Research Article

The Effect of Tuna Extract on Periarticular Tissue Catalase Activity and Malondialdehyde Levels in Animal Models of Adjuvant Arthritis

Fitri Handajani,[1]* Judya Sukmana[2]

[1]Biochemistry Departmen, [2]Pathology Anatomi Department Faculty of Medicine Hang Tuah University, Surabaya, Indonesia

*Corresponding author: fitrihandajanidr@gmail.com
Received 12 May 2023 ; Accepted 30 April 2024
https://doi.org/10.23886/ejki.12.389.16

Abstract

Tuna contains vitamins, minerals, amino acids, and fatty acids, which play a role in preventing oxidative stress. The pathophysiology of rheumatoid arthritis is often associated with oxidative stress due to increased ROS production. Adjuvant arthritis experimental animals are widely used for research on rheumatoid arthritis. This study aims to determine the effect of tuna fish extract on plasma malondialdehyde (MDA) levels and periarticular tissue catalase in adjuvant arthritis (AA) experimental animals with AA. The 24 experimental animals were divided into 3 groups, group 1, eight experimental animals without treatment; group 2, eight experimental animals AA-induced complete freund adjuvant (CFA); group 3, eight experimental animals AA treated with Tuna fish extract therapy, and at the end of the study the animals were sacrificed and examined for plasma MDA and periarticular tissue catalase levels. The results of the ANOVA statistical test showed a significant increase in periarticular tissue catalase activity (p=0.001) between the AA group and the treatment group. There was a considerable decrease in periarticular tissue MDA levels (p=0.001) between the adjuvant arthritis group and the treatment group with p value = 0.001. The Spearman correlation test between MDA levels and catalase activity shows -0.335, indicating an adequate and inverse correlation. The administration of tuna extract can prevent oxidative stress. It can be shown that increasing the antioxidant activity of catalase and reducing lipid peroxidation is measured by examining MDA levels.
***Keyword** : tuna extract, adjuvant arthritis, oxidative stress, malondialdehyde, catalase.*

Efek Ekstrak Tuna terhadap Aktivitas Katalase Jaringan Periartikular dan Kadar Malondialdehida pada Model Hewan Artritis Ajuvan

Abstrak

Tuna mengandung vitamin, mineral, asam amino, dan asam lemak yang berperan mencegah terjadinya stress oksidatif. Patofisiologi artritis rematoid sering dikaitkan dengan stres oksidatif akibat peningkatan produksi ROS. Hewan coba artritis ajuvan banyak digunakan untuk penelitian tentang rematoid artritis. Penelitian ini bertujuan untuk mengetahui pengaruh ekstrak ikan tuna terhadap kadar MDA plasma dan katalase jaringan periartikuler hewan coba artrtitis ajuvan (AA). Sebanyak 24 hewan coba di bagi 3 kelompok. Kelompok satu adalah yaitu 8 hewan coba tanpa perlakuan, kelompok dua adalah 8 hewan coba Artrtis Adjuvan yang diinduksi complete Freund adjuvant (CFA), kelompok 3 yaitu 8 hewan coba AA yang diberi perlakuan ekstrak ikan Tuna, dan pada akhir penelitian hewan coba dikorbankan kemudian diperiksa kadar MDA plasma dan katalase jaringan periartikuler. Uji Anova pada penelitian menunjukkan peningkatan aktivitas katalase jaringan periartikuler yang signifikan (p=0,001) antara kelompok AA dibandingkan kelompok perlakuan. Terdapa pula penurunan signifikan kadar MDA plasma (p=0,001) antara kelompok Arthritis adjuvant dibandingkan kelompok perlakuan.. Uji korelasi Spearman antar kadar MDA dan aktivitas katalase menunjukkan nilai -0,335 artinya terdapat korelasi yang cukup dan berkebalikan. Hasil tersebut menunjukkan bahwa pemberian ikan tuna mampu mencegah stres oksidatif dengan cara meningkatkan aktivitas antioksidan katalase dan menurunkan peroksidasi lipid yang diukur dengan pemeriksaan kadar MDA
***Kata kunci** : ekstrak tuna, artritis ajuvan, stres oksidatif, malondialdehid, katalase.*

