ACTA BIOCHIMICA INDONESIANA

RESEARCH ARTICLE

THE EFFECT OF MAJA FRUIT [Aegle marmelos (L) Correa] ON INFLAMMATION MARKERS INVOLVED IN THE AGING PROCESS

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ABSTRACT

Background: Aging is a process that every living creature will pass through and involves various factors. Increased inflammatory factors can be caused by hypoxia which forms Reactive Oxygen Species (ROS) and damages macromolecules and causes the acceleration of the aging process. Maja plants, empirically have long been used as medicinal plants, research on Maja leaves shows anti-inflammatory and antioxidant potential.

Objective: The purpose of this study was to find out and understand the potential of Maja fruit in the presence of inflammatory markers of IL-6 and TNF-α which are involved in the aging process.

Methods: This research was in vivo experimental, using male experimental animals Sprague Dawley rats divided into 8 groups (n = 4), and divided into 2 groups (fed with Maja ethanol extract (400 mg/ kg/day, 14 days) and not force-fed). Each group was divided into 4 subgroups (normoxia, hypoxia (O2 8%, N2 92%) for 3, 7 and 14 days). At the end of the trial period, animal blood was examined for IL-6 and TNF-α concentrations using the ELISA method.

Results: The results showed an increase in levels of IL-6 and TNF-α both in the group fed and not fed in line with the duration of hypoxia when compared with controls. However, groups that are not force-fed show a higher pattern compared to those who are force-fed.

Conclusion : The ethanol extract of Maja fruit can help slow down the aging process.

Keywords : Aging, Oxidative stress, Tumor necrosis factor-alpha, Interleukin 6, Hypoxia

INTRODUCTION

Aging is a process that every living creature will pass through. The process of collecting involves a variety of factors, several factors are programmed in the cell and some other factors are not (non-specific). Many aging theories try to explain how this process occurs, in an attempt to study normal (physiological) processes. The aging process involves various kinds of organs, resulting in a decline in function which can lead to a decrease in quality of life and disease. One theory of aging is the formation of free radicals that can cause cell damage.[1] Aging is also associated with an increase in the activity of inflammatory factors found in the blood, such as tumor necrosis factor-alpha (TNF-α), interleukin 6 (IL-6), cytokines antagonist, active phase proteins and neopterin. This increase in inflammatory factors in the elderly shows a pathological process caused by increasing age.[2]

Free radicals with oxygen molecular nuclei (O2) are known as Reactive Oxygen Species (ROS). Reactive Oxygen Species (ROS) can bind to lipids to form malondialdehyde (MDA) compounds, with proteins forming carbonyl compounds, with DNA or RNA forming 8-oxo-2'-deoxyguanosine or 8-oxo-guanosine.[3] ROS formation and antioxidant defense mechanisms in vivo cells. Antioxidants in the body will overcome the free radicals that are formed. On the other hand, increased production of ROS causes various diseases associated with reduced antioxidant defenses and tissue damage. Antioxidant defense systems are of two types, namely enzymatic and non-enzymatic.[4,5] Major antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) with the help of glutathione (GSH), glutathione reductase, glutathione S-transferase, and glucose 6 phosphate dehydrogenase.[6]

Antioxidants can also come from outside the body, for example from plants. Indonesia has various kinds of plants that can potentially become drugs, one of which is Maja fruit (Aegle marmelos (L) Correa). Maja fruit has been empirically long used as a medicinal plant. This fruit in certain groups of people is used as antidiarrheal and dysentery, treating burns, digestive problems, constipation, ulcers, antiviral, anti-parasitic, etc. The examination showed that Maja planted contain coumarin, alkaloids, polysaccharides, seed oil, tannins, carotenoids, polyphenols, etc.[7] From the research, Maja leaves could potentially be anti-inflammatory because they inhibit H1 antihistamine receptors, and can act as antioxidants because they have flavonoids, alkaloids, sterols, tannins, etc.[7,8]

The purpose of this study was to find out and understand the potential of Maja fruit (Aegle marmelos), the inflammatory markers of IL-6 and TNF-α involved in the aging process with Sprague Dawley animal models induced systemic hypoxia as a source of ROS.

