A Study on Brown Seaweed Therapy (*Sargassum* sp.) toward MDA Levels and Histological Improvement on Rat Foot Suffering Rheumatoid Arthritis

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ABSTRACT

Rheumatoid arthritis (AR), an autoimmune disease, is characterized by the inflammation in the joint area caused an excessive of free radicals. An excessive of free radicals in the body cause oxidative stress, that increasing the levels of malondialdehyde (MDA) as an indicator of lipid peroxidation and the decreasing levels of anti-oxidants. The treated with extract of brown seaweed (*Sargassum* sp.) intended to find out the MDA levels in serum and the histological of the foot joints rheumatoid arthritis rats. MDA levels are determined through a TBA test (*Thio Barbituric acid*), meanwhile the histological of the rat foot joints was determined by *Hematoxylen-Eosin* staining (HE). The results showed the brown seaweed extract therapy (*Sargassum* sp.) was significantly (p<0.01) reduce levels of MDA in the serum of 21.24% and improving histological foot joint rheumatoid arthritis rats.

Key word: Rheumatoid arthritis, Malondialdehyde, Herbal therapy, Brown seaweed

INTRODUCTION

Rheumatoid arthritis is an autoimmune disease that attacks the joints. Rheumatoid arthritis can produce a proliferative synovitis that often develop into the destruction of joint cartilage and underlying bone, causing disability due to arthritis [1]. Rheumatoid arthritis disease continues to grow such as in Korea (0.11%), China, Japan and Taiwan (0.3%), Australia, Malaysia (0.5%). Meanwhile, in America the number of people with rheumatoid arthritis in 1988 was 16.3 million people (11%), and every year the number of people with rheumatoid arthritis disease continues to increase and until 2005 the number of patients with rheumatoid arthritis has reached 66 million people, or nearly 1 from 3 people suffer arthritis [2]. In Indonesia, in 1980 to 2000 patients with rheumatoid arthritis have increased up to 9.99% of the entire Indonesian population with a 65-70 year life expectancy [3].

A variegated rheumatoid arthritis causes disease in an inflammatory process and degradation. An inflammation of the membrane lining the joints (synovial) caused by cytokine pro-inflammation and the high production of free radicals. The resulting of free radicals cause the onset of oxidative stresses, in which case an imbalance between of free radicals generated by antioxidant in the body so damage the cell membrane which is characterized by increased levels of malondialdehid (MDA), one of the indicators of lipid peroxidation. So take antioxidant compounds that come from outside the body in order to suppress the occurrence of elevated levels of MDA.
In general, this rheumatoid arthritis disease is treated with chemical medication that many complication like gastric disorders and swelling, weight gain, dispepsia, peptic ulcer and bleeding in the upper gastrointestinal tract [4]. Therefore, the research to develop the best treatment for rheumatoid arthritis is valuable. Here, the use of the brown seaweed (*Sargassum* sp.) as a herbal-based medicine is proposed.

Seaweeds are rich in antioxidants [5]. Polyphenols (flavonoids and fluorotanin) are an antioxidant found in brown seaweed (*Sargassum* sp.). The ability of polyphenols as antioxidants based on the presence of hydroxyl groups on aromatic compounds that can donate a hydrogen atom to free radicals and the ability of the electron delocalization as having conjugated double bonds, as well as the stability of resonance structures. Based on the test results of phytochemical ethanol extracts of brown seaweed *Sargassum* sp., indicated the existence of flavonoid (polyphenols), terpenoids, and alkaloids compounds. In another report, flavonoids of the *Sargassum* sp. can reduce levels of MDA concentration and increase the SOD levels [6]. Based on the description above, it is necessary to examine the role of brown seaweed extract (*Sargassum* sp.) in reducing the levels of MDA and repairing joint damage in rheumatoid arthritis rats.

