Cardiac Histopathology of Male Mice (*Mus musculus L.*) Given Maximum Physical Exercise and Gambir Catechin (*Uncaria gambir Roxb.*)

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Abstract. Physical exercise is an activity carried out to maintain body health and fitness. However, optimal physical exercise will harm the body because it produces free radicals that can damage cells and body tissues. Gambir is a plant whose main component is catechin which functions as an antioxidant. Antioxidants in gambir can prevent the progression of heart disease and lipid peroxidation that produces malondialdehyde (MDA). This study aims to see the histology of the heart muscle of mice (*Mus musculus L.*) given maximum physical exercise and catechin of gambir (*Uncaria gambir Roxb.*) with concentrations of 80%, 90%, and 95%. The research was conducted at the Laboratory of Zoology and Anatomical Pathology, Faculty of Medicine, Universitas Andalas. The method used in making cardiac histology is the paraffin method with Hemaktoxilin-Eosin staining. Data were analyzed descriptively. The results showed that maximum physical exercise caused heart muscle damage. Furthermore, the higher the gambir catechin concentration, the better inhibiting free radical damage formed due to maximum physical exercise. The conclusion is that gambir catechin with a concentration of 95% with a dose of 100 mg is the best concentration and dose in repairing damage to the heart muscle.

Keywords: Physical exercise · Gambir · Catechin

1 Introduction

Physical exercise is a structured, planned, and repetitive activity to maintain and improve health. Physical exercise can positively impact regulating metabolism, cognitive function, and cardiovascular function depending on exercise type, intensity, and duration [1, 2]. On the other hand, physical activity or exercise can be stressors for the body. For example, suppose the stress given to the body is carried out regularly, programmed and well measured. In that case, the body will form a coping mechanism that will turn the stress into a simulator that can improve physical fitness as a form of body adaptation.

On the other hand, physical exercise that is carried out with maximum and unmeasured intensity can disrupt body homeostasis and even damage the heart [3]. This is

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supported by research that shows changes in the histological picture of rat heart muscle given maximum physical exercise for ten days without rest. Damage to the heart muscle is characterized by ischemia and infarction [4].

The heart is an organ that can adapt quickly. The increased need for oxygen during physical activity increases oxygen delivery to the heart. Therefore, the heart will convert energy from fatty acids and glucose into mechanical energy so that energy is fulfilled for actin-myosin interactions in myofibrils. Maximum physical activity can increase oxygen consumption 100–200 times because of an increase in metabolism in the body. An increase in oxygen consumption by contracting muscles causes SOR (Reactive Oxygen Compounds) to occur due to an increase in electron leakage by mitochondria, resulting in increased production of free radicals that can cause cell damage [5].

Tissue damage caused by free radicals can be prevented by fulfilling enzymatic protection in cells to inhibit free radical propagation reactions and detoxify the formed free radicals. Naturally, in cells, various enzymatic and non-enzymatic antioxidants protect and defend cell organelles from the effects of free radical damage [6]. Although the body has a natural defense against free radicals, external antioxidant intake is needed under certain conditions, such as relatively strenuous physical exercise and insufficient endogenous antioxidants [4].

The province of West Sumatra produces about 90% of gambir and is exported by Indonesia to several countries, including Pakistan, Singapore, India and Bangladesh. Gambir plant (Uncaria gambir Roxb.) is a plant that contains high antioxidants. Gambir contains polyphenols such as alkaloids, terpenoids, flavonoids and other polyphenolic compounds. The flavonoid components in gambir consist of catechin (7–33%) pyrocatechol (20–30%) and quercetin (2–4%) [7]. Catechin is an anti-inflammatory, antioxidant, antitumor, antibacterial, and antiviral [8]. Various preclinical studies state that consuming catechin positively correlates with heart health through various mechanisms, namely antioxidation, antihypertensive, anti-inflammatory, antiproliferative, anti-thrombogenic and lowering fat levels [9]. Antioxidants in gambir can prevent the progression of heart disease and lipid peroxidation that produces malondialdehyde (MDA). Other benefits of gambir include as an anthelmintic, antibacterial, stimulant of In addition, the autonomic nervous system and as a stomach ulcer drug [10, 11].