MATERIAL AND METHODS

This study was an in vivo experimental study, using experimental animals of male Sprague Dawley rats carried out in the laboratory of Biochemistry and Molecular Biology, Faculty of Medicine, Tarumanagara University. Experimental animals were divided into 8 groups (n = 4). Group 1 (P1) was a group of mice that were not given Maja fruit extract and had normoxia. The next three groups (P2-P4), were groups of mice that were not given a Maja fruit

extract and had hypoxia for 3, 7 and 14 days. Group 5 (P5) was a group of mice that were given Maja extract and had normoxia. The next three groups (P6-P8) were groups of mice that were given Maja fruit extracts and had hypoxia for 3, 7 and 14 days. This research has obtained ethical approval from the Research Ethics Committee of the Trisakti University Medical Faculty with number 127 / KER / FK / II / 2018

The Maja fruit obtained from the Mekarsari fruit garden will be made simplicia and macerated using ethanol, which is then evaporated so that it gets a thick extract that will be fed to experimental animals at a dose of 400 mg/ kg/day for 14 days. Hypoxia is carried out using a hypoxic chamber and gas flowed with a composition of 8% oxygen (O2) and 92% nitrogen (N2). At the end of the trial period, the blood of the animal will be taken and the lysate is made. The lysate will be examined for IL-6 and TNF-α concentrations using the ELISA method using IL-6 rats and TNF-α ELISA kit (Abcam®). The results obtained were statistically processed with GraphPad Prism v.7.0 with an ANOVA one-way test, followed by Tukey's multiple comparisons test and unpaired t-test. The variables analyzed were considered significant if $p < 0.05$.

RESULTS

The results of examination of IL-6 concentrations in animal blood showed an increase in IL-6 concentration in the hypoxic group compared to controls, an increase in IL-6 concentration in line with the duration of hypoxia. Statistical test with ANOVA showed a significant difference ($p = 0.0448$) in the experimental group of animals which were not given a Maja fruit extract strain between 14 days versus hypoxia compared to controls (Tukey's, $p = 0.0293$) (Figure 1), whereas in the group given the strain Maja fruit extract does not show significant differences (Figure 2).

In the statistical test between the groups fed with those not fed on each treatment showed no significant differences (t-test, $p > 0.05$), it will still be seen that the group given the extract of Maja fruit extract showed more IL-6 concentrations low when compared to groups not given Maja fruit extract in each treatment group (Figure 3).

The results of examination of TNF-α concentrations in blood of experimental animals showed an increase in TNF-α concentration in the hypoxic group compared to controls, an increase in TNF-α concentration in line with the duration of hypoxia. Statistical tests with ANOVA showed a significant difference ($p = 0.0117$) in the experimental group of animals which were not given Maja fruit extract strains between hypoxia 7 (Tukey's, $p = 0.0276$) and 14 days (Tukey's, $p = 0.0250$) compared to controls (Figure 4), whereas in the group given the extract of Maja fruit extract did not show significant differences (Figure 5).

In the statistical test between groups fed with those not fed to each treatment showed no significant differences (t-test, $p > 0.05$), it would still be seen that the group given the extract of Maja fruit extract showed more TNF-α concentrations. low when compared to groups not given Maja fruit extract in each treatment group (Figure 6).

