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Broccoli Extract *(Brassica oleracea)* Decrease Periarticular Malondialdehyde Level and Disease Activity Score in Rats *(Rattus norvegicus)* with Adjuvant Arthritis

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Abstract: Rheumatoid arthritis is a chronic inflammatory disease characterized by inflammation, progressive joint destruction, significant disability, systemic manifestation and premature mortality. In rheumatoid arthritis, oxidative stress play important role in the mechanism leading to destructive sinovitis. Broccoli *(Brassica oleracea)* is rich in nutritional antioxidants ie. vitamin C and vitamin E, and non-nutritional antioxidants ie. carotenoid, phenolic substances and flavonoids. Broccoli is also rich in polyphenols, a group of phytochemistry considered to have the highest antioxidant content in the diet. Polyphenols inhibit lipid and other molecules oxidation through rapid hydrogen atom donation to free radicals which is likely quench oxidative stress. Three groups of male Wistar rats (*Rattus norvegicus*) were used in this study (n=8/group). The first group were non treated control. Adjuvant arthritis was induced in the other two groups by intradermal injection of 0,1 ml *Complete Freund's Adjuvant (CFA)* on the tail base. After 14 days, booster 0,1 ml CFA was given intradermally on the right and left dorsal feet. In one of the groups 2 gr/kgBW/day broccoli extract were administered per oral for 14 days since symptoms of adjuvant arthritis were appeared (21 days after adjuvant arthritic induction). At the end of experiment (on day 36), periarticular tissue MDA (Malondialdehyde ie. marker of oxidative tissue destruction) level and disease activity score were measured. The result of this study showed that broccoli significantly decreased periarticular tissue MDA level (p=0.001) in adjuvant arthritic rats. Broccoli also decreased disease activity score in adjuvant arthritic rats, but the level of reduction was not statistically significant (p=0.333). In conclusion this study showed that broccoli significantly decrease periarticular tissue MDA level and tends to decrease disease activity score of adjuvant arthritic rats. This outcome is likely due to the presence of antioxidants such as vitamin C, vitamin E, carotenoids and flavonoids in broccoli.

Key words: Malondialdehyde, disease activity score, adjuvant arthritis

1. Introduction

Rheumatoid arthritis is a chronic inflammatory disease characterized by progresive joint destruction, significant disability, systemic manifestation and premature mortality. [1] In this inflammatory process of rhematoid arthritis, oxidative stress and antioxidants play important role. [2] Reactive oxygen species has important role in the pathogenesis of rheumatoid arthritis. High reactive oxygen species production is known to cause oxidative tissue damage and inflammation. Increased reactive oxygen species production in rheumatoid arthritic patients is likely caused by TNF-α overproduction. [3] Cytokines that play direct role in the pathophysiology of rheumatoid arthritis are TNF-α, IL-1, IL-6 and IL-17.[4]

In rheumatoid arthritis oxidative stress plays important mechanism and is responsible for proliferative damage and synovitis. Moreover, oxidative stress also affects functional characteristic of synovial T lymphocytes resulted in alterations in signal transduction pathway of T cell receptors. Previous studies showed that oxygen radical derived from NADPH oxidases might have critical influence in arthritis development by increasing NADPH oxidases activity from circulating neutrophils and monocytes.[3]

Excess pro-inflammatory cytokine production induced neutrophil and macrophages activity to produce reactive oxygen species in synovial fluid which acts as mediator of tissue damage. The level of cytokine release in the synovium and in the plasma of systemic circulation can be used as biomarker of oxidative stress to estimate the level of inflammation in the joints. [5]

Malondialdehyde (MDA) is an end product of lipid peroxidation in the presence of cellular injury. Malondialdehyde formed through peroxidation of polyunsaturated fatty acids in the cells that has more than two double bonds such as linoleic acid, arachidonic acid and docosahexaenoic acid. Protein is easier to get damaged by MDA than free amino acid resulted in modification of many residues especially lysine and intramolecular as well as extra molecular cross link in the protein. MDA oxidized by aldehyde dehydrogenase to become semi aldehyde malonic acid which then decarbonized to become acetaldehyde which is a substrate for oxidation by aldehyde dehydrogenase enzyme to become acetate compounds.[6]

Broccoli is included in genus *Brassica* and is known to have a lot of bioactive non-enzymatic compounds. Broccoli is rich in nutritional antioxidant such as vitamin C and E as well as non-nutritional antioxidant such as carotenoids, a phenolic compound mainly flavonoids. Broccoli is also rich in polyphenols, a big group of phytochemicals which often considered to have the most antioxidants in diet. Polyphenol caused inhibition of lipid peroxidation and other molecules through rapid donation of hydrogen atom to free radicals. Intermediate compound from phenoxyl radical is quite stable thus inhibit the initiation of the next radical reaction. Flavonoids and their derivatives are the most prominent polyphenol group and the most ideal peroxyl radical scavenger, because of specific reduction towards alkyl peroxyl radicals make them an effective inhibitor of lipid peroxidation. [7]

This study aimed to determine whether administration of broccoli extract affects periarticular MDA level and the disease activity score in *Rattus norvegicus* with adjuvant arthritis.