Based on the above background, there has been no research on the histopathological picture of the heart of mice (*Mus musculus* L.) given maximum physical exercise and gambir catechin (*Uncaria gambir* Roxb.)

2 Methods

2.1 Place and Type of Research

The research was conducted in the Zoology laboratory, Faculty of Mathematics and Natural Sciences, Padang State University, Anatomical Pathology Laboratory, Faculty of Medicine, Andalas University. This research is an experimental study with a completely randomized design (CRD).

The test animals used were mice (*Mus musculus* L.), which were acclimatized for a week before receiving treatment. Mice were fed pellets and drank water. The cages used were trays with 30 cm x 20 cm x 11 cm. The mice were divided into 11 groups,

namely K1 not given gambir extract and not given maximum physical exercise (negative control), K2 not given gambir extract after being given maximum physical exercise treatment (positive control).), P1 was given 80% gambir catechin at a dose of 50 mg, P2 was given 80% gambir catechin at a dose of 100 mg, P3 was given 80% gambir catechin at a dose of 200 mg, P4 was given 90% gambir catechin at a dose of 50 mg, P5 was given 90% concentration of gambir catechin at a dose of 100 mg, P6 was given 90% gambir catechin at a dose of 200 mg, P7 was given 95% gambir catechin at a dose of 50 mg, P8 was given 95% gambir catechin at a dose of 100 mg, and P9 gambir catechin was given with a concentration of 95% at a dose of 200 mg. Maximum physical activity is carried out for 28 days with a maximum capacity of 5 times a week for 30 min.

2.2 Maximum Physical Treatment in Mice

The maximum physical exercise treatment given refers to the research of Ciulla et al., [9]. Mice are forced to swim in a container without a way out. During the maximum activity, the time needed is calculated. Mice will swim, dive and climb the walls of the container as a form of effort to get out of the container. When the mice appear to be resting and taking energy, the mice are submerged using tweezers. When the mice stopped their movements and only made movements to survive (maintaining their heads on the surface of the water), then the mice were considered to be doing a maximum activity.

2.3 Gambir Dose Calculation

It begins by suspending gambir powder into aquadest. The volume of drug administration (gambir suspension) (ml) is:

$$\frac{\text{Dose (mg/kg BB)} \times \text{BB (kg)}}{\text{Concentration (mg/ml)}}.$$

2.4 Cardiac Histology

Cardiac histology was made using the paraffin method and stained with Hematoxylin-Eosin.

2.5 Tissue or Organ Removal

Tissue removal is the first step in making these preparations, namely, dislocating the mice and performing surgery. The part taken is the heart of mice.

2.6 Fixation

Fixation aims to strengthen the tissue and prevent damage to the structure and active components of the tissue. The fixation solution used was 10% formalin.

2.7 Dehydration

Dehydration aims to remove the water in the tissue by using a medium that can fill the water in the tissue. Dehydration uses substances that have properties opposite to fixatives, namely serial alcohol solutions (70%, 80%, 90%, 95%) and absolute alcohol I, II, and III. The time required is: soaking in 70% alcohol for 3 days, 80% alcohol for 1 day, 90% alcohol for 1 day, and 95% alcohol for 1 day. Immersion in absolute alcohol I for 1 h, in absolute solution II for 1 h and absolute solution III for 1 h.

2.8 Clearing

The clearing process aims to remove the remnants of material remaining on the network, in this process using xylol I, II, III for 30 min-1 h.

2.9 Infiltration

The purification medium is replaced with the thawed planting medium in the infiltration process. The paraffin used are generally soft (melting point 48-500C), medium paraffin (melting point 52-540C) and hard paraffin (58-600C).