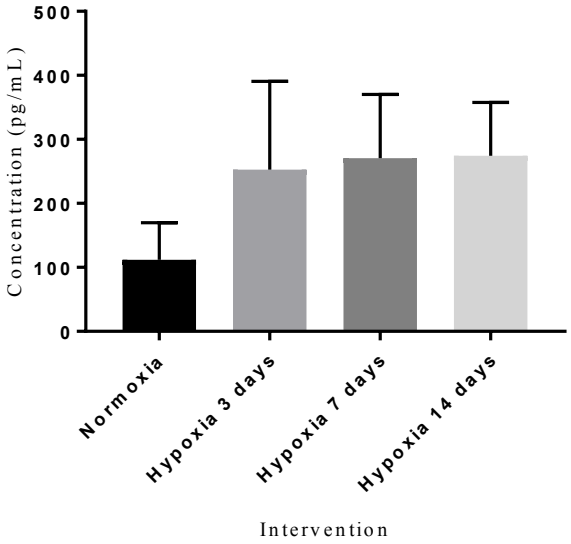

Figure 1. IL-6 Concentrations Without Given Maja Fruit Extract Group

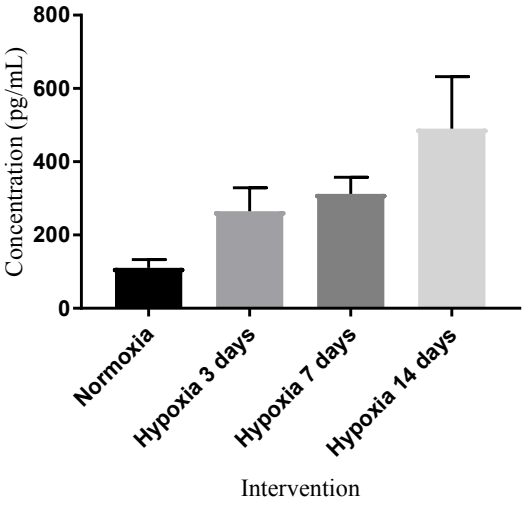

Figure 2. IL-6 Concentration After Given Maja Fruit Extract

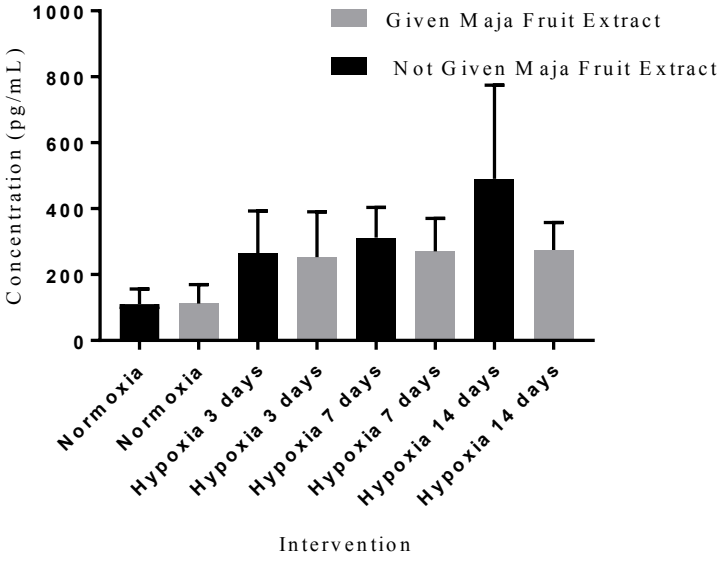

Figure 3. Comparison of IL-6 Concentrations Betwen Not Given and Given Maja Fruit Extract Groups