2. Experimental Method

Twenty four male Wistar rats, (125-170 grams, age 10-12 weeks) were housed at animal house in the Laboratory of Biochemistry, Faculty of Medicine, Universitas Hang Tuah, Surabaya, Indonesia. The study was conducted in accordance to Institution guidelines for animal research and Ethical Clearence Committe of Universitas Hang Tuah. Male Wistar rats (*Rattus norvegicus*) were used in this study (n=8/group). The control group was without treatment. In the second and third group adjuvant arthritis were induced by intradermal injection of 0,1 ml *Complete Freund's Adjuvant (CFA)* (Sigma F5881, Lot //SLBQ110V) on the tail base. After 14 days, booster 0,1 ml CFA was given intradermally on the right and left dorsal feet. In the third group rats were administered 2 gr/kgBW/day broccoli extract per oral for 14 days since symptoms of adjuvant arthritis were appeared (21 days after adjuvant arthritic induction). At the end of experiment (on day 36), periarticular tissue MDA (Malondialdehyde ie. marker of oxidative tissue destruction) level and disease activity score were measured.

2.1.Preparation of Broccoli Extract

Broccoli were washed with clean water and chopped in small pieces. The pieces were dried in the oven for 2-3 days with temperature of 40^0 C maximum. The dry pieces were mascerated in ethanol. The mass were then moved into a funnel and filtrated using 4 layers of No. 1 Whatman filter paper until filtrate and residue were obtained. The filtrate were then concentrated using *Vaccum Rotary evaporator* at temperature of 40^0 C.

2.2. *Measurement of Periarticular MDA Tissue*

One hundred mg periarticular tissue were placed in a reaction tube in 550 µl H_2O, homogenized and 100 µl trichloroacetate acid was added. After being vortex, 250 µl HCl was added, vortex again and 100 µl sodium thiobarbiturate was added, then centrifuged at 500 rpm (rotation per minute) for 10 minutes. The supernatan were taken and filtered with glass wool. The result was then heated up in water bath at 100^0 C for 20 minutes and left at room temperature. One ml H_2O was added and was read using spectrophotometer at wavelength 531 nm. The results were interpolated using standard curve.

2.3. Measurement of Disease Activity Score

Disease activity score were measured by observing the severity of the disease clinically. Measurement score used was the 1994 modified system of Santos and Tipping [8] where:

1 = No inflammation and movement restriction.
2 = Inflammation on particular joint of distal back foot but no movement restriction.
3 = Moderate inflammation on back foot joint and knee with movement restriction.
4 = Severe inflammation on back foot joint and knee with significant movement restriction.

3. Results and Discussion

The results showed reduction of arthritis severity in adjuvant arthritis rats receiving broccoli extract (Fig. 1C) in comparison to adjuvant arthritic rat group that did not received broccoli extract (Fig. 1B).

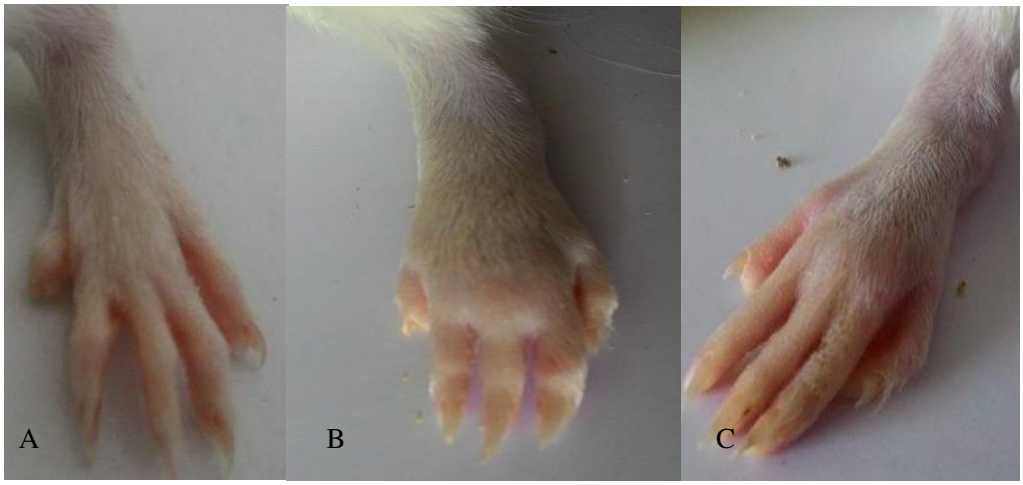

Fig. 1. Normal untreated rat foot (A), rat foot from adjuvant arthritic group (B) and rat foot from adjuvant arthritis receiving broccoli extract (C)