2.10 Embedding

The infiltrated organs are implanted in a mould and filled with hard paraffin, placed at room temperature until the paraffin hardens and then transferred to the refrigerator. After hardening, the paraffin is cut and pasted on the block.

2.11 Sectioning

The incision is made using a microtome by attaching the object to the block holder, then adjusting and directing the cutting surface with a microtome knife. The tissue was cut to a thickness of 4μm. Good cut results are put into a water bath that has been filled with distilled water and heated to a temperature of 38–40 0C. Before being affixed to the slide, the slide is first smeared with egg white. This is so that the pieces are easily attached to the object glass and are not easily separated during the colouring process. Then the tissue is placed in an incubator to make the attachment more perfect.

2.12 Staining

The dye used was Hemaktocillin-Eosin, and the series of preparations were selected and arranged in a preparation rack. The next step was deparaffinization using xylol I, xylol II and xylol III solutions with 5 min of immersion for each solution. The next stage was rehydration using absolute alcohol solution I, absolute II and absolute II for 3 min, 70% alcohol for 3 min. Minutes, 80% alcohol for 3 min, 90% alcohol for 3 min. Then wash with tap water for 5–10 min twice. Then enter the Hemaktocillin staining stage for 5 min. Then soaked again using tap water, put into the Eosin solution for 5 min, and washed again.

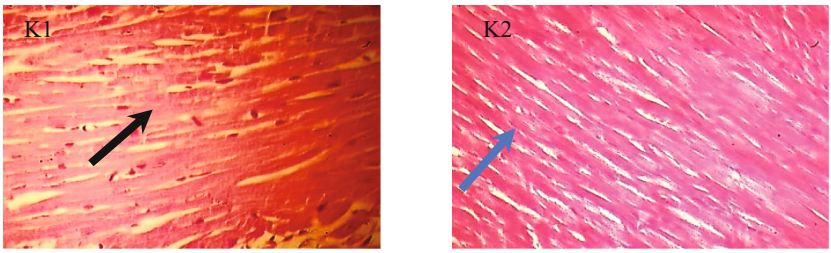

Fig. 1. Histology of the heart of mice (Mus musculus L.) negative control (K1) and positive control (K2)

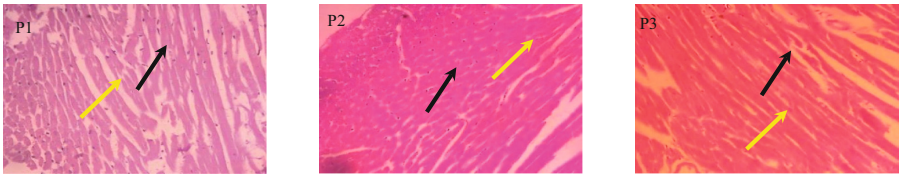

Fig. 2. Histological description of the heart of mice (Mus musculus L.) given gambir catechin with a concentration of 80% with dose levels of 50 mg (P1), 100 mg (P2) and 200 mg (P3). The black arrows show the necrotic cell nuclei and the yellow arrows show the hypertrophied and hyperplastic heart muscle.

The next stage is dehydration, namely removing water from the tissue using 70% alcohol, 80% alcohol, 90% alcohol for a few seconds, absolute alcohol I for a few seconds, absolute alcohol II for 1 min, absolute alcohol III for 3 min, xylol I, xylol II and Xylol III for 3 min each. Finally, the preparations were mounted and observed under a microscope.

2.13 Data Analysis

The data obtained were analyzed descriptively by looking at and comparing the histopathological differences in the heart muscle of Mice (Mus musculus L.)

3 Result and Discussion

The negative control heart muscle (K1) cross-section was not treated. Cross-sectional pattern of cardiac myocytes and normal cell nuclei shown by black arrows. Positive control (K2) was only given maximal physical exercise Cardiac muscle was necrotic. The nucleus of the cell undergoes karyorexia. The blue arrow indicates the occurrence of hypertrophy and hyperplasia, namely an increase in the number of cardiac muscle cells so that they are denser.

