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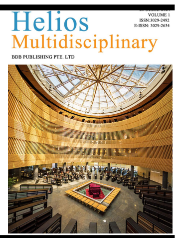

Effect of Probiotic Lactobacillus Casei on Secretory Immunoglobulin A (S-IgA) Levels in Rats Norvegicus Strain Wistar Post Ovariectomy

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ABSTRACT

Estrogen promotes the growth of Lactobacillus colonies in the vagina, converting glycogen in superficial cells into lactic acid, resulting in a low vaginal pH that inhibits pathogenic bacteria. Post-menopause, Lactobacillus growth declines, leading to thinner secretions, higher vaginal pH, and a weakened immune system. This study investigates the effect of Lactobacillus casei extract on secretory immunoglobulin A (IgA) levels, vaginal acidity, and beneficial bacteria in the vaginal mucosa. An experimental post-test-only control group design was employed with 24 female rats aged 8-10 weeks, weighing 180-220 grams. Following ovariectomy and a 14-day acclimation, the rats were divided into four groups: a control group (K) with no treatment and three treatment groups (P1, P2, P3) receiving Lactobacillus casei extract at doses of 2ml, 2.25ml, and 2.5ml, respectively, administered orally for 14 days. On day 15, vaginal acidity was measured using a swab method, and IgA levels were assessed via ELISA; gram-positive staining was performed for bacterial identification. One-way ANOVA revealed that Lactobacillus casei extract significantly increased IgA levels and reduced vaginal acidity ($p = 0.003$ and $p = 0.000$). The reduction in acidity is attributed to Lactobacillus converting glycogen into lactic acid, maintaining vaginal acidity, while S-IgA on the mucosal surface acts as an opsonin and antigen-presenting cell to inhibit pathogenic growth. The study concludes that Lactobacillus casei extract can elevate secretory IgA levels and normalize vaginal pH.

1. Introduction

Menopause is a condition of a woman who has not menstruated for 12 months, blood FSH levels > 40 IU / ml and estradiol levels < 30 pg / ml (Sarwono P, 2003). Menopause is also called a transition period from a productive period slowly to a non-productive period caused by a decrease in estrogen and progesterone hormones due to decreased ovarian function (Dwi L, 2021).[1]

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Some experts in the field of menopause state that menopause occurs between the ages of 45 and 55 years. However, menopause can occur earlier before the age of 45 to 55 years which is called premature menopause. One of the causes of premature menopause is ovarian removal surgery (ovariectomy) (Dwi L, 2021).[2]

In menopausal women, hypo-estrogenic occurs, namely low estrogen <30 pg/ml due to decreased ovarian function. Hypo-estrogenic in menopausal women causes many complaints, both physical and psychological. Physical and psychological complaints experienced by menopausal women include: vaginal dryness, less elastic vagina, increased vaginal acidity (pH), vaginal atrophy, pain during sexual intercourse (drypareunia), decreased libido, frequent urination (urinary incontinence), decreased ovarian function, metabolic disorders, namely increased cholesterol levels, increased LDL and decreased serum HDL, hot flushes, insomnia, anxiety (depression), decreased memory and irritability (Dwi L, 2021; Atika P, 2021).

Lactobacillus casei probiotics that are converted into lactic acid can stimulate the immune system with peptidoglycan and lipopolysaccharide compounds in the cell wall (Suroso, 2014). Based on research, IgA production will increase if you get lactic acid bacteria. Lactic acid bacteria of the Lactobacillus group can provide health effects such as increasing local immune responses and increasing IgA production. IgA production is then excreted into the intestinal lumen to prevent the attachment of pathogenic microorganisms (Isolauri, 2021).

2. Research Objectives

General Objectives

To determine the effect of administering probiotic extract of *Lactobacillus casei* strain shirota on increasing S-IgA in post-ovariectomy rats.

Specific Objectives

1 To compare S-IgA levels in normal rats and various doses of probiotic extract *Lactobacillus casei* strain shirota.

2 To compare S-IgA levels in post-ovariectomy rats and various doses of probiotic extract *Lactobacillus casei* strain shirota.

