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The Effect of Uncaria Gambier Roxb Extract on Levels of F2-Isoprostanes in the Submaximal Exercise

Syahrastani*, Elsa Yuniarti and Dwi Hilda Putri

Abstract--- *Athletes always do strenuous physical exercises to prepare themselves to face a championship or a match in a short time. The exercise that fatiguing will increase the formation free radicals that can be damaged the cell membrane with peroxidation process lipid, so F2-isoprostane will formed. This process can be prevented by giving antioxidant from Uncaria gambierRoxbextract that containning highly level of catechins. This study is aimed to determine the effect of Uncaria gambierRoxb extract on levels of F2-isoprostan from Education Center and Sport Training Student in West Sumatera, who perform submaximal exercises. This study was experimented as pre-test and post-test group design to 17 students of football in PPLP West Sumatera. Uncaria gambierRoxb extract 500 mg are given 2 hour before submaximal exercises. The method to examine the F2-isoprostanes is ELISA. Statical analysis was paired samples t-test and the results obtained statistically significant when p <0,05. There is different effect of F2-isoprostanes levels before and after the administration of Uncaria gambierRoxb extract was 48,8+16.9 pg/ml vs 35,3+15,1 pg/ml, p<0.001. Giving Uncaria gambierRoxb extract may affecting the levels of F2-isoprostanes who perform submaximal exercise with a significant.*

Keywords--- *Uncaria Gambierroxb Extract, Submaximal Exercise, F2-Isoprostanes.*

I. INTRODUCTION

Athletes tend to do strenuous physical exercises to prepare themselves in short time when facing a championship. These strenuous exercises may not bring the maximum results and may pose high risk to the athletes that can lead to injury. These excessive physical exercises can be in forms of heavy training type, high intensity of exercises, long duration of exercises, and intensive training frequency [1].

Excessive physical exercises can cause adverse effect on homeostasis state of the body, which ultimately also affect body's systems work [2,3]. The high metabolic rate, a lack of oxygen supply, and an increase in lactic acid during strenuous exercise will stimulate the production of free radicals in form of Reactive Oxygen Species (ROS) [4,5].

Increased levels of F2-isoprostane that occurs as the harbinger of an increase of free radicals induced by lipid peroxidation during submaximal exercise and may cause direct damage to the endothelial cells of the blood vessels [6].

The increase in the number of free radicals will exceed the ability of the immune system and can not be neutralized by antioxidants in the body and it causes oxidative stress [7]. Therefore, when ROS levels increase

Syahrastani, Senior Lecturer, Department of Physical Education, Faculty of Sport Science, Universitas Negeri Padang, Indonesia. E-mail: syahrastani@yahoo.com*
Elsa Yuniarti, Lecturer, Department of Biology, Faculty of Mathematics and Science, Universitas Negeri Padang, Indonesia.
Dwi Hilda Putri, Lecturer, Department of Biology, Faculty of Mathematics and Science, Universitas Negeri Padang, Indonesia.

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beyond the antioxidant defense system of the body, the body needs to intake of antioxidants from the outside [7].

Intake of antioxidants can be obtained from the daily diet contained in vegetables, fruits, and spices that contain vitamin C, vitamin E, and flavonoids. Several flavonoid compounds are used as antioxidants are phenols and amines derivatives. Antioxidant phenols consists mostly of natural antioxidants and a number of antioxidant synthesis [8]. One of polyphenol compounds that functions as antioxidants is Gambier.

Gambier (Uncaria gambier Roxb) is a site-specific plant and main commodity of West Sumatra province. But until now, Gambier plant has not been optimally utilized by Indonesian people as a medicinal herb. Gambier leaf extract contains most of the Catechin and Tannic acids of Catechu which belong to flavonoids and antioxidants. Catechin is a secondary metabolite naturally produced by plants and includes the flavonoid. This compound has antioxidant activity because of its phenol group [8].

Several research have shown Catechin, in Gambier's leaf extract, has very powerful antioxidant activity. Through this research, it is expected that the extracts administered before submaximal exercises will suppress the occurrence of lipid peroxidation process which is illustrated by decreased levels of F2-isoprostane plasma of PPLP students doing submaximal physical exercises.

This research proposal aims to reveal the effect of administering Gambier's leaf extracts on submaximal exercises in minimizing damage impact of muscle tissue and vascular endothelium on athletes which is shown by F2-isoprostane level of PPLP West Sumatra students.

II. RESEARCH METHODS

This study is an experimental study with pre and post-test one-group design. In this study, first performed Gambier isolation leaf extract (Uncaria gambier Roxb) in the biochemistry laboratory of Department of Biology UNP. Leaves that are used are the leaves of fresh Gambier, steamed for 1 hour and then pressed by machine to separate between the precipitate and filtrate. The precipitate thus fractionized with ethyl acetate then evaporated. Later on, the results of the fractionation of ethyl acetate is washed with distilled water. After the rinsing process finished, it is dried with a freeze dryer and be the leaf extract of Gambier that will be used. Gambier leaf extract packed in capsules according to the dosage based on age and weight of each sample.

In this study, the samples are PPLP West Sumatra students who meet the inclusion and exclusion criteria. The inclusion criteria are male, BMI-ideal, and willing to participate in research. Exclusion criteria are being ill, have a history of metabolic disease, taking medication within 1 week old, active smokers, strenuous activity 24 hours before the intervention. Samples did submaximal exercise first by using a multi-stage test run (20-Meter Shuttle Test) to achieve maximal oxygen uptake (VO2mak) (Physical Therapy, 2000). After that, blood samples were taken from the cubital vein in the elbow crease as much as 5cc then added with EDTA in centrifuge at 3000 rpm for 15 minutes then partial of the plasma stored in a 80°C freezer. And 100 µL plasma of F2-isoprostane was measured before administration of Gambier leaf extract capsules. The examination of F2-isoprostane levels used F2-isoprostane Human Kit by the method of double antibody sandwich ELISA. Gambier leaf extract supplementation on a sample given 2 hours prior to submaximal exercise. Immediately after the second submaximal exercise, blood

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samples were taken back in the same way to measure F2-isoprostane plasma after administration Gambier leaf extract capsules. Examination of F2-isoprostane levels used F2-isoprostane Human Kit by the method of double antibody sandwich ELISA.

