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Seluang Fish (*Rasbora Spp*.) Oil Decreases Inflammatory Cytokines Via Increasing Vitamin D Level in Systemic Lupus Erythematosus

Radiyati Umi Partan[1], Rachmat Hidayat[2*], Nurwan Saputra[1], Febry Rahmayani[1], Hari Prapto[1], Timotius Wira Yudha[2]

[1]Internal Medicine Department, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia; [2]Biology Department, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia

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***Correspondence:** Rachmat Hidayat. Biology Department, Faculty of Medicine, Universitas Sriwijaya, Palembang, Indonesia. E-mail: dr.rachmat.hidayat@gmail.com

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Abstract

BACKGROUND: Systemic Lupus Erythematosus (SLE) is an autoimmune disorder mediated by inflammatory cytokines. Decreasing vitamin D levels is a common feature in SLE patients. Vitamin D has the capacity in suppressing inflammatory cascade. Seluang fish (*Rasbora spp*.) contained a high level of vitamin D with the potential as a new therapeutic modality.

AIM: This study aimed to assess the efficacy of Seluang fish oil against proinflammatory cytokines, vitamin D levels, and clinical conditions of SLE.

METHODS: A randomised, double-blind, clinical trial study design was conducted. The subjects were 16 SLE subjects treated with 500uL Seluang fish oil capsules and 16 SLE subjects with placebo capsules. Measurement of vitamin D, IL-1, IL-6 and IL-17 levels were performed with ELISA. Clinical assessment of SLE was performed with MEX-SLEDAI. Bivariate analysis, T-test, was performed. Data were presented in the form of mean ± SD.

RESULTS: The administration of Seluang fish oil was clinically able to show efficacy assessed by the MEX SLEDAI score. Significant results were also shown by increased vitamin D levels and reduced levels of IL-1, IL-6 and IL-17, in Seluang fish oil group.

CONCLUSION: Seluang fish oil possessed the efficacy of reducing the inflammatory response in SLE patients by increasing serum vitamin D levels.

Introduction

Systemic Lupus Erythematosus (SLE) is one of the threatening autoimmune diseases that impend global health. The prevalence of SLE in the Asia-Pacific Region is 4.3-45.3 per 100,000 populations. Meanwhile, the prevalence of SLE in Indonesia is 0.5%, and the prevalence is escalating from year to year. There are 16.000 new SLE cases in the United States. This shows the number of new SLE cases is very significant. The cases of SLE require solemn emphasis from the pathophysiological aspects to a comprehensive therapeutic approach [1].

SLE is a prototype of an autoimmune disorder characterised by antibody production towards components of the cell nucleus. The pathological conditions found in SLE patients are related to the inflammatory process, vasculitis, immune complex deposition and vasculopathy. The inflammatory process is the main pathological condition in SLE disorders [2]. SLE begins with the formation of antibodies against the cell components of the individual itself, and Anti-double Stranded DNA (ds-DNA) is found in 95% of SLE cases.

The presence of ds-DNA is triggered by an increase in the B cell population, due to a decrease in the production of T cells, triggered by the production of IL-2. Increased IL-2 will promote the activation of Th-17 cells to produce IL-17. This condition will activate the inflammatory cascade, which is mediated by interleukin-1 (IL-1), IL-6 and Tumor Necrosis Factor-Alpha (TNF-α), which are responsible for the damage in various target organs and eventually will lead to target organ failure [3].

Vitamin D is a steroid hormone playing a role in the regulation of cell growth, proliferation, apoptosis and regulation of the immune system. Vitamin D deficiency is one of the clinical conditions that further aggravate clinical SLE. Decreasing vitamin D levels is a common feature in SLE patients. Administration of immunosuppressant drugs (glucocorticoids) will trigger a decrease in vitamin D levels. Nevertheless the drug is the standard drug consumed by SLE patients. Vitamin D has the capacity in suppressing the immune response so that it will suppress inflammatory cascade [4]. Vitamin D supplementation was able to optimally demote clinical problems in SLE patients [5]. Therefore, vitamin D supplementation in SLE patients becomes strategic management.

Exploration of the vitamin D source modality is a strategic step for SLE management. Seluang fish (*Rasbora spp.*) is an endemic freshwater fish in South Sumatra, Indonesia. Seluang fish is the first level consumer in its food chain. As a first level consumer, it is safe from the accumulation of heavy metal contamination commonly found in top consumer fish. It is rich in oil that lines up the fish body. Previous studies showed that Seluang fish oil contained vitamin D (cholecalciferol) in the amount of 2043.34 IU/mL [6]. With a massive vitamin D content, Seluang fish oil possesses the potential to be further investigated regarding its efficacy in SLE cases.