3 To determine the most optimal dose of probiotic extract *Lactobacillus casei* strain shirota that can increase S-IgA in post-ovariectomy rats.

3. Methods

Research design

This study was conducted with a true experimental design model, post-test only control group type, where the model animals will be divided into several treatment groups. There are 4 treatments for model animals, namely standard diet (K), standard diet + *Lactobacillus casei* strain shirota Probiotic Extract, with various doses (P1, P2, P3). This study aims to determine the effect of the dose of *Lactobacillus casei* strain Shirota Extract as a probiotic on changes in S-IgA in the vaginal mucosa of Post ovariectomy rats.

Study sample

Samples were taken randomly from an accessible population, namely Rattus norvegicus strain Wistar post ovariectomy aged 8-10 weeks (according to the age of the experiment), with the condition that it meets the inclusion and exclusion criteria.

Inclusion and exclusion criteria

Inclusion criteria including body weight: 180-220 grams, healthy, during 3 days of pre-treatment there was no weight loss so that the total weight was not below 180 grams, Post Ovariectomy Rats. Exclusion criteria include rats died due to treatment, behavior changes (not wanting to eat, weak).

Study variables

Independent variable: administration of *Lactobacillus casei* strain Shirota Probiotic Extract to the treatment group, Treatment group I: 100 ml x 0.018 = 2, thus treatment group I, dose: 2 ml/head/day, Treatment group II: (100+150): 2 ml x 0.018 = 2.25 thus treatment group II, dose 2.25 ml/head/day, Treatment group III: 150 ml x 0.018 = 2.7 = 2.5, thus treatment group III, dose 2.5 ml/head/day.

Dependent variable: pH and S-IgA in the vaginal mucosa of ovariectomized female Rattus norvegicus strain Wistar.

Controlled variables: gender, body weight, healthy condition, age, feed, cage conditions (individual cages).

Data analysis

In the comparative test of the mean of the measured variables between the control group and the other three treatment groups, the Anova One Way test will be used in the data analysis technique. This test is used to determine which sample group is significantly different from the other sample groups due to different treatments. In this study, the Anova One Way test was used to compare the mean of the response variables between the control group (mice), group P1 (mice + ELcsS dose 1), group P2 (mice + ELcsS dose 2) and group P3 (mice + E LcsS dose 3). This is to determine the effect of treatment on the response variables and prove the research hypothesis. The study is considered significant with a 95% confidence level if $p \leq 0.005$, if there is a difference in the group is significant. The regression correlation test was conducted to determine the effect of *Lactobacillus casei* Strain Extract at various doses on S-IgA levels. All test results will be computerized with the SPSS 24 program.

4. Results

The process of testing the effect of giving *Lactobacillus casei* strain Shirota extract on immunoglobulin A levels was carried out using one way ANOVA. As explained in the research method, the treatments given included giving *Lactobacillus casei* strain Shirota extract 2 ml/head/day (P1), *Lactobacillus casei* strain Shirota extract 2.25 ml/head/day (P2), and *Lactobacillus casei* strain Shirota extract 2.5 ml/head/day (P3) and control (K). Descriptively, the average immunoglobulin A levels in each treatment are explained in the Figure 1.