Introduction

Fish muscle tissue is the most consumed part of fish. Big eye tuna (*Thunus obesus*) has a dominant muscle tissue network. Muscle tissue in tuna contains many essential amino acids that are easy to digest and rich in taste. The amino acid content in tuna reaches 26% by weight; these amino acids can play a role in the healing process. Tuna also contains fatty acids as precursors for forming eicosanoids which help regulate prostaglandin synthesis. Tuna fish contains many vitamins, minerals, arachidonic acid, and omega-3 fatty acids that act as antioxidants. Omega-3 and omega-6 fatty acids have a role in reducing the production of inflammatory cells, and proinflammatory cytokines and reducing the number of enzymes that degrade cartilage by inhibiting the activation of the transcription factor NF-kB. [1–3]

The benefit of tuna as a nutrition is that it meaty flavor, is cheap, easy to get is available throughout the season, and contains compounds that are important for health. Several studies show that the nutritional content of tuna can act as an antioxidant and anti-inflammatory. This advantage attracts researchers to look for alternative therapies for arthritis with pathophysiology related to decreased antioxidant activity and oxidative stress due to chronic inflammatory processes in arthritis patients. [4]

Rheumatoid Arthritis is a disease whose etiology is still unclear, with symptoms of systemic and local chronic inflammatory processes. The pathogenesis of this disease is believed to be due to the formation of free radicals (ROS) associated with the inflammatory process. In rheumatoid arthritis patients, there is an increase in ROS and inflammatory processes due to the activation of the disease. An increase in ROS that endogenous antioxidants cannot compensate for will cause oxidative stress and damage. One marker of oxidative damage is lipid peroxidation, which can measure using levels of malondialdehyde (MDA).[5–7]

Rheumatoid arthritis is an autoimmune disease that often occurs in middle-aged women and results in joint damage and damage to several vital organs in the body. This situation decreases sufferers' quality of life; thus, compelling, efficient, and relatively affordable treatment is needed. People living with arthritis experience a reduction in endogenous antioxidant levels, and an inflammatory process occurs, resulting in cytokine secretion. The inflammatory process that occurs results in the formation of free radicals so that the body is dampened with endogenous antioxidants, which will decrease the activity and number of endogenous antioxidants in the body, such as catalase. [6,8–10]

This study utilized an animal adjuvant arthritis model as an experimental animal for rheumatoid arthritis. This animal model is similar to rheumatoid arthritis (RA) in humans. This procedure facilitates the understanding of mechanisms, pathophysiology, and the development of new therapies that can be used in rheumatoid arthritis.[11,12] This study aims to determine whether tuna fish extract affects MDA levels and catalase activity of articular tissue in experimental animals.

Methods

This study used animal models of adjuvant arthritis, namely experimental animals induced with complete Freund adjuvant (CFA) so that signs of arthritis would occur. The experimental animals were divided into three groups: the group without treatment consisted of 8 Rattus norvegicus, the group induced by CFA according to the procedure consisted of 8 *Rattus norvegicus*, and the treatment group comprised of 8 *Rattus norvegicus*, which was induced with CFA after signs of arthritis, were given tuna extract orally for seven days. Then the experimental animals were sacrificed and examined for MDA levels and catalase activity.[1]

Adjuvant Arthritis Induction Procedure using CFA

The experimental animals were injected intradermally at the base of the rat's tail with 0.1 ml of CFA. After 14 days, a booster of 0.1 ml of CFA was given intradermally divided into the right and left legs. After seven days, symptoms of adjuvant arthritis will appear in the form of swelling, redness, and pain in the leg joints.[13] This arthritis model, called Adjuvant-Induced Arthritis (AIA), has been widely used as a model for AR.[13,14]

Examination of Periarticular Tissue Catalase by Spectrophotometry Method100 mg of periarticular tissue was added with 900 µl phosphate buffer, homogenized, and centrifuged at 3000 rpm for 10 minutes. Then the supernatant was taken and added with 1 ml of 60 mM H_2O_2. The results obtained were read with a spectrophotometer at a wavelength of 240 nm. The results obtained are interpolated on the standard curve.[14]

Measurement of Plasma MDA Levels by Spectrophotometric Method

The procedure for measuring plasma MDA levels: 10 µL BHT reagent is put into a centrifuge tube, then 250 µL calibrator or sample is added to the tube. The tube that was filled with the solution

was added 250 µL of acid reagent and 250 µL of BHT reagent, then vortexed for 5 seconds and incubated for 60 minutes at 60o C. Centrifuged at 10,000 rpm for 2-3 minutes. The solution was transferred into cuvettes, and the absorbance was measured with a spectrophotometer. The absorbance results are calibrated with a standard curve.[15]

Results

In Table 1 it was found that there was an increase in the mean MDA levels in Group 2 compared to Group 1, and there was a decrease in the mean MDA levels in Group 3 compared to Group 2 Variable data on MDA levels for each group can be seen in the Table 1.

