

Effect of Tambelo (*Bactronophorus thoracites*) Extract for Body Weight on Male Mice (*Mus musculus*) During Physical Activity

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Abstract. Tambelo (*Bactronophorus thoracites*) is a marine invertebrate that lives as a mangrove borer which has decomposed. These animals by the Mimika community are known to have the potential to increase physical fitness if they have carried out excessive physical activity. Testing of compounds that have the potential to improve fitness in tambelo is not widely known. Therefore this study aims to identify the potential of tambelo extract in improving physical fitness in men carried out in vivo by using male mice test animals. In this study, the experimental animals used were male Swiss Webster mice. Mice were divided into three groups, namely the akuabidestilata and two groups treated with a dose of 1 or 3 g/kg b.w. Mice are treated with physical activity, in the form of a running test and followed by a swimming test. The parameters is body weight measured using analytical scales. The results showed the influence of tambelo extract in providing internal energy, this was indicated by the addition of body weight to the body of mice during physical activity. Based on the results of the study it can be concluded that the womb provides energy sources both internally and externally in mice during physical activity

I. Introduction

Physical activity is every movement of the body by skeletal muscles and requires energy expenditure, both internal energy and external energy. [1] Internal energy usually comes from reserves of energy sources in the form of glycogen, fat and protein. External energy comes from food eaten or supplements consumed to improve physical fitness. If the body lacks activity, it can be at risk of experiencing chronic diseases that can cause death globally [2]. On the other hand, excessive physical activity and lack of energy supply can cause the body to experience fatigue. Fatigue is a complex phenomenon that can be defined as the inability to maintain muscle strength, thereby reducing

performance during prolonged exercise [1]. One of the factors that causes fatigue is the lack of external energy supply, thus depleting internal energy reserves. The energy produced comes from food or supplements consumed.

Experts in the field of medicine try to create drugs (supplements) that help improve fitness, but supplements produced if consumed for a long time have the potential to have side effects. The side effects caused trigger other health problems, such as having liver or kidney dysfunction [2]. Therefore, WHO recommends the use of herbal medicine or traditional medicine because it is believed to be able to have a positive effect but reducing it does not even have side effects. In Indonesia, the use of natural materials as traditional medicine has been carried out by ancestors since centuries ago. Herbal medicines have been widely accepted in almost all countries in the world. In general, herbal medicines that are consumed come from animals and plants, both on land, river and sea. Marine organisms are able to produce chemical compounds, to be able to defend themselves from predatory attacks. These chemical compounds have the potential to produce [3]. The group of marine organisms that have been used as natural medicinal ingredients include sponges, sea cucumbers and mollusks.

Natural medicinal ingredients include sponges, sea cucumbers and mollusks. The type of mollusk used as herbal medicine by the Mimika Regency community is tambelo (*Bactronophorus thoracites*). Tambelo is a type of marine invertebrate and lives on mangrove trees that have decomposed (weathered). The Kamoro tribe, Mimika Regency, believes that tambelo can potentially be one of the herbal supplements that can stimulate physical fitness in men. But information about tambelo's potential to improve physical fitness in men is not widely known. One way to find out the potential of tambelo extract in improving physical fitness in men is done in vivo using test animals. Therefore the purpose of this research is to identify the potential of tambelo extract in improving physical fitness in men carried out in vivo by using male mice test animals.

2. Methodology

2.1 Time and Place of Research

This research was conducted from January 2016 to April 2017. Tambelo samples were taken from Timika, Papua. Tambelo extraction and physical activity testing in mice located at SITH ITB Animal House, SITH ITB Animal Development Laboratory and PAU ITB.

2.2 Materials and Tools

Tambelo, methanol (MeOH) solvent, aquabidestile, 30% KOH solution, Accutrend Plus strip (blood lactic acid, blood triacylglyceride, and blood glucose), aquades, 0.2% anthrone-sulfuric acid, 95% ethanol, ice cubes, tissue, gloves, syring to 1 ml. Coolbox, maserator, rotary evaporator, mortar, freedry, centrifugation, measuring cup (5 ml, 100 ml and 1L), mice cage, drinking bottle, oral sonde, stirring rod, stopwatch, glass swimming pool (40x40x50), plastic rotaroad ($r = 5$ cm) given dynamo and connected to electric current, headlamp, black plastic, digital camera, Accutrend Plus Lactate, ballast 5% of mice body weight, spectrophotometer, water bath, vortex, 5 ml and 12 ml test tubes, pipettes eppendorf (20 μ L, 100 μ L and 1000 μ L), yellow and blue eppendorf tips, a set of surgical instruments (scissors, tweezers, scalpels, surgical boards, pins) analytic scales, phalone bottles (10 ml and 50 ml), vial bottles 5 ml.

2.3 Test animals

The test animals used were male mice (*Mus musculus*) Swiss Webster strain, aged 6-8 weeks, with a mean range of body weight ± 20 -25 g.