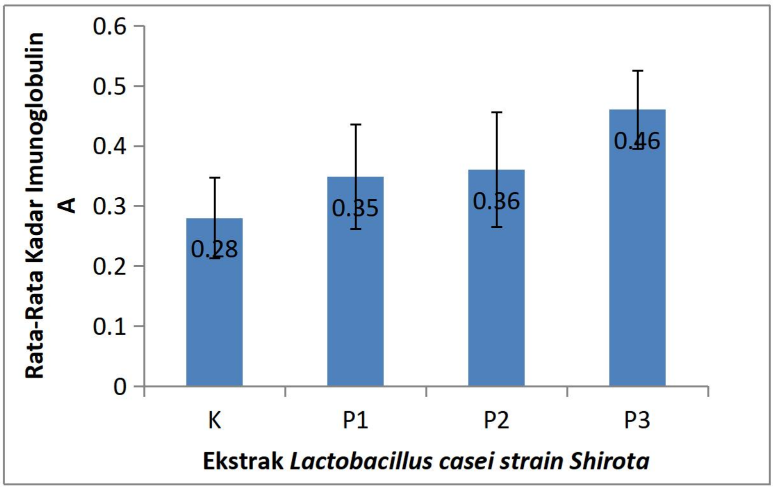

Figure 1. Average levels of immunoglobulin A for each treatment

Based on Figure 1 above, the control group had the lowest average immunoglobulin A levels. Meanwhile, in mice given *Lactobacillus casei* strain Shirota extract with several dose levels, it was shown that the average immunoglobulin A levels were higher than the control group. This indicates an increase in immunoglobulin A levels. To test whether there was a significant increase in immunoglobulin A levels or not, testing was carried out using ANOVA. The following are the results of the ANOVA test for immunoglobulin A levels (Table 1).**Table 1.** ANOVA test for immunoglobulin A levels

Intervention	Mean ± SD	p-value
K	0.28 ± 0.067	0.006
P1	0.348 ± 0.087	
P2	0.36 ± 0.095	
P3	0.46 ± 0.065	

Based on Table 1 above, the source of treatment diversity has a p-value of 0.006 and is smaller than α = 0.05. So from this test it can be concluded that there is a significant effect of giving various doses of *Lactobacillus casei* strain Shirota extract on increasing immunoglobulin A levels. Or in other words, there is a significant difference in immunoglobulin A levels at each treatment level. To further determine the difference in the average immunoglobulin A levels at each treatment level, a further post hoc test was carried out using the LSD test. The following are the results of the 5% LSD test (Table 2).

Table 2. The results of the 5% LSD test

Comparative		Mean	p-value
K	P1	-0.068	0.154
	P2	-0.080	0.082

	P3	-0.180	0.001
P1	P2	-0.012	0.805
	P3	-0.112	0.029
P2	P3	-0.100	0.040

From the results of the post hoc test using 5% LSD, in the comparison of the control group (K) with the administration of *Lactobacillus casei* strain Shirota extract at a dose of 2 ml/head/day (P1) and 2.25 ml/head/day (P2), a p-value of > 0.05 was obtained. This shows that the administration of *Lactobacillus casei* strain Shirota extract at a dose of 2 ml/head/day (P1) and 2.25 ml/head/day (P2) has not been able to significantly increase the levels of immunoglobulin A in mice. While the comparison of the control group (K) with the administration of *Lactobacillus casei* strain Shirota extract at a dose of 2.5 ml/head/day (P3), a p-value of < 0.05 was obtained. This shows that the administration of *Lactobacillus casei* strain Shirota extract at a dose of 2.5 ml/head/day was able to significantly increase the levels of immunoglobulin A in white mice. Comparison between administration of *Lactobacillus casei* strain Shirota extract at a dose of 2 ml/head/day (P1) with administration of *Lactobacillus casei* strain Shirota extract at a dose of 2.25 ml/head/day (P2) showed a p-value > 0.05. This means that there is no significant difference in the average levels of immunoglobulin A. Comparison between administration of *Lactobacillus casei* strain Shirota extract at a dose of 2 ml/head/day (P1) with administration of *Lactobacillus casei* strain Shirota extract at a dose of 2.5 ml/head/day (P3) showed a p-value < 0.05. Comparison between administration of *Lactobacillus casei* strain Shirota extract at a dose of 2.25 ml/head/day (P2) with administration of *Lactobacillus casei* strain Shirota extract at a dose of 2.5 ml/head/day (P3) also showed a p-value < 0.05. This shows that there is a significant difference in the average immunoglobulin A levels between P1 and P3 and P2 and P3.

