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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 Ranuh¹, Alpha Fardah Athiyah¹, Andy Darma¹, Wibi Riawan²,
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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-20506 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-20506 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-20506 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

(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 *Escherichia 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 2.67×10^9 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 μ m, 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

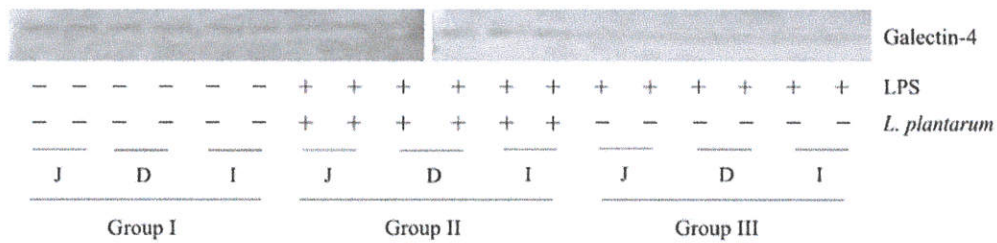


Figure 1. *L. plantarum* restored amount of Galectin-4 protein in duodenum, jejunum, and ileum of LPS-induced rats. Duodenum, jejunum, and ileum lysates of LPS-induced and/or *L. plantarum*-treated rats were subjected to western blot analysis to detect Galectin-4. J: jejunum, D: duodenum, I: ileum.

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 Galectin-4 was observed in all duodenum, jejunum, and ileum of Group I (Figure 1). However, Galectin-4 protein was not clearly detected in Group II. LPS diminished Galectin-4 protein in all duodenum, jejunum, and ileum. With the treatment of *L. plantarum*, Galectin-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.

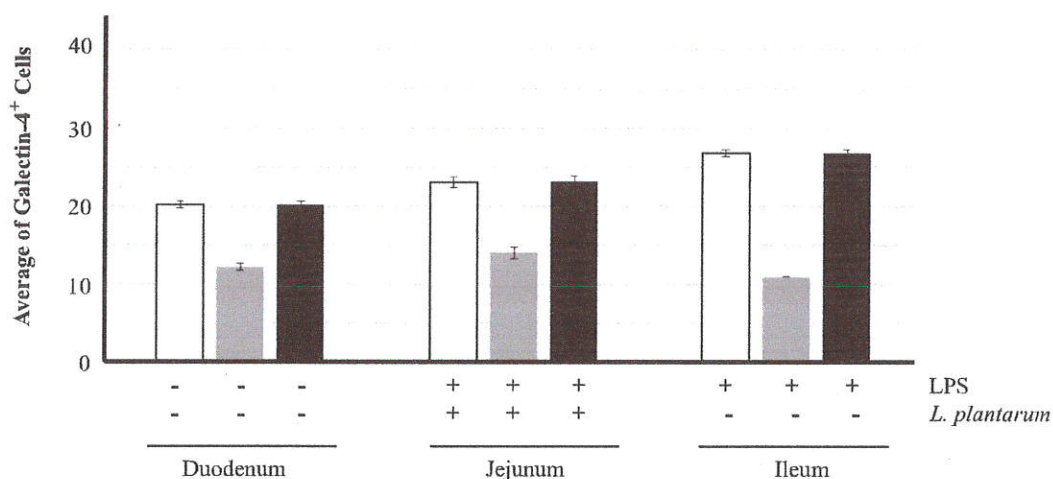


Figure 2. *L. plantarum* restored number of Galectin-4⁺ cells in duodenum, jejunum, and ileum of LPS-induced rats. Galectin-4-immunohistochemically-overexpressed cells in duodenum, jejunum, and ileum of LPS-induced and/or *L. plantarum*-treated rats were examined and counted.

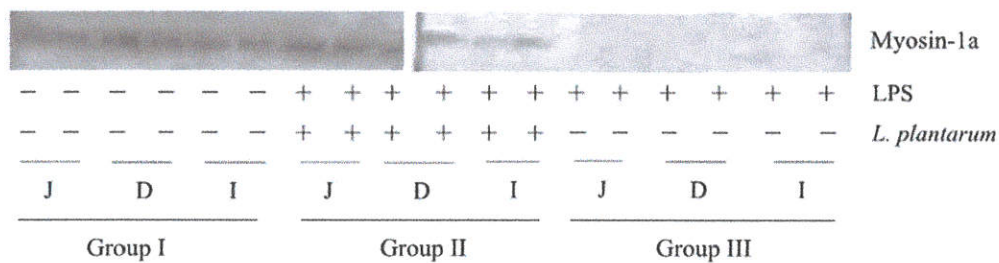


Figure 3. *L. plantarum* restored amount of Myosin-1a protein in duodenum, jejunum, and ileum of LPS-treated rats. Duodenum, jejunum, and ileum lysates of LPS-induced and/or *L. plantarum*-treated rats were subjected to western blot analysis to detect Myosin-1a. J: jejunum, D: duodenum, I: ileum.