2.4 Test Animal Acclimation

Before testing, acclimation to experimental animals was carried out for seven days to adjust mice by changing the daily behavior of mice where mice will be active during the day (when it's dark). Acclimation of mice is done by maintaining in the room with 12 hours of bright automatic lighting and

12 different dark hours with normal light periods (light starts at 7:00 a.m. and dark starts at 7:00 a.m.). Mice are kept in cages with husks that are equipped with eating places and drinking bottles. Each cage contains 3 mice. Feeding and watering are carried out in ad libitum, and weighing of all experimental animals is carried out during maintenance.

2.5 Determination of Groups and Number of Test Animal Repeat

This study uses a Completely Randomized Design (CRD). 24 mice were used, divided into four groups with each group consisting of six replications (calculation for each number of replications based on the formula [4]

; with T = number of groups and n = number of replications for each group. Mice are divided into four groups, namely

1. Solvent control, namely the group given aquabidilata
2. Tambelo extract dose 1 g / kg b.w
3. Tambelo extract dose 3 g / kg b.w

2.6 Collection of Tambelo

Tambelo is obtained from the Bruguera sp. Mangrove tree trunk. and Avicennia sp. which has decayed in the mangrove area of Timika Pomako, Papua. Tambelo was taken and then stored in a coolbox containing ice cubes for further extraction of methanol.

2.7 Making tambelo methanol extract [5]

Five kg of fresh tambelo are taken and cleaned by removing the teeth and pallet so that only the commonly consumed part is obtained, the abdomen. After cleaning it was obtained two kg then dried using freeze-dry at -50°C for 1x24 hours after which tambelo was crushed with mortar then weighed. 400g of tambelo were macerated using methanol (MeOH) solvents with a ratio of 1: 5 (400: 2000ml) at room temperature for 3x24 hours with filtration carried out every 1x24 hours. The resulting filtrate is combined and concentrated with a rotary evaporator. Concentrated extracts were obtained by GC-MS test while to determine chemical composition and analysis of amino acids using HPLC analysis. For the test preparation, the concentrated extract of tambelo is dried using freeze-dry and crushed and then dissolved with aquabidestile for later use.

2.8 Dose Determined

The dosage is determined based on the amount of tambelo meal consumed by the community from the lowest to the highest consumption of food [6]. The results of the lowest and highest human conversion to mice and ascorbic acid doses were determined based on adult consumption per day. The dose of tambelo extract and ascorbic acid was converted to mice based on conversion tables, so that the lowest dose of tambelo extract was obtained 1 g / kg bw and the highest dose was 3 g / kg bw, 130 doses of ascorbic acid mg / kg bw and the solvent control used is aquabidestilate.

2.9 Exposure to test preparations

Exposure of the test preparation, ascorbic acid and aquabidylata was carried out orally gavage in mice for 28 consecutive days. The injection volume given is 0.1 / 10 g b.b, then the mice are left for 30 minutes before testing physical activity in the form of running test and swimming test.

2.10 Testing of physical activity

2.10.1 Running Test [7] and Swim Test [8]; [9]

Mice are made running on a plastic rotarod with a diameter of 5 cm that is paired with dynamo and connected to an electric current. Rotarod is made at speeds of 30 and 40 rpm and is followed by a swimming test in a glass bath that has been provided with a water depth of 40 cm and a water temperature of 28°C . Testing of physical activity is divided into two, namely moderate activity and

severe activity. Moderate activities are characterized by lower frequencies in shorter duration of time carried out in weeks I and II which aim to exercise muscle strength. Heavy activity is characterized by a higher frequency and a longer duration which aims to maintain muscle strength. Testing of physical activity is carried out consecutively for four weeks, namely :

1. Week I and II of mice run at rotarod at 30 rpm for 20-30 minutes followed by swimming test for 10-15 minutes (without addition of 5% load on tail)
2. Weeks III and IV of mice run on rotarods with a speed of 40 rpm for 30-120 minutes and are followed by swimming tests to fatigue which is indicated by the heads of mice below the water surface for ± 7 minutes (addition of 5% load on the tail).

2.11 Measurement body weight (bw) of mice

Measurement of mice BB was carried out every day before being given treatment for 28 consecutive days. Mice are weighed using analytical scales.

2.12 Data Analysis

The data obtained were analyzed by SPSS 16 using the analysis of variant test (ANOVA) with further tests using the Duncan test at a significant level of 0.05

3. Results and Discussion

Based on the research conducted, the mean body weight of mice in each treatment group during physical activity is shown in Figure 1.