Table 1. MDA Levels for Each Group

No	Group 1(nmol/g)	Group 2 (nmol/g)	Group 3(nmol/g)
1	164	2179	2751
2	233	1591	2220
3	201	1969	1120
4	209	2666	2098
5	341	3187	1388
6	252	2754	1224
7	303	2899	885
8	254	2334	1515
Mean	244.625	2459.875	1650
Standard Deviation	56.88	524.54	640.57

In Table 2 it was found that there was a decrease in the mean catalase activity levels in Group 2 compared to Group 1, and there was an increase in the mean catalase activity in Group 3 compared to Group 2.

Table 2. Catalase Activity of Periarticular Tissue

No	Group 1(U/L)	Group 2(U/L)	Group 3(U/L)
1	362.67	220.70	358.33
2	325.67	344.17	347.30
3	313.00	288.67	354.67
4	343.00	342.67	359.33
5	349.67	315.67	355,00
6	335.67	284.33	376.33
7	342.67	241.67	358.00
8	422.00	271.67	384.00
Mean	349.29	288.69	361.62
Standard Deviation	32.97	44.50	12,21

The results of the ANOVA test between groups on MDA levels and catalase activity showed significant differences between groups and continued with the least significant difference (LSD) test. The results of the Spearman correlation test between MDA levels and catalase activity show -0.335, which means a good and inverse correlation.

Table 3. Correlation between MDA Levels and Catalase Activity based on LSD Results

Group		MDA levels	Catalase activity
1	2	0.001*	0.001*
	3	0.001*	0.460
2	3	0.003*	0.001*

Note : * =p<0,05 significant
Group 1 : Without treatment
Group 2 : AA
Group 3 : Treatment (AA+ tuna fish extract)

Discussion

The Role of Tuna Fish Extract on Periarticular Malondialdehyde (MDA) Levels in Adjuvant Arthritis.

Oxidative stress biomarkers one of which is MDA, are used as an alternative to assess disease activity in rheumatoid arthritis patients. Oxidative stress is a state of increased levels of oxidants that cannot be dampened by antioxidants in the body. The impact of oxidative stress causes oxidative damage. If this occurs in the lipid membrane, lipid peroxidation will form, which can be measured by MDA levels. Increased MDA levels positively correlated with pro-inflammatory cytokines, ROS levels, and disease activity in patients with adjuvant arthritis.[10,16,17]

In patients with rheumatoid arthritis, there is excessive activation of TNF-α which triggers an increase in the production of ROS and is followed by a decrease in antioxidants in the body. If this situation continues, it will cause an imbalance between oxidants and antioxidants in the form of oxidative stress resulting in oxidative damage. One of the effects of oxidative stress is the occurrence of lipid peroxidation, which MDA levels can measure. Lipid peroxidation can occur in all cell membranes, organelles, and lipoproteins. Oxidative stress plays an essential role in patients with rheumatoid arthritis, which underlies the occurrence of proliferative synovitis damage, so it can be assumed that in the periarticular tissue of patients with rheumatoid arthritis, there is an increase in ROS levels which triggers lipid peroxidation.[5,7,18]

Research conducted by Kostoglou-Athanassiou et al,[10] shows that omega-3 polyunsaturated fatty acids are precursors for forming eicosanoids which act as anti-inflammatories. In rheumatoid arthritis patients, omega 3 reduces inflammation by reducing pro-inflammatory cytokines, reducing antigen presentation via MHC II, and modulating T-cell differentiation. These three processes result in modulating the autoimmune inflammatory response. Omega 3 ameliorates or prevents experimental arthritis and may decrease disease activity in rheumatoid arthritis.[10]

Omega-3 inhibits NF-kB activation, thereby inhibiting the expression and production of proinflammatory cells, which reduces the secretion of proinflammatory cytokines in the form of TNFα, interleukin 1β, and interleukin 6. Inhibition of NF-kB activation by omega-3 also decreases the number of macrophages in the joints so that the production of free radicals free by proinflammatory cells such as macrophages can be prevented. This inhibits oxidative damage through lipid peroxidation in the joint periarticular tissue.[10,14,20]

In this study, it was proven that there was an increase in periarticular tissue MDA levels in the AA experimental animal group compared to the untreated animal group. This proves that there is an increase in lipid peroxidation in the joints of arthritic animals due to increased free radicals and inflammation in AA experimental animals. Administration of tuna fish extract to the treatment group significantly reduced MDA levels in the periarticular tissue of AA animals. This shows that probably the content of omega 3 in tuna extract can prevent lipid peroxidation in periarticular tissues [3,10]