**EXPERIMENTAL**

**Experimental Animals and Research Design**

A number of 18 rats (*Rattus norvegicus*) (male, body weight 200g) were housed at room temperature in the animal house in the Laboratory of Cellular and Molecular Biology, Mathematics and Sciences Faculty, Brawijaya University Malang and were exposed to alternate cycles of 12 h light and darkness. The rats were grouped into three groups: a healthy group, rheumatoid arthritis group, and rheumatoid arthritis group which was treated with brown seaweed extract (*Sargassum* sp.). The rheumatoid arthritis rats were injected with 0.1 mL CFA at the base of the tail intradermal and incubated for fourteen days. Then they were injected with CFA at intradermal of right and left foot of the rats and incubated for the next seven days. Rats were treated with the extract of brown seaweed (*Sargassum* sp.) at a dose of 100 mg/Kg BW of rats were given oral therapy for 14 days, and on the 15th day, rats were dissected. Rats were killed by neck dislocation, and rats’ serum and feet were taken. The serum was taken from the tail, and the rats’ feet were also taken, and the skin were slashed and washed with 0.1% NaCl and immersed in 4% PFA for seven days. All conditions and handling of the animals were conducted following the protocols approved by the Ethical Clearance Committee of Brawijaya University (159-KEP-UB).

**PROCEDURE**

**MDA levels Measurement Using Thiobarbituric Acid test**

The rats serum 100 µl was added with 550 µl of aquadest, 100 µl TCA 100%, 250 µl HCl 1 N and 100 µl Na-thio 1%. The mixture was homogenized with a vortex. The mixture was centrifuged at 550 rpm for 15 minutes, and the supernatant was taken. The resulted solution was incubated in water bath at 100°C for 20 minutes, and left to room temperature and measured using UV-Vis spectrophotometer at 532 nm.

**Histological Features using Hematoxylen-Eosin Staining Method**

Prepate of the rats foot put into 1-3 xylol respectively for 5 minutes, and was put into the variation of ethanol which was started from absolute ethanol 1-3, ethanol 95%, 80%, and 70% respectively for 5 minutes, and was soaked in aquadest for 5 minutes. Then, it was
put into hemotoxylene dyes for ± 10 minutes to penetrate the equipment color. After that, it was washed over flowing water for 30 minutes, and rinsed with aquadest before continued to a colouring with eosin dye. The colour was resulted using eosin stained by inserting the equipment into the eosin alcohol for 5 minutes, then soaked in the aquadest to release the excess of eosin. Moreover, in the dehydration process, the equipment was inserted in the graded ethanol 80%, 90%, and 95% to the 1-3 absolute of ethanol. Then, in the clearing process, it was done by putting it in the xylol 1, 2 and was further dried. Finally, the result was mounted using entellan. The dried and stained ultrathin sections were observed using a microscope (Olympus BX53) with a magnification of 600 times.

RESULTS AND DISCUSSION
Levels of Malondialdehyde (MDA) in Rats Serum

Extract of brown seaweed (*Sargassum* sp.) can reduce levels of MDA (Table 1 and Figure 1). The results showed the increasing levels of MDA in the group of rats with rheumatoid arthritis (0.488 ± 0.015 ppm) higher than the healthy group (0.259 ± 0.009 ppm). While in the group of rats with rheumatoid arthritis treated using extract of brown seaweed (0.314 ± 0.017 ppm) showed the decreasing levels of MDA compared to the rats with arthritis itself. This indicate the therapy of brown seaweed at a dose of 100 mg/Kg BW provide a positive influence on the decreasing of the levels of MDA rheumatoid arthritis rat serum. Statistical analysis results also showed a significant influence on the group rheumatoid arthritis rat serum toward the rheumatoid arthritis rat serum treated with brown seaweed extract (*Sargassum* sp.) (P < 0.01).

| Group                               | MDA Levels Average (µg/mL) | MDA Improvement Levels toward Control (%) |
|-------------------------------------|----------------------------|------------------------------------------|
| Healthy (Negative Control)          | 0.259±0.009                | 0                                        |
| Sick (Positive Control)             | 0.488±0.015                | 88.41                                    |
| Treatment (brown seaweed water extract (*Sargassum* sp.) | 0.314±0.017 | 21.24                                    |

MDA levels in rat serum (*Rattus norvegicus*) decreased after treatment with extracts of brown seaweed (21.24%). Decreasing of MDA level possible was caused by an antioxidant nutrient found in the brown seaweed such as polyphenol compounds, flavonoids, alkaloids, terpenoids and tannins. Theoretically, inhibition mechanism of lipid peroxidation by extracts of brown seaweed as antioxidant compound can counteract free radicals in the system. Brown seaweed has antioxidant content of polyphenols group (fluorotanin and flavonoids), fucoidan, fukosantin, α-tocopherol, alginate, and iodine [7]. Compounds from polyphenol groups, fukosantin, α-tocopherol and carotenoids have antioxidant activity that useful to counteract free radicals. Malondialdehyde which is the product of peroxidation can be used as an indicator for occurring of a lipid disorder. Therefore, it can be concluded the high levels of malondialdehyde in the rheumatoid arthritis rat’s serum as an indication of the high levels of sinovial membrane tissue disorder which were happened due to the reactive oxygen compounds.