This study aimed to assess the efficacy of Seluang fish oil against proinflammatory cytokines (IL-1, IL-6, IL-17), serum Vitamin D levels, and MEX-SLEDAI clinical scores in SLE patients.

Material and Methods

Subjects

A randomised, double-blind, clinical trial study design was conducted. This study was conducted in the Rheumatology Outpatient Internal Medicine Department, Dr Moh. Hoesin General Hospital Palembang, South Sumatra, Indonesia. This study was by the Declaration of Helsinki of the World Medical Association and had received ethical approval by the Ethics Committee Faculty of Medicine, Universitas Sriwijaya, Dr Moh Hoesin General Hospital Palembang. Informed consent was done for all study subjects. The subjects of this study were 32 patients who met the criteria of American College of Rheumatology (ACR) 1997 classification, consisting of 16 SLE subjects who were treated with 500 uL Seluang fish oil capsules, one capsule per day, for 90 days and 16 SLE subjects who received placebo capsules. Study subjects were between 15-50 years, with no comorbidities or severe clinical conditions.

Measurement of proinflammatory cytokines and vitamin D levels

Three ml blood was withdrawn from each study subject, which was then inserted into the EDTA tube, then centrifuged at a speed of 5000 rpm for 10 minutes. The supernatant was obtained and stored at -20°C. Measurement of Vitamin D, IL-1, IL-6 and IL-17 levels was performed with Enzyme-linked Immunosorbent Assay (ELISA) technique Cloud-Clone (Cloud-Clone Corp., Texas, USA), by the work protocol issued by the manufacturer.

Clinical assessment of SLE

Clinical assessment of SLE was performed with the Systemic Lupus Erythematosus Disease Activity Index scoring system (SLEDAI) using data from medical records and laboratory findings. This scoring consisted of 24 items with a total score of 0-105.

Statistical analysis

Data analysis was carried out using SPSS 24.0 software (SPSS Inc., Chicago, USA). Data were presented in the form of mean ± SD. Bivariate analysis, T-test, was conducted to analyse mean differences between the treatment and control groups. Statistical significance was of p-value < 0.05.

Results

Table 1 exhibited that the clinical conditions of the study subjects between the treatment group and the control group were not statistically different. The subjects showed the equality of clinical conditions in SLE so that the possibility of bias in this study could be prevented.

Table 1: Baseline Characteristics of Study Subjects

Characteristics	Treatment	Placebo	P-value
	Mean ± SD	Mean ± SD	
Age (years)	30.31 ± 9.32	26.75 ± 8.96	0.227 *
Duration of SLE (months)	18.63 ±13.54	21.09 ± 4.80	0.677*
Haemoglobin (g/dL)	12.42 ± 1.89	11.69 ± 1.25	0.210*
Leukocyte (x 10^3) (/µL)	8.6 ± 3.28	8.69 ± 2.84	0.936*
Platelet (x 10^3) (/µL)	294.8 ± 56.34	330.81 ± 114.39	0.985*
Blood sugar (mg/dL)	106.5 ± 33.08	105.38 ± 19.89	0.692*
Ureum (mg/dL)	23.13 ± 7.81	25.5 ± 11.42	0.748*
Creatinine (mg/dL)	0.693 ± 0.117	0.683 ± 0.286	0.126*
SGOT (AST) (U/L)	20.31 ± 10.24	20.88 ± 7.35	0.416*
SGPT (ALT) (U/L)	22.38 ± 15.66	22.00 ± 9.68	0.521*
Calcium (mg/dL)	1.14 ± 0.08	1.17 ± 0.06	0.383*
dsDNA (ng/mL)	434.4 ± 55.46	423.0 ± 52.64	0.318*
Vitamin D (ng/mL)	44.81 ± 7.09	46.91 ± 9.16	0.474*
IL-17 (pg/mL)	497.19 ± 148.34	515.57 ± 160.93	0.739*
IL-1 (pg/mL)	69.81 ± 18.09	72.05 ± 19.62	0.739*
IL-6 (pg/mL)	413.97 ± 95.09	425.75 ± 103.16	0.739*
MEX SLEDAI	3.31 ± 2.30	3.69 ± 2.41	0.656*

*Unpaired T Test; p = 0.05.