Effect of Giving Tuna Fish Extract on Catalase Activity in Periarticular Tissue of AA Experimental Animals

In patients with rheumatoid arthritis, there is a chronic inflammatory process in several systems, and the most common is the inflammatory process in the joints. This will result in an increase in total oxidant status and a decrease in total antioxidant status and cause oxidative stress. Oxidative stress is an essential pathophysiology in the development and severity of joint damage in patients with rheumatoid arthritis. Free radicals formed due to oxidative stress will have an indirect impact on the occurrence of joint damage through inflammatory processes and cellular immune responses in people with rheumatoid arthritis. This directly results in joint cartilage degradation by attacking joint proteoglycans and inhibiting their synthesis. This situation will affect the antioxidant status in the body. [20,21]

Catalase is an endogenous antioxidant in the form of an enzyme that catalyzes the reaction of hydrogen peroxide into water and oxygen. In patients with rheumatoid arthritis, antioxidants, including catalase, will experience an overall decrease. In experimental animals, with adjuvant arthritis, catalase levels are reduced due to oxidative stress, so many antioxidants are used to reduce the formation of free radicals. In this study, the administration of tuna extract reduced the inflammatory process that occurred so that the production of free radicals would decrease and only a small amount of antioxidants were used to reduce oxidants. This condition causes that there are still quite a lot of available antioxidants, including catalase.[5,22]

The nutritional composition of tuna meat consists of protein, fatty acids, and minerals. The nutritional content of tuna, which plays a role in reducing the severity of disease in AA, is vitamin A, vitamin C, omega 3, omega 6, and selenium. Vitamin A and vitamins contained in meat act as antioxidants that can reduce free radicals formed due to inflammatory processes, which result in oxidative stress. This can be prevented, and oxidative damage can be avoided. Selenium is a component of enzymatic antioxidants. Consuming tuna, which contains much selenium, can increase the activity of enzymatic antioxidants (gluthathione, superoxide dismutase, and catalase). [23] Tuna fish also contains potential antioxidants, so experimental animals with adjuvant arthritis, given tuna fish extract, will experience an increase in antioxidant activity, including the antioxidant catalase. This study showed that the administration of tuna fish extract increased the antioxidant activity of catalase.[2,3,24]

The limitation of this research is that the author only measures antioxidant catalase activity and lipid peroxidation by MDA level, so it cannot show the overall antioxidant activity status. Spearman correlation test for MDA levels and periarticular tissue catalase activity in this study showed the number -0.335, which means that there is a good correlation, and the reverse means that if there is an increase in MDA levels, it will be followed by a decrease in catalase activity. This indicates that an increase in MDA indicates lipid peroxidation due to increased free radicals and oxidative stress. A decrease will follow this condition in catalase activity which acts as an endogenous anti-oxidant because it has been widely used to reduce free

radical contamination. The limitation of this study is the research only measures antioxidant catalase activity and lipid peroxidation by MDA level, so it cannot show the overall antioxidant activity status.

Conclusion

Treatment of Tuna fish extract in AA animals can reduce MDA levels and increase catalase activity in animal models of AA and have a good dan reverse correlation.