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 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

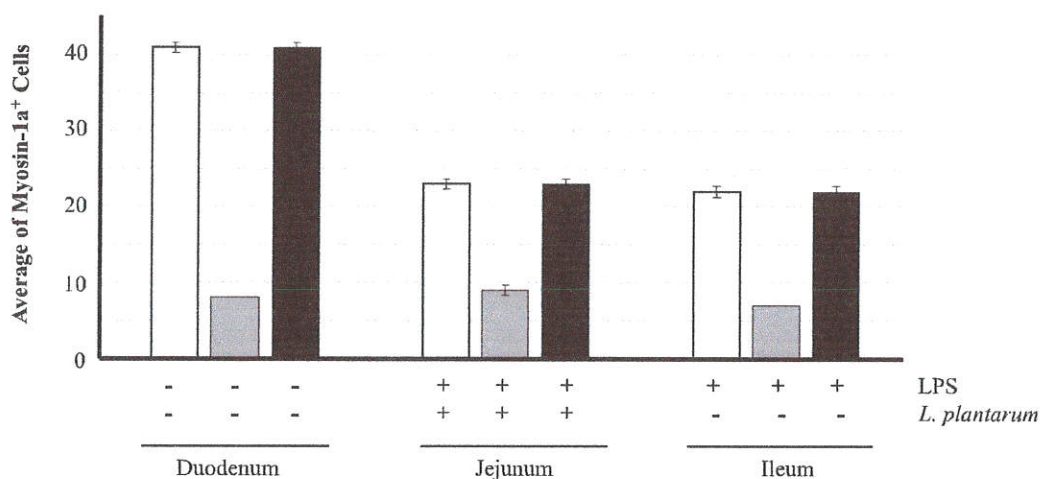


Figure 4. *L. plantarum* restored number of Myosin-1a⁺ cells in duodenum, jejunum, and ileum of LPS-treated rats. Myosin-1a-immunohistochemically-overexpressed cells in duodenum, jejunum, and ileum of LPS-induced and/or *L. plantarum*-treated rats were examined and counted.

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.