From this test, it was shown that the addition of a dose of *Lactobacillus casei* strain Shirota extract of 2.5 ml/head/day was able to increase immunoglobulin A levels significantly. The highest immunoglobulin A levels were produced by administering *Lactobacillus casei* strain Shirota extract of 2.5 ml/head/day. This shows that administering *Lactobacillus casei* strain Shirota extract of 2.5 ml/head/day was able to increase immunoglobulin A levels optimally.

Lactobacillus can stimulate the immune system and increase vaginal acidity levels (pH). This is in line with the results of the author's research which found that the increase in IgA in the treatment groups was significantly different, in the administration of *Lactobacillus casei* extract dose I (2 ml/mouse) there was an increase in IgA levels compared to the control group (K), as well as dose II (2.25 ml/mouse) had an increase in IgA levels that were almost the same as group I compared to the control group (K), treatment dose I and treatment dose III (2.5 ml/mouse). Treatment dose III has the most optimal effect for increasing immunity, this is indicated by the increase in IgA levels. This phenomenon is a non-linear fact due to the increase in dose from dose II to dose III.

At the time of immunoglobulin A (S-IgA) sampling, by scraping the vaginal mucosa of mice which was too small and little, so that in the P2 treatment the 5th sample results could not be read, because the mucosal sample was too small. This study is likely the first study to be conducted so that there is the most appropriate method in taking secretory immunoglobulin A samples from the vaginal mucosa of rats.

5. Discussion

Lactobacillus casei probiotics that are converted into lactic acid are able to stimulate the immune system with peptidoglycan and lipopolysaccharide compounds in the cell wall (Suroso, 2014). Based on research, IgA production will increase if you get lactic acid bacteria. Lactic acid bacteria of the *Lactobacillus* group can provide health effects such as increasing local immune responses and increasing IgA production. IgA production is then excreted into the intestinal lumen to prevent the attachment of pathogenic microorganisms (Isolauri, 2021).

Based on research conducted by Octaviyanti in 2009, it was stated that the level of vaginal acidity (pH) in women in the 41-45 age group was pH ≤ 5 (47.7%) and pH> 5 (53.3%) and *Lactobacillus* colonies in the ≥ 46 age group were low, namely 3.4%.

Several literatures prove that the Probiotic *Lactobacillus casei* given orally will enter the digestive tract and colonize in the rectum then cross to the vaginal epithelial cells which will affect the vaginal microflora (Strus M, 2021).

The results of research by Gorodeski G, 2005, stated that the luminal pH in the vagina is produced by the cultivation of doderlein lactobacilli, which states that this bacillus produces hydrogen peroxide and secretes protons into the surrounding environment, which can acidify the pH of the vaginal lumen.

6. Conclusion

Probiotic Extract *Lactobacillus casei* strain shirota can reduce vaginal acidity levels with an increase of 1ml/head/day of -0.302 with an R-Square value of 43.3%. Probiotic Extract *Lactobacillus casei* strain shirota can increase secretory immunoglobulin A (S-IgA) levels with an increase of 1ml/head/day of 0.054, with an R-Square value of 33.4%.

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References

Chee, W. J. Y., Chew, S. Y., & Than, L. T. L. (2020). Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. *Microbial Cell Factories, 19*(1), 1–24. https://doi.org/10.1186/s12934-020-01464-4

Franić, D., & Fistonić, I. (2019). Laser therapy in the treatment of female urinary incontinence and genitourinary syndrome of menopause: An update. *BioMed Research International, 2019*, Article 1576359. https://doi.org/10.1155/2019/1576359

Giri, S. S., Sukumaran, V., Sen, S. S., & Park, S. C. (2018). Use of a potential probiotic, Lactobacillus casei L4, in the preparation of fermented coconut water beverage. *Frontiers in Microbiology, 9*(AUG), Article 1976. https://doi.org/10.3389/fmicb.2018.01976

Harbige, L. S., Pinto, E., Allgrove, J., & Thomas, L. V. (2016). Immune response of healthy adults to the ingested probiotic Lactobacillus casei Shirota. *Scandinavian Journal of Immunology, 84*(6), 353–364. https://doi.org/10.1111/sji.12495