References

1. Handajani F, Prabowo S. Sedimentation rate and arthritis inflammatory score in adjuvant arthritis model. Int J of Pharmtech Res. 2019;12:85–90. doi: 10.20902/IJPTR.2019.120310
2. Peng S, Chen C, Shi Z, Wang L. Amino acid and fatty acid composition of the muscle tissue of Yellowfin Tuna (Thunnus Albacares) and Bigeye Tuna (Thunnus Obesus). J Food Nutr Res. 2013;42–5. doi: 10.12691/jfnr-1-4-2
3. Prospective T. Potential Source of Tuna Fishes Therapeutic Effect Health Benefits 2021;2020–1.
4. Govindbhai Vagh R, Bhola D V, Chudasama BG, Vagh RG. Therapeutic prospective of tuna fish [Internet]. 2021. Available from: www.bioticainternational.com
5. Mateen S, Moin S, Khan AQ, Zafar A, Fatima N. Increased reactive oxygen species formation and oxidative stress in rheumatoid arthritis. PLoS ONE. 2016;11:1–15. doi: 10.1371/journal.pone.0152925
6. Scherer HU, Häupl T, Burmester GR. The etiology of rheumatoid arthritis. J Autoimmun. 2020;110:102400. doi: 10.1016/j.jaut.2019.102400
7. Yoo S jin, Go E, Kim Y eun, Lee S, Kwon J. Roles of reactive oxygen species in rheumatoid arthritis pathogenesis. J Rheum Dis. 2016;23:340-47. doi: 10.4078/jrd.2016.23.6.340
8. Atzeni F, Talotta R, Masala IF, Bongiovanni S, Boccassini L, Sarzi-Puttini P. Biomarkers in rheumatoid arthritis. Isr Med Assoc J. 2017;19:512–6.
9. Zhou L, Feng JT, Zhang L, Kuang Y. Clinical significance of serum total oxidant/antioxidant status for the disease activity in active rheumatoid arthritis. Int J Clin Exp Pathol. 2017;10:8895–900.
10. Kostoglou-Athanassiou I, Athanassiou L, Athanassou P. The effect of omega-3 fatty acids on rheumatoid arthritis. Mediterr J Rheumatol. 2020;31:190–4. doi: 10.31138/mjr.31.2.190
11. Asquith DL, Miller AM, McInnes IB, Liew FY. Animal models of rheumatoid arthritis. Eur J Immunol. 2009;39:2040–4. doi: 10.1002/eji.200939578
12. Quiñonez-Flores CM, González-Chávez SA, Del Río Nájera D, Pacheco-Tena C. Oxidative stress relevance in the pathogenesis of the rheumatoid arthritis: a systematic review. BioMed Res int. 2016;2016:6097417. doi: 10.1155/2016/6097417
13. Handajani F, Prabowo S. Sargassum duplicatum extract reduced artritis severity score and periarticular tissue matrix metalloproteinase- 1 (MMP-1) expression in ajuvan artritis exposed to cold stressor. Sys Rev in Pharm. 2020;11:302–7. doi: 10.31838/srp.2020.6.48
14. Prabowo S, Handajani F. The benefits of mackerel fish (Scomberomorus commersonii) extract to decrease inflammatory process. Res J Pharm Technol. 2021;14:1651–7. doi: 10.5958/0974-360X.2021.00293.6
15. Handajani F, Aryati A, Notopuro H, Aulanni'am A. Prophylactic Sargassum duplicatum inhibit joint damage in adjuvant arthritic rats exposed to cold stress through inhibition of NF-KB activation. Int J ChemTech Res. 2016;9:151–9.
16. Frijhoff J, Winyard PG, Zarkovic N, Davies SS, Stocker R, Cheng D, et al. Clinical relevance of biomarkers of oxidative stress. Antioxid Redox Signal. 2015;23:1144–70. doi: 10.1089/ars.2015.6317
17. Fonseca LJS Da, Nunes-Souza V, Goulart MOF, Rabelo LA. Oxidative stress in rheumatoid arthritis: what the future might hold regarding novel biomarkers and add-on therapies. Oxid Med Cell Longev. 2019;2019:7536805. doi: 10.1155/2019/7536805
18. Pisoschi AM, Pop A. The role of antioxidants in the chemistry of oxidative stress: a review. Eur J Med Chem. 2015;97:55-74. doi: 10.1016/j.ejmech.2015.04.040
29. Calder PC. Omega-3 fatty acids and inflammatory processes: from molecules to man. Biochem Soc Trans. 2017;45:1105–15. doi: 10.1042/BST20160474
20. Sies H. Oxidative stress : a concept in redox biology and medicine. Redox Biology. 2015;4:180–3. doi: 10.1016/j.redox.2015.01.002
21. Elsayed Azab A, A Adwas Almokhtar, Ibrahim Elsayed AS, A Adwas A, Ibrahim Elsayed Ata Sedik, Quwaydir FA. Oxidative stress and antioxidant mechanisms in human body. J App Biotechnol & Bioeng. 2019;6:43–7. doi: 10.15406/jabb.2019.06.00173
22. Liu Z, Ren Z, Zhang J, Chuang CC, Kandaswamy E, Zhou T, et al. Role of ROS and nutritional antioxidants in human diseases. Front Physiol. 2018;9:477. doi: 10.3389/fphys.2018.00477
23. Sadauskiene I, Liekis A, Staneviciene I, Naginiene R, Ivanov L. Effects of long-term supplementation with aluminum or selenium on the activities of antioxidant enzymes in mouse brain and liver. Catalysts. 2020;10:585. doi: 10.3390/catal10050585
24. Nurilmala M, Hizbullah HH, Karnia E, Kusumaningtyas E, Ochiai Y. Characterization and antioxidant activity of collagen, gelatin, and the derived peptides from Yellowfin Tuna (Thunnus albacares) skin. Mar Drugs. 2020;18:98. doi: 10.3390/md18020098