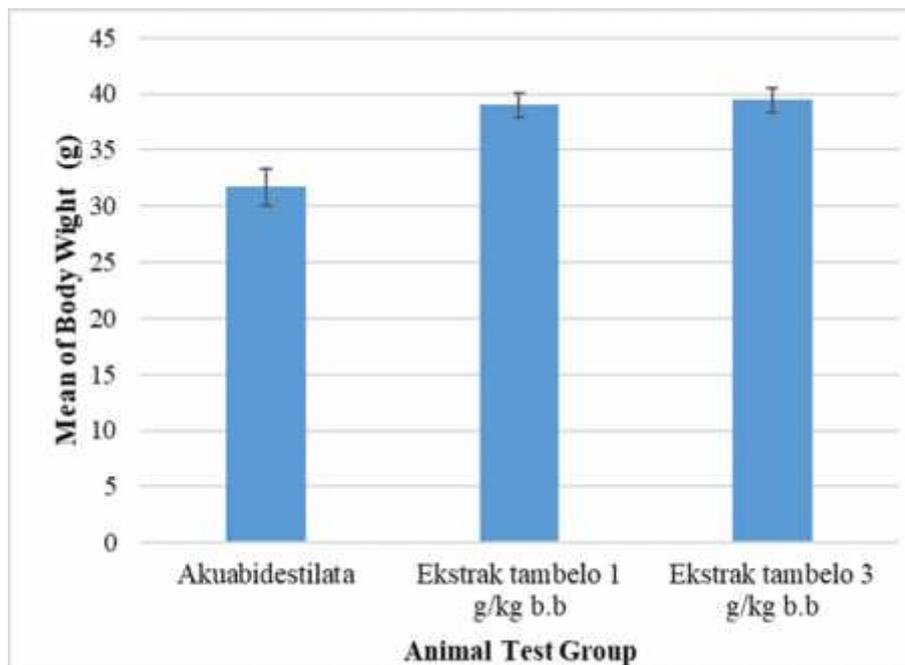


Figure 1. The mean BB of each test group is significantly different ($p < 0.05$, mean \pm SD, $n = 6$)

Fig. 1 shows that there is a significant difference in BB for each group of test animals ($p < 0.05$). BB for tambelo extract group 1 or 3 g / kg b.b was significantly higher when compared with the group of akuabidestilata and ascorbic acid. This shows that tambelo extract is able to provide an energy source both external and internal energy which is characterized by a high BB in the tambelo extract group compared to the group of aquabidestile and ascorbic acid. When doing physical activity, the body needs energy. The main energy source in the body comes from glucose. Glucose will be broken

down into energy in the form of ATP. Glucose can be obtained from food or supplements consumed. Excessive glucose in the body is stored in the form of glycogen both muscle glycogen and liver glycogen and fat in the form of triglycerides.

[10] explained that tambelo extract given to mice can increase and provide external and internal energy storage in the form of glucose, glycogen and triglycerides. It is assumed that the height of BB in the tambelo extract group was influenced by the content of the compounds contained in the tambelo extract. [10] also explains that there are 16 types of amino acids and eight types of fatty acids, namely four saturated fats and four unsaturated fats. The highest amino acid contained in tambelo extract is alanine. In this study, alanine is thought to play a role in regulation of glucose use as an energy source when carried out physical activity. Alanine contained in muscle cells is passed into the blood and liver. In the liver, alanine is converted to pyruvate and NH_2 . Pyruvate is converted into glucose into the bloodstream and then passed into muscle cells to be used as an energy source during physical activity. Excess deposits of glucose are stored in the form of glycogen in both the muscles and liver. Besides being stored in the form of glycogen, glucose can be stored as fat through the process of gluconeogenesis. Therefore BB in the tambelo extract group is higher compared to the other groups.

[11] also explained that tambelo n-hexane extract contained 15 types of fatty acids while [10] explained that in addition to amino acids, methanol (MeOH) extract of tambelo also contains eight types of fatty acids, this is because of the different types of solvents used. The n-hexane solvent is a type of non-polar compound solvent while the methanol solvent is a type of solvent that is capable of dissolving polar compounds. The type of fatty acid contained in tambelo extract can be used as an energy source during physical activity. [10] explained that mice induced by tambelo methanol extract and carried out physical activity had a higher blood triglyceride concentration (below the upper / normal threshold) compared to the solvent control group (akuabidestilata). Triglycerides are one form of fat deposits in the body that can be used as an energy source. When physical activity is carried out, the body will need considerable amounts of energy. The energy obtained comes from food or supplements and is used by the body in the form of glucose while glucose / carbohydrate deposits are in the form of glycogen, fat deposits in the form of triglycerides. The high fatty acids contained in tambelo extract were able to provide a source of energy in the form of triglycerides so that the body weight of the tambelo extract group was higher compared to the group of aquabidestile.

4. Conclusion

Based on the results of the research conducted it can be concluded that tambelo extract is able to provide external and internal energy sources such as glucose, glycogen and fat in the form of triglycerides characterized by high BB in the tambelo extract group compared to other groups.

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