All data obtained are recorded in a special sheet, computerized and presented in the form of table or graph. Mean differentiation between the plasma level of F2-isoprostane between before and after administration of Gambier leaf extract capsule at submaximal exercise statistically tested using paired sample t-test with a significant value of $p < 0.05$.

III. RESULTS

Experimental studies have been conducted with research designs of pre and post-test group design on 17 West Sumatra PPLP soccer students.

Table 1: Characteristics of Research Subjects

Variabel	n	Mean	SD	Min – Mak
Student age (yr, mo)	17	16,7	0,5	15,8-17,4
height (cm)		172,8	4,9	163-183
weight (kg)		61,1	4,4	53-69
BMI (kg/m^2)		20,5	1,5	18,3-23,5
VO_2max (ml/kg/minute)		43,5	2,4	40,2-48,7

From the evaluation criteria VO2 max according to the AHA (American of Heart Association) the average value of VO2max in the good category (43-52 ml / kg BW / minute). From the average value of BMI can be in the normal category (18.5-24.9 kg / m2) according to WHO. However, according to the American Academy of Pediatrics (AAP) BMI is not recommended for assessing athlete's body composition because there can be a high interpretation of BMI in athletes not describing an overweight condition because the athlete's muscle mass is greater. Therefore, to assess the body composition of an athlete, measurements of% fat, fat mass, FFM, prediction weight, fat mass prediction, fat to gain27. The measurement can be done using a Body Composition Analyzer but this research was not carried out.

Table 2: Distribution of the Average Levels of F2-Isoprostans of PPLP Students Who did Submaximal Exercises Before and After Administration of Gambier Leaf Extract

	n	Mean (pg/ml)	SD (pg/ml)	p
Before treatment	17	48,8	16.9	0,001
After treatment	17	35,3	15,1	

The statistical test results obtained value of 0.001, it can be concluded that there is a significant difference between F2-isoprostane levels before and after administration of Gambier leaf extract. This shows that catechins can inhibit the lipid peroxidation process that occurs in cell membranes.

IV. DISCUSSION

Study conducted by Watson on 17 athletes whose blood had drawn at rest, after submaximal and high-intensity exercises and 1 hour of recovery, the levels of F2-isoprostane as markers of oxidative stress were higher after

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submaximal (38%), high-intensity exercises (45%), and 1 hour of recovery (31%) [9]. Robinson also got the same results in six male patients with Chronic Fatigue Syndrome (CFS) and 6 healthy controls showed that there was no difference between interleukin-6 (IL-6) and soluble interleukin-6 receptor (sIL-6R) in CFS patients at rest or during exercises. On the other hand, F2-isopoprostane plasma remained elevated at rest and after submaximal exercises for patients with CFS [10].

Increased levels of F2-isoprostane happens because of excessive physical activities that increase muscle contraction, metabolic rate, and temperature. Increased temperature and metabolic rate will lead to an increase in oxygen consumption in the vessels, so oxygen will decrease and an increase in free radical formation. The increase in free radicals causes damage to the endothelial cell membranes, where the amount of free radicals that are formed will be equivalent to the process of lipid peroxidation that occurs in the cell membranes that can be assessed with the formation of F2-isoprostane compounds.

In this study, Gambier leaf extract containing Catechin that functions as anti-inflammatory is given to suppress the production of F2-isoprostane thus obtain meaningful results. This study is backed by research conducted by Loke [11]. He found that polyphenols including Catechin, Quercetin and Teaflavin can reduce F2- isoprostane levels [11].

The same study was conducted by Natalie Eich in 2009 to 16 patients who suffered from oxidative stress. After having breakfast, patient had blood drawn to determine early plasma level of Epicatechin and then given 1 gram of grape extract containing Epicatechin and had their blood drawn four times with interval of 1 hour. There was a decrease of F2-isoprostane levels in the first three hours and an increase in the fourth hour [12]. In contrast to study conducted by Murphy [13], where the provision of Catechin in 32 healthy people showed no significant changes in the F2-isoprostane levels compared with controls. This was due to the process of lipid peroxidation in healthy people still can be neutralized by endogenous antioxidant so that F2-isoprostane levels were unchanged.

Research on the use of antioxidants are still studied and researched. Some research shows that the use of antioxidants will only increase levels in blood plasma but their effect on the body is still doubtful because of pharmacokinetics of these substances makes it not absorbed and excreted by body through urine or feces [14].

Several studies on the use of antioxidants in athletes are still underway because some theories say that while athletes produce much free radicals when exercising but the body has homeostatic mechanisms to neutralize these free radicals by increasing the production of endogenous antioxidants as the body's natural defenses against free radicals. Some literature says that it does not really matter to recommend antioxidant supplements to athletes, better to eat foods that contain antioxidants in your diet every day [15].

V. CONCLUSION AND SUGGESTION

There is a significant effect of Gambier leaf extract administration on submaximal physical exercise on F2-isoprostan levels. This study still has weaknesses, including a small number of samples and not comparing with a control group who were not given Gambier leaf extract. Further research is needed on the dose and duration of Gambier leaf extract administration.

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