The administration of Seluang fish oil was clinically able to show efficacy compared to placebo, which was assessed by the MEX SLEDAI score.

Significant results were also shown by vitamin D levels, which exhibited that Seluang fish oil was able to increase serum vitamin D levels compared with placebo. Increased levels of vitamin D in the group receiving Seluang fish oil was followed by a decrease in inflammatory markers, namely a significant reduction in levels of IL-1, IL-6 and IL-17, compared to the placebo group (Table 2).

Table 2: Proinflammatory Cytokines and Vitamin D Levels

Measurement	Treatment	Placebo	P-value
	Mean ± SD	Mean ± SD	
Vitamin D (ng/mL)	89.81 ± 7.11	47.12 ± 3.16	0.005*
IL-17 (pg/mL)	245.31 ± 21.34	518.87 ± 65.33	0.001*
IL-1 (pg/mL)	39.21 ± 7.09	72.65 ± 18.71	0.003*
IL-6 (pg/mL)	213.47 ± 21.09	431.75 ± 34.46	0.001*
MEX SLEDAI	1.31 ± 0.30	3.56 ± 1.41	0.006*

*Unpaired T Test; p = 0.05.

Discussion

This study revealed the efficacy of Seluang fish oil as one of the newest therapeutic approaches to SLE. High level of vitamin D (cholecalciferol) is believed to be the biological plausibility of the efficacy of Seluang fish oil against SLE. Vitamin D is a sterol group compound which is important in the regulation related to the immune system [7].

Vitamin D is a signalling molecule that activates vitamin D receptors, which are part of intracellular receptors. Activation of the vitamin D receptor will suppress the regulator protein Nuclear Factor-Kappa Beta (NF-kB) gene expression so that NF-kB gene expression process does not occur and hence NF-kB protein is suppressed [8]. NF-kB is a transcription factor that plays a role in the expression of proinflammatory cytokine genes. NF-kB suppression causes a decrease in IL-2 gene expression and suppression of IL-2 cytokines. The occurrence of suppression of IL-2 causes a decrease in T lymphocyte cell proliferation, which will be followed by a decrease in lymphocyte cell proliferation. IL-2 is believed to play a major role in autoimmune disorders [9].

Proliferation suppression of lymphocyte cells causes suppression of auto-antibody production of cell components. This decrease in auto-antibody production will cause a decrease in cascade inflammatory activation and thus the pro-inflammatory cytokines, such as IL-1, IL-6 and IL-17 [10]. Activation of proinflammatory cytokines will cause oxidative stress in cells. Activation of oxidative stress causes caspase cascade activation, which will lead to cell death. Extensive cell death in the organ, causing damage to target organs, such as the kidneys, heart, lungs and blood vessels [11]. IL-6 is an inflammatory cytokine produced by various cells, such as monocytes, fibroblasts, endothelial cells and B and T lymphocyte cells. These cytokines regulate differentiation of lymphocyte B cells. Differentiation of B cells leads to the initiation of antibody production, Ig G. The increase in IL-6 certainly promotes the production of autoantibodies in SLE patients, which in turn worsens the clinical condition of SLE patients [12], [13]. IL-17 is a cytokine produced by lymphocyte T cells, a type of T-helper cell 17. IL-17 is believed to be an important inflammatory cytokine that plays a role in the process of organ destruction in SLE cases. Increased production of IL-2, will cause proliferation of T cells, including T-helper 17 cells [14], [15], [16].

Another study conducted on the SLE mice showed that vitamin D3 supplementation possessed good efficacy in suppressing the inflammatory process. Vitamin D3 suppressed the production of IL-17, IL-23 and gamma interferon. Where an increase in IL-17, IL-23 and gamma interferon would lead to cell damage, especially the kidneys and blood vessels in mice [4, 10]. The above study certainly supported the results of this study, where high vitamin D contained in Seluang fish oil was believed to play a major role in the efficacy of Seluang fish oil in SLE cases.

In conclusion, Seluang fish oil possessed the efficacy of reducing the inflammatory response in SLE patients by increasing serum vitamin D levels.