Lipid damage consists of three phases; that are initiation, propagation, and termination. Initiation process is the process when a hydrogen atom is removed from the lipid
molecules. Some compounds can react with hydrogen atoms forming hydroxyl radical (•OH), alkoxy (RO), peroxyl (ROO) and may also HO₂ but not including H₂O₂. Membrane lipids generally are phospholipid consist of unsaturated fatty acids in which peroxidation is easily occur due to the issuance of methylene group (-CH₂-) from the hydrogen atom contains only one electron. So, there are carbon atoms with no pair of electrone. The existence of a double bond in the fatty acid weakened the CH bonds on the carbon atom adjacent to the double bonds. It eased the transfer of a hydrogen atom [8]. When there is sufficient oxygen concentration lipid radicals, then it react with the oxygen to form a peroxyl radical (ROO•). This formation occurs in the propagation stage. At the termination, peroxyl radical (ROO•) attacks the other hydrogen atoms originating from other lipid molecules that are close by and produce lipid peroxides and peroxyl radicals or interact with other antioxidants [9]. This process causes the synovial membrane compliers cells dead, and thus damaging the synovial membrane.

**Figure 1.** Comparison of the average value of serum levels of MDA in healthy rats (*Rattus novergicuss*), rheumatoid arthritis rats, and therapies with extract brown seaweed rats (*Sargassum* sp.)

**Effect at Brown Seaweed Extracts Therapy (*Sargassum* sp.) toward Histological Features of Rheumatoid Arthritis Foot**

The results in Figure 2 showed that the extract of brown seaweed (*Sargassum* sp.) at a dose of 100 mg/Kg BW reduce the formation of panus at rheumatoid arthritis joint. At the foot of healthy rats (A), it shows that the histology of the foot joints are in good condition and perfect and the joint surface looks flat and organizes properly. Whereas, in rats treated with brown seaweed (C), the joints had been repaired and the results are the normal rats. In untreated rheumatoid arthritis rats (B), the joint surfaces are not perfect and uneven, where the joint surface looks flat and irregular. Joint damage is caused by the formation of panus thereby increasing the production of free radicals. This of free radical compounds can trigger the formation of antibodies, by modifying the protein aggregates that can activate phagocytic cells and cause inflammation. Formation of antibodies against autoantigens or antigens from infectious gene (referred to rheumatoid factor) can lead to the formation of immune complexes which in turn can lead to the complexes activation and phagocytic [10].

Joint damage in rheumatoid arthritis usually starts from the periphery in which the synovial tissue normally forms a bond with joint cartilage. There is a growth of inflamed synovial tissue, in and around cartilage. The excessive tissue grows is called Panus. Panus often have vascularization and contain iron deposit. Most of the cells found in panus were large and mononuclear, and many of which have fibroblastic picture as well. Most of macrophages derived from monocytes of blood vessels drawn by chemotactic factors produced in the joint. Cytokines produced by macrophages included TNF-α. TNF-α induced activation of NF-kB in the sinoviosit, which led to an increase in the production of various
kinds of products, including IL-1, IL-8, and monocyte chemoattractant protein-1 (MCP-1). IL-1 and TNF-α also increased bone resorption [11].

**Figure 2.** HE staining results on Rheumatoid Arthritis Foot (A= Leg joints of control rats (healthy); B= rats foot joint suffering rheumatoid arthritis; and C= foot joints suffering rheumatoid arthritis plus of extract brown seaweed treatment (*Sargassum* sp.). A 100x magnification. Arrows indicate the occurrence of structural changes in hyaline cartilage (cartilage) on treatment.

**CONCLUSIONS**

Based on the research conducted on rheumatoid arthritis rats treated with extract of brown seaweed (*Sargassum* sp.), it was found that seaweed extract therapy (*Sargassum* sp.) with dose of 100 mg/Kg BW decrease the levels of MDA in rats for rheumatoid arthritis 21.24% and showed histological of rheumatoid arthritis rat leg joints.

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