Hill, D., Sugrue, I., Tobin, C., Hill, C., Stanton, C., & Ross, R. P. (2018). The Lactobacillus casei group: History and health-related applications. *Frontiers in Microbiology, 9*(SEP), Article 2107. https://doi.org/10.3389/fmicb.2018.02107

Huang, C. H., Li, S. W., Huang, L., & Watanabe, K. (2018). Identification and classification for the Lactobacillus casei group. *Frontiers in Microbiology, 9*(AUG), Article 1974. https://doi.org/10.3389/fmicb.2018.01974

Kato-Kataoka, A., Nishida, K., Takada, M., Kawai, M., Kikuchi-Hayakawa, H., Suda, K., Ishikawa, H., Gondo, Y., Shimizu, K., Matsuki, T., Kushiro, A., Hoshi, R., Watanabe, O., Igarashi, T., Miyazaki, K., Kuwano, Y., & Rokutan, K. (2016). Fermented milk containing Lactobacillus casei strain Shirota preserves the diversity of the gut microbiota and relieves abdominal dysfunction in healthy medical students exposed to academic stress. *Applied and Environmental Microbiology, 82*(12), 3649–3658. https://doi.org/10.1128/AEM.04134-15

Megawanti, P. (2014). Wen Shixin 1 Wei Dongxue 2 Liu Bo2 Zhao Jin 1 *. *2*(150), 59–61.

Nowroozi, J., Mirzaii, M., & Norouzi, M. (2004). Study of Lactobacillus as probiotic bacteria. *Iranian Journal of Public Health, 33*(2), 1–7.

Paul, A. K., Paul, A., Jahan, R., Jannat, K., Bondhon, T. A., Hasan, A., Nissapatorn, V., Pereira, M. L., Wilairatana, P., & Rahmatullah, M. (2021). Probiotics and amelioration of rheumatoid arthritis: Significant roles of Lactobacillus casei and Lactobacillus acidophilus. *Microorganisms, 9*(5), Article 1070. https://doi.org/10.3390/microorganisms9051070

Qian, Z., Zhao, D., Yin, Y., Zhu, H., & Chen, D. (2020). Antibacterial activity of Lactobacillus strains isolated from Mongolian yogurt against Gardnerella vaginalis. *BioMed Research International, 2020*, Article 3548618. https://doi.org/10.1155/2020/3548618

Stanaway, J. D., Afshin, A., Gakidou, E., Lim, S. S., Abate, D., Abate, K. H., Abbafati, C., Abbasi, N., Abbastabar, H., Abd-Allah, F., Abdela, J., Abdelalim, A., Abdollahpour, I., Abdulkader, R. S., Abebe, M., Abebe, Z., Abera, S. F., Abil, O. Z., Abraha, H. N., … Murray, C. J. L. (2018). Global, regional, and national comparative risk assessment of 84 behavioural, environmental and occupational, and metabolic risks or clusters of risks for 195 countries and territories, 1990–2017: A systematic analysis for the Global Burden of Disease Study 2017. *The Lancet, 392*(10159), 1923–1994. https://doi.org/10.1016/S0140-6736(18)32225-6

Talib, N., Mohamad, N. E., Yeap, S. K., Hussin, Y., Mubin Aziz, M. N., Masarudin, M. J., Sharifuddin, S. A., Hui, Y. W., Ho, C. L., & Alitheen, N. B. (2019). Isolation and characterization of Lactobacillus spp. from kefir samples in Malaysia. *Molecules, 24*(14), Article 2606. https://doi.org/10.3390/molecules24142606

Yan, R., Wang, K., Wang, Q., Jiang, H., Lu, Y., Chen, X., Zhang, H., Su, X., Du, Y., Chen, L., Li, L., & Lv, L. (2022). Probiotic Lactobacillus casei Shirota prevents acute liver injury by reshaping the gut microbiota to alleviate excessive inflammation and metabolic disorders. *Microbial Biotechnology, 15*(1), 247–261. https://doi.org/10.1111/1751-7915.13750