

The Effect of Aerobic Exercise against D-galactose and AlCl₃-induced Hepatosteatosi s in Mus Musculus C57BL/6J

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ABSTRACT

In the 21st century, sedentary habits and consumption of caramelized food packed in aluminium foil made the oxidative state on the body. There are differences in opinions about aerobic exercise and its effects on inflammation and oxidative stress. This research aims to compare the liver histologic pattern between the group which was given aerobic exercise and not given after being induced with D-galactose and AlCl₃..: This research used an experimental method using two groups of Mus musculus C57BL/ which was injected with D-galactose (90 mg/kg body weight) and AlCl₃ (40 mg/kg body weight). The control group was only injected with those substances. The aerobic group was intervened with swimming for 30 minutes each day (6 days a week). After being sacrificed, HE staining was done in the liver specimens to evaluate the bile duct proliferation and steatosis changes. There were significant differences in biliary duct proliferation ($p = 0.043$) and steatosis changes ($p = 0.043$) in an aerobic group compared to the control group. Aerobic exercise which was conducted 30 minutes for 6 days a week showed more bile duct proliferation and increased steatosis changes.

1. Introduction

The liver is a complex organ with various homeostatic functions, such as a site for drug metabolism and as an excretion pathway of endogenous and exogenous compounds (Beckwitt, 2018). Drug and toxic substance poisoning are common in recent decades, but can be prevented (Orsini). Medicines, household products, plants, cleaning products, and cosmetics are toxic substances that often cause poisoning (Nistor, 2018).

In the 21st century, foods that contain high levels of sugar such as fast foods, caramelized foods, and products processed using *aluminum foil* are common. D-galactose as a form of monosaccharide from sugar products is used to form energy and macromolecules synthesis in the body obtained from lactose (dairy products) (Ohlsson, 2017). The ability of D-galactose to bind with free amino acids (glycation) will produce AGEs (*Advanced Glycation End Products*). AGEs bind to the RAGE (receptor the *Receptor for Advanced Glycation End Products*) in the liver, causing elevation of free radicals in cells and activating intrinsic signaling pathways such as PKC, PI3K/Akt, Src/RhoA, JAK/STAT, MAPK/ERK, and NADPH oxidase cause inflammation, cell apoptosis, and accelerate the progression of *non-alcoholic fatty liver disease* (NAFLD) (Asadipooya, 2019).

Aluminum chloride (AlCl₃) is a hepatotoxin that is usually found in medicines, food, untreated water, cosmetics, cooking utensils, and others. This is proved by elevation of ALT (*Alanine aminotransferase*), AST (*Aspartate aminotransferase*), ALP (*Alkaline phosphatase*), bilirubin, total cholesterol, triglycerides, and blood sugar. (Ighodaro, 2012) On biochemical examination it can also be found lower levels of GSH (*Glutathione*) and SOD (*Superoxide dismutase*) which are natural antioxidants in the liver. (Kalaiselvi, 2014) Chronic exposure to aluminum can cause liver damage (Geyikoglu, 2013).

Apart from intoxication, oxidative stress is also an important factor in liver damage. Aerobic exercise is believed to produce a

hepatoprotective response to tissue damage by increasing antioxidants (Nikbin, 2020). There was an increase in antioxidant levels, such as *heat shock protein 70* (HSP70) and *glutathione peroxidase* (GPx), as well as a decrease in oxidative stress biomarkers such as malondialdehyde (MDA) during aerobic exercise (Ahmadian, 2014). With an increase in antioxidants and a decrease in oxidative stress, it is expected to decrease the inflammatory response that occurs, especially in the liver. In contrast, several studies have showed an elevation of inflammation markers, such as interleukin-6 (IL-6) and *tumor necrosis factor- α* (TNF- α), (Sloan 2018) as well as *reactive oxygen species* (ROS) (SHI, 2007). at the time of aerobic exercise.

There have been many studies related to aerobic exercise that has been assessed biochemically and biomolecularly, but not many have been studied in the fields of histology and histopathology. The effects of aerobic exercise on inflammation and oxidative stress still show differences of opinion. Therefore, researchers wanted to see a picture of the biomolecular changes at the cellular level by assessing differences in liver histology in samples induced by D-galactose and AlCl_3 with aerobic exercise treatment compared to samples only induced by these toxic compounds.

2. Materials and Methods

2.1 Experimental animals and research design

This research is an experimental study, induction, and intervention of experimental animals was carried out at the Laboratory of Anatomic Pathology, University of Indonesia, Jakarta, Indonesia, and the preparations was carried out at the Laboratory 20 to 25 grams, are maintained at 20 to 25 °C on a 12-hour controlled light/dark cycle. Laboratory standard cages are used to breed experimental animals with access *ad libitum* to food and drink. All experimental animal procedures were carried out in accordance with the Helsinki declaration. This research was approved by the Research Ethics Committee of the Faculty of Medicine, Atma Jaya Catholic University, Jakarta, Indonesia (No: 10/12/KEP-FKUAJ/2016). The Mice were divided into 2 research groups. Group 1 (control) consisted of three mice induced by oxidative stress without aerobic exercise treatment and group 2 (aerobic) consisted of three mice induced by oxidative stress with aerobic exercise treatment (swimming for 30 minutes for 6 days) at the same time as chemical induction.

3. Methods

3.1 Treatment Of experimental animals

Hepatitis was induced experimentally using D-galactose (Sigma-Aldrich Co., St. Louis, MO, USA) (dose 90 mg/kg) and AlCl_3 (Sigma-Aldrich Co., St. Louis, MO, USA) (dose of 40 mg/kgBW) intraperitoneally and using intervention (control or aerobic exercise) every day for 6 days and sacrificed on the 7th day. The mice were anesthetized using ether then sacrificed and the liver tissue was taken.

