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Effect of Dangke on Changes in Neutrophil to Lymphocyte Ratio in Obese White Rats

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ABSTRACT

Introduction: Obesity is associated with inflammation. Previous research has found that obesity determined by Body Mass Index (BMI) and Waist Circumference (LP) is associated with inflammation. Dangke contains *Indigenos Lactic Acid Bacteria*. The presence of lactic acid bacteria in fermented products has very positive benefits for health. The function of papaya sap which is used in making dangke, can act as an antibacterial and anti-inflammatory ingredient.

Methods: Pure experimental research with a design, namely Pre-Post Randomized Clinical Trial Design. Data analysis used the independent T-test/Mann-Whitney test with a value <0.05 .

Results: The difference in the mean levels of Neutrophil to Lymphocyte (NLR) in the Intervention and control groups obtained a p-value of 0.94 which means that there is no relationship between control and intervention on NLR levels. The average change in NLR levels in the control group p-value of 0.61 which means there is no significant change in NLR levels in test 1 and test 2. The average change in NLR levels in the intervention group before and after the intervention was given a p-value of 0.07, which means there was no significant change in NLR levels in the intervention group.

Conclusion: This study showed that intervention with Dangke did not yield a significant alteration in the NLR levels within the animal model of obesity.

Keywords: Dangke; neutrophil lymphocyte ratio; obesity; inflammation



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Introduction

Obesity is associated with inflammation. Waist circumference (LP) and body mass index (BMI) show that obesity is associated with inflammation. An imbalance between incoming and outgoing energy intake is the main cause of obesity. Too much food intake causes high energy intake, but low metabolism and physical activity in the body cause low energy output. Body fat, skinfold thickness, and other measurements of abdominal fat were also positively correlated with inflammation. Many diseases are caused by obesity. The morbidity and mortality rate in obese sufferers is very high. Obesity involves systemic inflammation due to the accumulation of excess adipose tissue and is generally associated with negative health impacts. The adverse health impacts associated with obesity occur primarily in people with fat distribution in the upper body.⁽¹⁾⁽²⁾

The obesity epidemic in the world with more than 1 billion adults who are overweight, 300 million adults are obese. The prevalence of obesity in the world increased by 8.7% - 13.1%, increasing to 10.5% -15.1% in women, and by 6.6% - 11.1% in men. In addition, prevalence in Asian countries that are members of the World Health Organization increased by 1.9–4.7% from 2000 to 2016, 2.7–6.1% in women, and 1.1–3.3 % in men.^{(3),(4)}

The prevalence of overweight o obesity in the Indonesian adult population increased from 2007 to 2018. The prevalence of obesity was around 8.6% to 11.5% from 2007 to 2013 and around 13.6% in 2018. However, from 2007 to 2018, namely 10.5%, 14.8%, and 21.8% respectively. Based on gender, the prevalence of obesity in adult women (>18 years) was 32.9% in 2013, an increase of 18.1%. The prevalence of obese adult male population in 2013 was 19.7%, higher than in 2007 (13.9%) and 2010 (7.8%)⁽⁴⁾.

There are keys to the human defense system when inflammation occurs, namely neutrophils and lymphocytes. Several studies suggest that increases in neutrophils, lymphocytes, and total leukocytes are associated with metabolic disorders caused by obesity. NLR is the ratio of neutrophils to lymphocytes and has been shown in several studies to be a marker of inflammation associated with metabolic syndrome, insulin resistance, and obesity^{(4),(5)}.

Neutrophil To Lymphocyte (NLR) is measured as the ratio of the absolute number of neutrophils to the number of lymphocytes, which is a new inflammatory biomarker that is used as a prognostic factor for various diseases and NLR as a marker for assessing the severity of inflammation in obesity. The higher the NLR level, the higher the value of neutrophil cells in the body and the more severe the inflammation^{(6),(7)}.

Dangke is a buffalo or cow's milk product that is traditionally produced in South Sulawesi. Its distinctive characteristics are its oval shape, chewy texture, and strong milky aroma and taste. Danke contains indigenous lactic acid bacteria (BAL indigenos). The presence of lactic acid bacteria in fermented products has a very positive impact on health. Danke is made by coagulating milk with papaya sap. The benefits of papaya sap used in danke production have been reported to have anti-inflammatory and antibacterial properties^{(8),(9),(10)}.

Methods

The research method used was pure experimental with a design, namely Pre-Post Randomized Clinical Trial Design. This experiment aims to see the treatment before and after giving dangke to the NLR levels of obese white mice. The rats used were 16 wistar strain rats. How to fatten the rats by feeding fat feed. How to measure NLR by using routine blood tests.

Results

Table 1 Results of Analysis of Differences in Average NLR

| Treatment Category | N | Mean±SD | P value |
|--------------------|---|-----------|---------|
| Control | 8 | 0.28±0.14 | 0.94 |
| Intervention | 8 | 0.27±0.12 | |

Table 1 shows that in the intervention and control groups after being given the intervention, there is a difference in the average NLR in the intervention and control groups after being given the intervention, where the NLR level obtained a p-value of 0.94, which means there is no relationship between the intervention and the NLR level.