Broccoli extract treatment significantly decreased (p=0.001) periarticular tissue MDA level in rats wih adjuvant arthritis (1923.88 nmol/g $\pm$ 361.298 nmol/g vs 957.13 nmol/g $\pm$ 111.338 nmol/g) after 14 days treatment. Mean and standard deviation of periarticular tissue MDA level were shown in Table 1.

Table 1. Mean and standard deviation of periarticular tissue MDA level

Group	Mean $\pm$ Standard Deviation
I	279.63 $\pm$ 116.727 nmol/g
II	1923.88 $\pm$ 361.298 nmol/g
III	957.13 $\pm$ 111.338 nmol/g

Note: Goup I : Rats without treatment; Group II : Rats with adjuvant arthritis ajuvan; Group III : Rats with adjuvant arthritis and received broccoli extract for 14 days.

Broccoli extract treatment resulted in the decrease of disease activity score but statistically not significant (p=0.333) in rats with adjuvant arthritis (3.63 vs 3.38) after 14 days treatment. Mean of disease activity score were shown in Table 2.

Tabel 2. Mean of disease activity score

Group	Mean
I	1
II	3.63
III	3.38

Note: Goup I : Rats without treatment; Group II : Rats with adjuvant arthritis ajuvan; Group III : Rats with adjuvant arthritis and received broccoli extract for 14 days.

Rheumatoid arthritis is a condition that induce oxidative stress. In rheumatoid arthritis sufferers, there are 5 fold increase in the production of reactive oxygen species in blood mitochondria and monocytes compared to normal individuals. It is likely that the level of oxidative stress is a measure of specific pathogenic image of rheumatoid arthritis. Free radical indirectly affect joint damage as they play as second messenger in the process of inflammation and immunologic respond in rheumatoid arthritis. Exposure of T cells to increased oxidative stress made them refractory towards several stimulation including growth and death and potentially increase abnormal immune response. [2]

Free radicals degrade joint cartilage directly, by damaging proteoglycans and their syntheses. In rheumatoid arthritis there are also oxidative damage in hyaluronic acid and and oxidative damage of protein and DNA. Furthermorethere there is also lipoperoxidation that resulted in increase the level of MDA. [2]

Broccoli contain a lot of bioactive compounds such as vitamin C, anthocyanin, phenolic compounds and flavonoids. [9] Broccoli is rich of nutritional antioxidants such as vitamin C and E as well as non-nutritional antioxidants such as carotenoid, phenolic compounds especially flavonoid. Vitamin C including ascorbic acid and its oxidative products, dehydroascorbic acid play important role in intracellular and extracellular redox reaction. Vitamin C protects cell death and directly cleans superoxide radicals, singlet oxygen, and acts as chain breaker of lipid peroxidation. Vitamin C works together with vitamin E to regenerate oxidized α-tocopherol that bind in the cell membrane and to develop antioxidant network. [7]

Broccoli is rich of polyphenol and has the most antioxidant in the diet. Polyphenol caused inhibition of lipoperoxidation and other molecules through quick donation of hydogen atom to free radicals. Intermediate compounds of phenoxyl radicals are quite stable thus inhibit the initiation of further radical reactions in chain reaction. This antioxidant will protect joints from further oxidative tissue damage. Flavonoid and their derivates are the biggest group of polyphenol and the most prominent and the most ideal scavenger of peroxyl radicals which make them effective inhibitor of lipid peroxidation. Broccoli contains flavonol and anthocyanin that function as a potent antioxidants. [7] So, antioxidants in the boccoli may quench free radicals formed in the adjuvant arthritic rats and reduce oxidative damage in the joint tissues.

It is suggested that by administering broccoli extract for 14 days to adjuvant arthritic rats, the high level of antioxidants in broccoli inhibited the occurence of oxidative stess. As a result oxidant compounds were reduced and lipid peroxidation were also reduced in periarticular tissue, so that MDA level in the periarticular tissue were lowered significantly. When oxidative stress were reduced, joint damage were also reduced and as a result the disease severity in the joints pictured by disease activity score were reduced as well, although it was not statistically significant.

4. Conclusions

Broccoli decreased significantly periarticular tissue MDA level and tends to decrease disease activity score in adjuvant arthritic rats. The outcome is likely due to the presence of antioxidants such as vitamin C, vitamin E, carotenoids and flavonoids in broccoli.

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