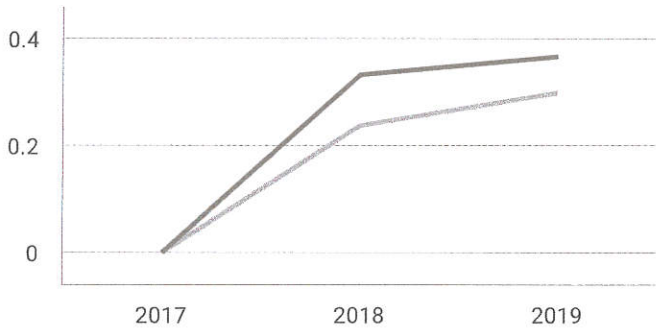




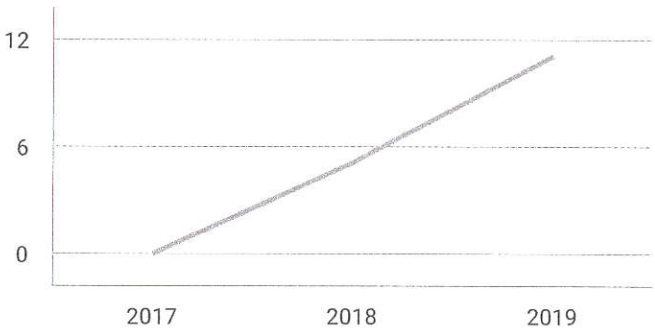
Total Cites Self-Cites



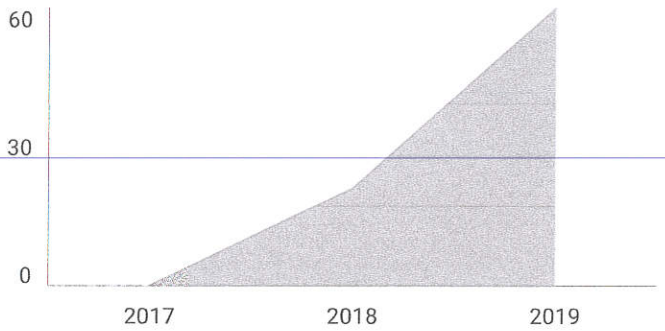
External Cites per Doc Cites per Doc



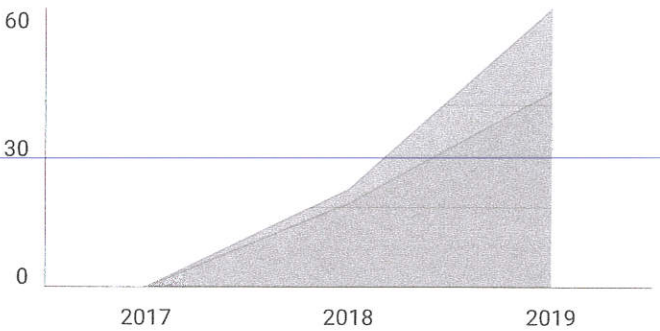
% International Collaboration



Citable documents Non-citable documents



Cited documents Uncited documents



← Show this widget in your own website

Just copy the code below and paste within your html code:

```
<a href="https://www.scimag
```