A physical exercise carried out optimally will trigger an imbalance in the production of free radicals with the body's antioxidants, which is called oxidative stress. As a result, oxygen consumption will increase by 20 times in the body and 100 times in muscle

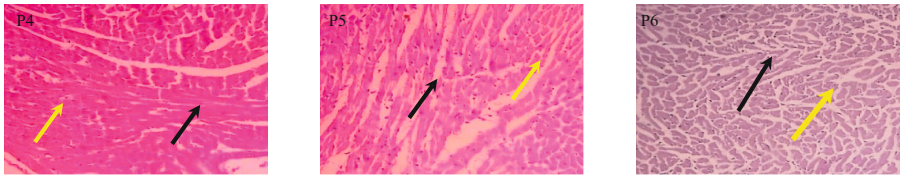

Fig. 3. Histological description of the heart of mice (Mus musculus L.) given gambir catechin with a concentration of 90% with a dose level of 50 mg (P4), 100 mg (P5) and 200 mg (P6). The black arrows show the necrotic cell nuclei and the yellow arrows show the hypertrophied and hyperplastic heart muscle.

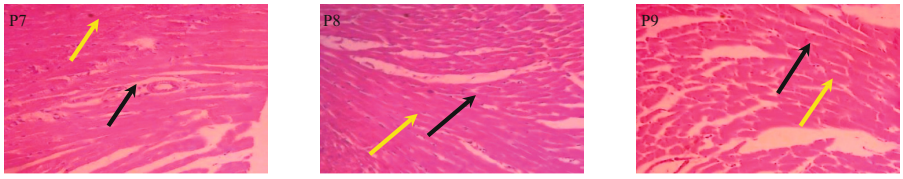

Fig. 4. Histological description of the heart of mice (Mus musculus L.) given gambir catechin with a concentration of 95% with dose levels of 50 mg (P7), 100 mg (P8) and 200 mg (P9). The black arrows indicate normal cell nuclei and the yellow arrows indicate that the heart muscle is hypertrophied and hyperplastic.

fibres during maximum physical exercise. This increase in oxygen consumption triggers an increase in the production of free radicals that can cause cell damage [8].

Physical exercise that is carried out continuously causes adaptation and hemostasis in the body. Changes in the physiological structure, namely the heart muscle structure, are responded by neuromuscular and are characterized by changes in the number and diameter of muscle fibres (hypertrophy) due to increased composition and muscle tone [3]. Physical exercise that is carried out continuously causes adaptation and hemostasis in the body. Changes in the physiological structure, namely the heart muscle structure, are responded to by neuromuscular and characterized by changes in the number and diameter of muscle fibres (hypertrophy) due to increased muscle composition [12].

In addition, the heart muscle also undergoes necrosis. Necrosis is characterized by typical nuclear changes, namely pyknosis, karyolysis and karyorrhexis. Pyknosis is characterized by a shrinking nucleus, dark colour, irregular borders and the presence of chromatin agglomeration on Hemaktocillin-Eosin (HE) staining. In the karyocresic stage, the nucleus disintegrates into fragments of chromatin substance. When chromatin becomes lysed, it is referred to as karyolysis [13]. Cardiac muscle fibres or myocytes are stable cells that cannot regenerate if they undergo necrosis because they do not have satellite cells such as skeletal muscle. Necrosis in the heart muscle will be replaced by connective tissue, affecting heart function. After comparison, it is seen that the higher the concentration of gambir catechin used, the better in repairing damage due to maximum physical exercise.

4 Conclusion

Based on the research that has been done, it can be concluded that the 95% concentration of gambir catechin with a dose of 100 mg is the best concentration and dose in repairing damage to the heart muscle.

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