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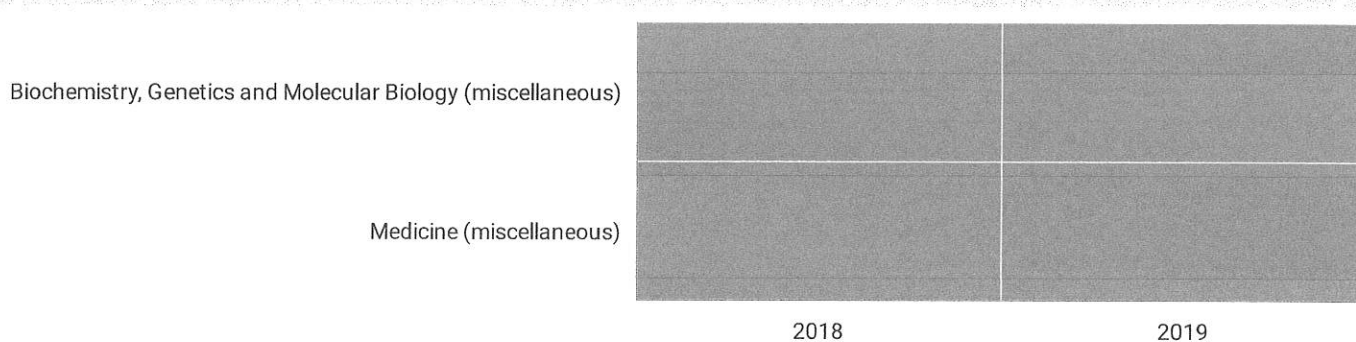
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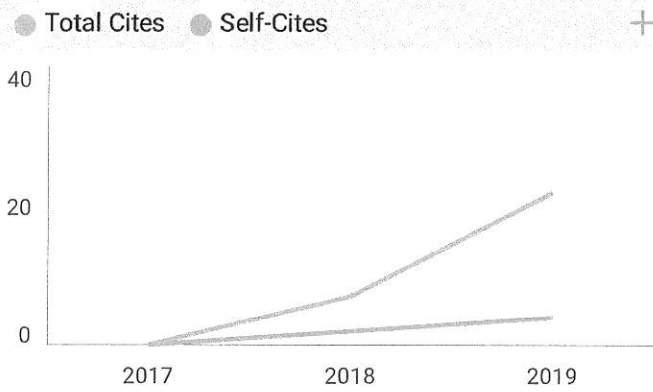
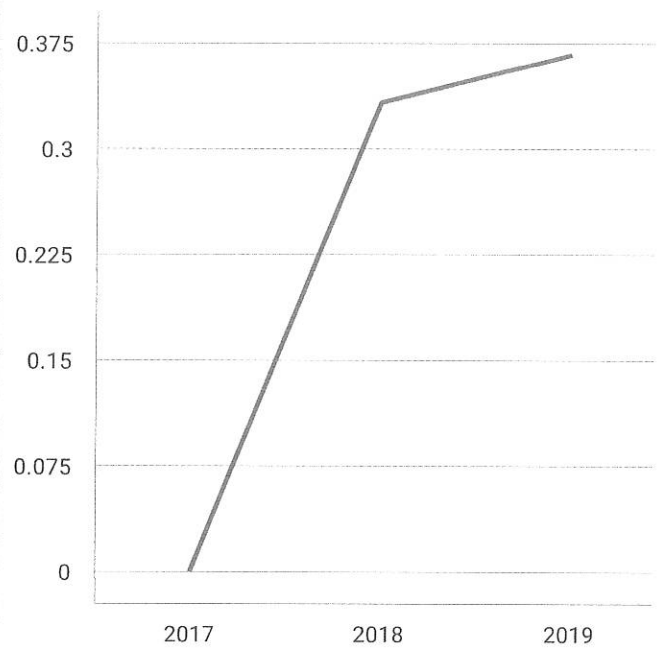
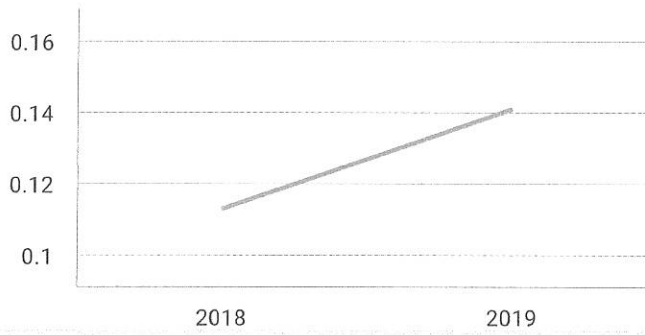
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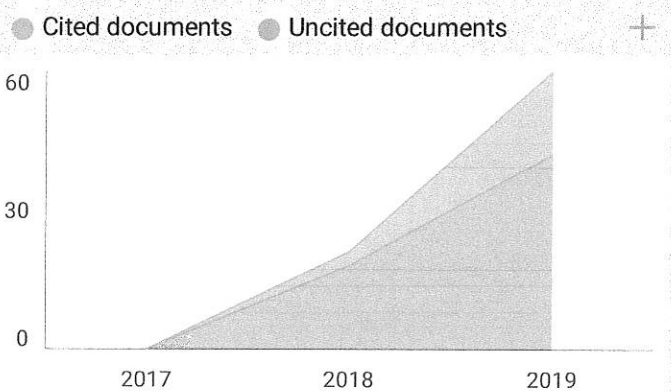
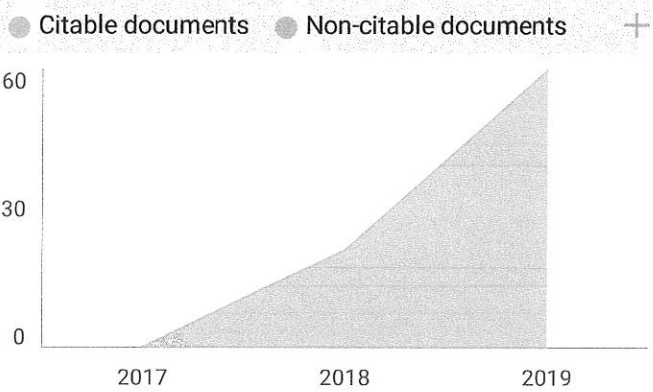
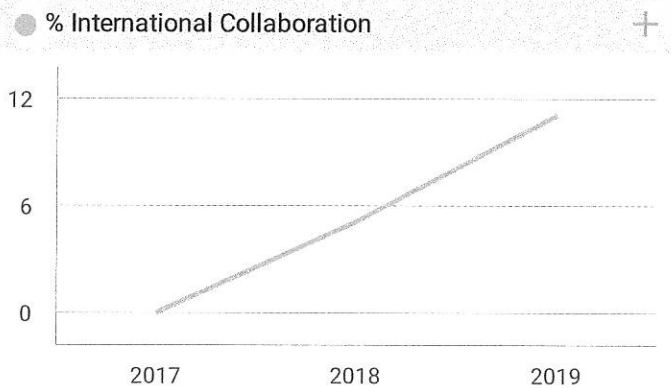
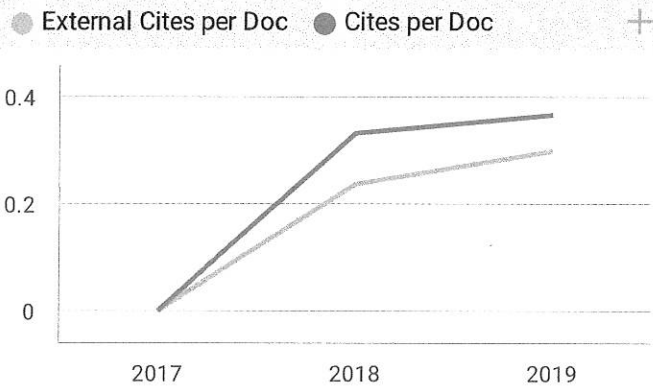


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