Table 2 Results of Analysis of Changes in Average NLR in the Control Group

| Treatment Category | N | Mean±SD | P value |
|--------------------|---|-----------|---------|
| Test 1 | 8 | 0.23±0.16 | 0.61 |
| Test 2 | 8 | 0.28±0.14 | |

Table 2 shows the average change in NLR in the control group. NLR levels obtained a p-value of 0.61, which means there was no significant change in NLR levels in test 1 and test 2.

Table 3 Results of analysis of changes in average NLR in the intervention group before and after being given the intervention

| Treatment Category | N | Mean±SD | P value |
|--------------------|---|-----------|---------|
| Before | 8 | 0.18±0.08 | 0.07 |
| After | 8 | 0.12±0.12 | |

Table 3 shows the average change in NLR in the intervention group before and after being given the intervention, where the NLR level obtained a p-value of 0.07, which means there was no significant change in NLR levels before and after the intervention in the intervention group.

Discussion

NLR has been included as a marker of inflammation, believed to reflect the balance between innate (neutrophils) and adaptive (lymphocytes) immune responses. Previous research shows that an increase in NLR is associated with an increase in the concentration of various pro-inflammatory cytokines which can cause cellular DNA damage. However, despite research studying the relationship between these parameters and various chronic diseases with inflammation there are only a few papers discussing their relationship with obesity and diet quality ⁽¹⁾.

From the research results, it was found that the average NLR level in the intervention group before administration was 0.18 ± 0.08 and after administration was 0.12 ± 0.12 . Meanwhile, in the control group, the average NLR level before administration was 0.23 ± 0.16 and after administration was 0.28 ± 0.14 . In this study, giving dangke did not have a significant impact on NLR levels because the results of the study for the intervention group consisting of 8 mice showed an increase in NLR levels in 6 mice, a decrease in NLR levels in 1 mouse, and NLR levels remained the same in 1 mouse. mice, while in the Control group consisting of 8 mice there was an increase in NLR levels in 5 mice, and a decrease in NLR levels in 3 mice. The intervention group's NLR levels increased by 5 mice and the control group's NLR levels increased by 6 mice, which is only a difference of 1 point, so giving dangke to mice did not affect NLR levels.

This research is in line with Igor N which can be used as a synbiotic supplement combined with foods containing low carbohydrates and high protein. The results of the research from the 2 groups, namely intervention and placebo, showed that there was no significant difference in BMI and weight loss for 3 months. The study shows that there are limitations in the use of supplements used to adjust the gut microbiota by inhibiting microbial fermentation associated with foods high in protein and low in carbohydrates related to metabolism that requires further research ⁽¹¹⁾.

This research is not in line with Payam Syarifan's research findings published in the Journal of Health,

Population, and Nutrition regarding "Efficacy of Low Fat Milk and Yogurt Fortified with Vitamin D3 on Systemic Inflammation in Adults with Abdominal Obesity." Here, the results showed that serum levels of neutrophils, lymphocytes, platelets, and red blood cell distribution (RDW) before and after treatment were significantly lower in the fortified dairy group. The results showed that serum neutrophil, lymphocyte, platelet and RDW levels before and after intervention were significantly lower in the fortified milk product group ($p < 0.05$). The values of neutrophils to lymphocytes (NLR), platelets to lymphocytes, and RDW to platelets (RPR) decreased significantly in the enriched group ($p < 0.05$). There were significant differences between groups. It has been shown that administration of probiotics has been shown to have an effect on the immune response, increase lactose tolerance, prevent diarrhea, have anti-inflammatory effects, and even reverse intestinal dysbiosis associated with obesity ⁽¹²⁾.

In the *danke* there is (BAL indigenos). BAL is milk which also has the potential to be a probiotic for the development of functional foods. BAL is generally marketed as bacteria that contain probiotics, especially from the genera *Bifidobacterium* and *Lactobacillus*. Probiotics are proven to have benefits for health, reducing (BMI) and body weight because probiotics can prevent toxic substances, proteins, lipopolysaccharides from passing through the epithelium, and can reduce intestinal permeability, causing reduced energy absorption, and can provide an anti-inflammatory mechanism that can reduce fat in the body. adipocyte cells as an anti-obesity effect. NLR was included as an inflammatory marker thought to reflect similarities between adaptive immune (lymphocyte) and innate immune (neutrophil) responses. Previous research shows that an increase in NLR is associated with an increase in various pro-inflammatory cytokines because they can damage DNA in cells. However, although there are studies investigating the relationship between these parameters and various chronic diseases involving inflammation, few publications address the relationship with obesity and diet quality ^{(1),(11),(13)}.

NLR is a more sensitive parameter for bacterial infection than an increase in the number of red blood cells. The prospective longitudinal observational study reports the association between clinical severity and lymphocytopenia in patients with severe sepsis and septic shock in an oncology intensive care unit ^{(14),(15)}.