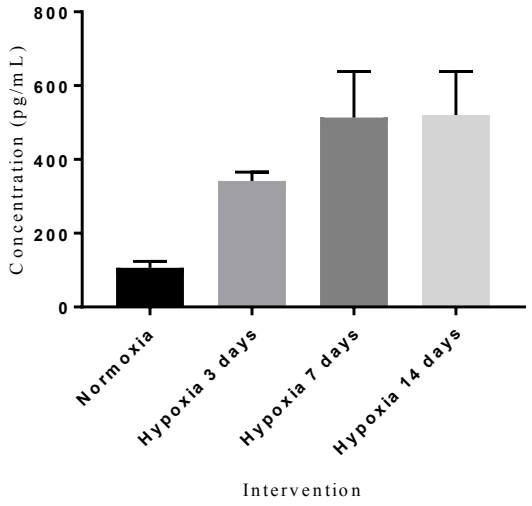

Figure 4. TNF-α Concentrations Without Given Maja Fruit Extract Group

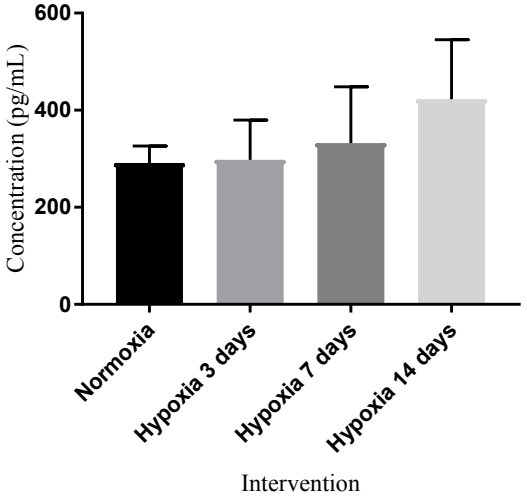

Figure 5. TNF-α Concentration After Given Maja Fruit Extract

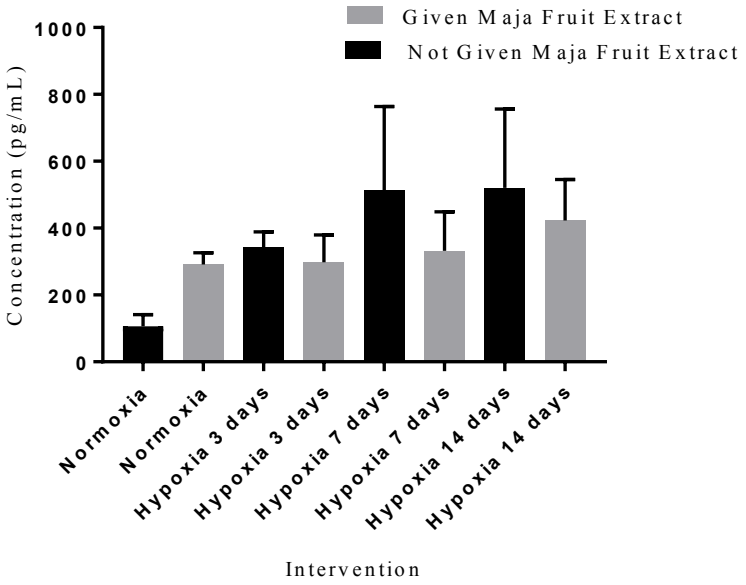

Figure 6. Comparison of TNF-α Concentrations Betwen Not Given and Given Maja Fruit Extract Groups

DISCUSSION

Our data shows that hypoxic treatment will cause an inflammatory process in line with the study of LI et al[9] which showed an increase in IL-6 concentration in hypoxic-induced animal serum. Maja fruit extract can suppress the formation of IL-6 in line with Behera's JP[10] research, which states that water extracts from immature Maja fruit can suppress the occurrence of the inflammatory process. IL-6 with CRP is one of the most commonly used inflammatory markers and can be used as a predictor of mortality or complications of a disease. Kuznicka et al's study showed an

increase in IL-6 and CRP in the elderly according to their age.[11,12]

Our study also shows that the Maja fruit extract can suppress the formation of TNF-α in accordance with the research of Arul et al[13], which states that the leaves of the Maja plant have anti-inflammatory abilities both acute and chronic. Sedger and McDermott's study found that TNF-α is an acute phase protein for atherosclerotic patients in the elderly.[2,14]

CONCLUSION

Our data clearly showed there is an increase in inflammatory processes due to hypoxia and the ethanol extract of Maja fruit (Aegle Marmelos) can suppress the formation of TNF-α and IL-6. So that the ethanol extract of Maja fruit can help slow down the aging process.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Acknowledgments

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