References

1. Ministry Of the Health Republic Of Indonesia. Lupus. Ministry of the Health Republic of Indonesia. Jakarta, 2017.

2. Finzel S, Schaffer S, Rizzi M, Voll RE. Pathogenesis of systemic lupus erythematosus. Z Rheumatol. 2018; 77(9):789-98. https://doi.org/10.1007/s00393-018-0541-3 PMid:30291433

3. Ohl K, Tenbrock K. Inflammatory Cytokines in Systemic Lupus Erythematosus. J Biomed Biotechnol. 2011; 2011:1-14. https://doi.org/10.1155/2011/432595 PMid:22028588 PMCid:PMC3196871

4. Bishak YK, Ghavamzadeh S, Shokri A, Payahoo L, Khalili L, Hassanalilou T. Role of vitamin D deficiency in systemic lupus erythematosus incidence and aggravation. Autoimmune Highlights. 2017; 9(1):1-10. https://doi.org/10.1007/s13317-017-0101-x

5. Handono K, Tanuwijaya LK, Fitri LE, Kalim H. The Correlation between Serum Concentration of Vitamin D with Vitamin D Receptor Expression and Disease Activity in Indonesian Patients with Systemic Lupus Erythematosus: Preliminary Study. J Trop Life Sci. 2016; 4(2):113-8. https://doi.org/10.11594/jtls.04.02.06

6. Partan RU, Hidayat R. The Potential Role of Seluang Fish Oil (Rasbora spp.) in Improving Calcium, Zinc and Vitamin D3 Deficiencies. IJOO. 2017; 11(2):285-91.

7. Condur L, Chirila S. The relationship between vitamin D and cardiovascular risk. Medic.ro. 2018; 2(1):8-12. https://doi.org/10.26416/MED.122.2.2018.1657

8. Dankers W, Colin EM, Van Hamburg JP, Lubberts E. Vitamin D in autoimmunity: Molecular mechanisms and therapeutic potential. Front Immunol. 2017; 7:697. https://doi.org/10.3389/fimmu.2016.00697 PMid:28163705 PMCid:PMC5247472

9. Cannell JJ, Grant WB, Holick MF. Vitamin D and inflammation. Dermatoendocrinol. 2015; 6(1):e983401.

https://doi.org/10.4161/19381980.2014.983401

10. Reihani H, Rastin M, Sahebari M, Mahmoudi M, Ghoryani M, Abdollahi N, Tabasi NS, Rabe SZT, Arab FL, Faraji F. Effects of Vit D on Th17 cells and related cytokines in lupus erythematosus patients. Front Immunol Conference Abstract: 15th International Congress of Immunology (ICI), 2013.

11. Salem ML, El-Badawy A. Programmed death-1 / programmed death-L1 signaling pathway and its blockade in hepatitis C virus immunotherapy. World J Hepatol. 2015; 7(23):2449-58. https://doi.org/10.4254/wjh.v7.i23.2449 PMid:26483866 PMCid:PMC4606200

12. Sabry A, Elbasyouni SR, Sheashaa HA, Alhusseini AA, Mahmoud K, George SK, Kaleek EA, Abo-Zena H, Kalil AM, Mohsen T, Rahim MA, El-Samanody AZ. Correlation between levels of TNF-α and IL-6 and hematological involvement in SLE Egyptian Patients with lupus nephritis. Int Urol Nephrol. 2007; 38(3-4):731-7. https://doi.org/10.1007/s11255-006-0047-9

13. Umare V, Pradhan V, Nadkar M, Rajadhyaksha A, Patwardhan M, Ghosh KK, Nadkarni AH. Effect of proinflammatory cytokines (IL-6, TNF, and IL-1β) on clinical manifestations in indian SLE patients. Mediators Inflamm. 2014; 2014:1-8. https://doi.org/10.1155/2014/385297 PMid:25548434 PMCid:PMC4273527

14. Tahernia L, Namazi S, Rezaei N, Ziaee V. Cytokines in systemic lupus erythematosus: their role in pathogenesis of disease and possible therapeutic opportunities. Rheumatol Res. 2017; 2(1):1-9. https://doi.org/10.22631/rr.2017.69997.1010

15. Raymond W, Eilertsen GO, Griffiths S, Nossent J. IL-17A levels in systemic lupus erythematosus associated with inflammatory markers and lower rates of malignancy and heart damage: Evidence for a dual role. Eur J Rheumatol. 2017; 4(1):29-35. https://doi.org/10.5152/eurjrheum.2017.16059 PMid:28293450 PMCid:PMC5335884

16. Saber NZ, Maroof SH, Soliman DA, Fathi MS. Expression of T helper 17 cells and interleukin 17 in lupus nephritis patients. Egypt Rheumatol. 2017; 39(3):151-7. https://doi.org/10.1016/j.ejr.2017.01.005