NLR is a parameter used to determine the inflammatory response and immune system in the body, especially those related to certain diseases or conditions. The NLR value is calculated by dividing the number of neutrophils (a type of white blood cell) by the number of lymphocytes (another type of white blood cell). An increase in NLR indicates inflammation or stress and a decrease in NLR indicates a less effective immune response. The value of NLR levels in mice is not significant or does not show the expected results, including: errors in blood collection methods or blood analysis methods, diseases suffered by mice, administration of drugs, inadequate nutrition, genetic factors, and environmental factors such as stress, temperature, light, and the social environment of mice can also influence the immune

system response and NLR levels of mice.

Conclusion

This study is the first study with the results of research on the effect of dangke on changes in NLR levels in obese white rats concluded as follows. There is no significant relationship in NLR levels in both the control group and the intervention group to the administration of dangke. Therefore, further research is needed to produce more accurate findings. For researchers, it is hoped that the dose of dangke administration needs to be increased so that the results of NLR levels in the research sample can be more significant changes, the treatment of rats during the study is more concerned both from high-fat feeding both the environment in white rats.

Conflicts of Interest

There is no conflict of interest

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References

1. Parveen KZ, AvabrathaRodríguez-Rodríguez E, López-Sobaler AM, Ortega RM, Delgado-Losada ML, López-Parra AM, Aparicio A. Association between neutrophil-to-lymphocyte ratio with abdominal obesity and healthy eating index in a representative older Spanish population. *Nutrients*. 2020;12(3). doi:10.3390/nu1203085
2. Novri, SD Risk Factors for Obese Women in Lower Middle Socioeconomic Status, Thesis, Health Nutrition Study, Muhammadiyah University of Gresik. 2021;2(2):163-168.
3. Suryaalamsah Ii, Akbar Z, Harisatunnasyitoh Z, Et Al. Increasing Knowledge About Overweight and Obesity among Caregivers at the IGBS Darul Marhamah Islamic Boarding School, Jatisari Village, Cileungsi District, Bogor Regency, West Java. *Proceedings of the National Seminar on Community Service LPPM Umj*; 2019.
4. Wijayanti E, Retnoningrum D, Hendrianintyas M. The relationship between inflammatory markers and hemoglobin in obesity at the Faculty of Medicine, Diponegoro University for the period May-September 2018. *DiscoverSys Digest of Medical Sciences*. 2019;10(1):242-246. doi:10.1556/ism.v10i1.347
5. Sofia Yusnur Rafida, Hairuddin, Muhammad Hasan. Relationship Between Neutrophil-to-Lymphocyte Ratio (NLR) Values and Procalcitonin (PCT) Levels in COVID-19 Patients in Jember Regency. *Andalas Medical Faculty Journal*; 2022.
6. Aydın M, Ahsen Yilmaz, Feti Tulubas. Neutrophil lymphocyte ratio in obese adolescents. *Northern Clinics of Istanbul*. Published online 2019. doi:10.14744/nci.2015.25238.
7. Harun, A. 2022. Systematic Review: The Role of Neutrophil Cells in the Metabolic Disease Diabetes Mellitus Type 2. *Faculty of Health Sciences*. Aisyiyah University Yogyakarta; 2022.
8. Nugraha KA. Bacteriostatic and Bactericidal Agents in Dangke, Thesis, Fkg Hasanuddin University Makassar; 2021.

9. Rifah HA, Budiartman S, Harsi DK. Antibacterial Activation of Dried Papaya Sap Against *Staphylococcus Aureus* in Dangke. *Journal of Technology and Food*. Bogor Agricultural Institute. 2019;25(1).
10. Sulmiyati S, Said NS. Characteristics of Buffalo Milk Dangke with the Addition of Dry Papain Crude. *agriTECH*. 2019;38(3):345.
11. Bila Yunisa Tri Utami S, Ary Lantika U, Rachmi A. Scoping Review: Effectiveness of Consuming Probiotics on Body Weight in Overweight and Obese Adults. *Bandung Conf Ser Med Sci*. 2022;2(1):402–9.
12. PayamSharifan. Effectiveness of Low Fat Milk and Yogurt Enriched with Vitamin D3 on Systemic Inflammation in Adults with Abdominal Obesity. *Journal of Health, Population and Nutrition*. 2022;41(8).
13. Nurwilda Kaswi. The Effect of Milk Type on Lactic Acid Bacteria Species in Dangke from Enrekang Regency. Faculty of Science and Technology, Uin Alauddin Makassar; 2020.
14. YuliatiWidiastuti. The Effect of Probiotic and Selenium Supplementation on the Immune Response of NLR (Neutrophil Lymphocyte Count Ratio), Hemoglobin and Albumin in Wistar Rats Induced by *Mycobacterium Tuberculosis*. Diponegoro University Faculty of Medicine; 2019.
15. Nishimura Mie. Effects of Yogurt Containing *Lactobacillus Plantarum* HOKKAIDO on Immune Function and Stress Markers. Hokkaido Information University, Ebetsu, Hokkaido, Japan. 2019;6(3):275-277.