3.2 Analysis of histopathologic

Liver specimens was fixed in formalin *phosphate-buffered saline* (PBS) 10% for the analysis of histopathologic, and then embedded in paraffin, cut with a thickness of 5 to 10 μm , and stained with hematoxylin and eosin (H & E) using standard techniques, then observed under the microscope. Signs of liver damage (biliary duct proliferation, degree of steatosis) were assessed by a pathologist from the Department of Anatomic Pathology, Faculty of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia in every 10 fields of view with 100x magnification.

Biliary duct proliferation was assessed in the portal area. Normally, one to two bile ducts should be found in each portal vein. Observations are made on at least 10 portal veins. The bile ducts are considered proliferative if there are more than two bile ducts per portal vein. Degree of steatosis assessment is done by looking for fat infiltration in the form of microsteatosis and macrosteatosis at 10 large visual fields and classified as 0=no fatty infiltration, 1=<5% hepatocytes affected, 2=5-33% hepatocytes affected, 3=34-66% hepatocytes affected, 4=>66% hepatocytes affected. The average of biliary duct proliferation and the degree of steatosis of 10 visual fields in each rat were recorded for statistical analysis.

The results were analyzed using SPSS software version 24.0 (SPSS Inc., Chicago). The differences in the degree of bile duct proliferation, the degree of steatosis, the degree of fibrosis, and the number of pseudoglandular found were analyzed using an independent t-test if the distribution was normal or using the Mann-Whitney test if an abnormal distribution was found. The differences are significant statistically if $p < 0.05$.

4. Results

The differences in markers of liver damage between the two groups can be seen in Table 1. There was a significant elevation in bile duct proliferation ($p = 0.043$) and degree of steatosis ($p = 0.043$) in the aerobic group (Fig. 1) compared to the control group (Fig. 2).

Table 1. Histological Changes in Both Groups.

Markers of liver damage	Control group	Aerobic group	P-value
Biliary duct proliferation	1.2 (0.9 – 1.2)	3.3 (2.9 – 3.3)	0.043
Steatosis	1.3 (1.0 – 1,3)	2.6 (1.6 – 2.6)	0.043

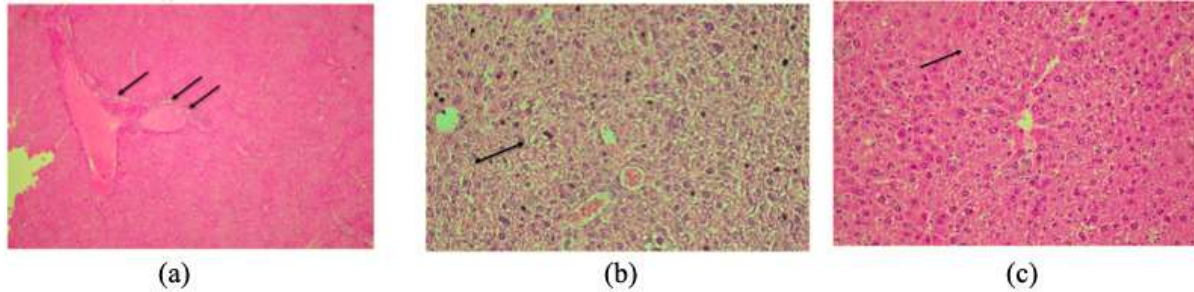


Figure 1.

Histological cross-section of the liver in aerobic group mice. (a) bile duct proliferation is seen (black arrow), (b) hepatocyte steatosis is seen with *ballooning hepatocytes* (black arrow), and (c) hepatocyte macrosteatosis is shown (black arrow). (400x magnification).

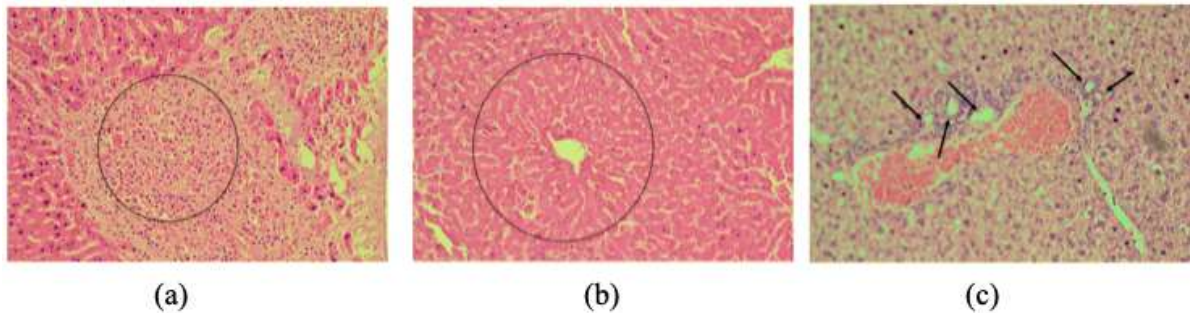


Figure 2. The histological cross-section of the liver in the control group mice. (a) inflammatory cell infiltration and blanching of hepatocytes are seen, (b) hepatocytes have lost their nucleus, and (c) bile duct proliferation is seen (black arrow). (400x magnification).

5. Discussion

Biliary duct proliferation is a response to alcohol, toxins, or drugs that cause extrahepatic obstruction and liver damage (Hall, 2017). This is followed by changes in the extracellular matrix around the newly formed bile ducts resulting from the production of cytokines, chemokines, growth factors, and angiogenic factors by reactive ductular cells (Pinzani, 2018). Proliferation in these ducts can induce fibrosis directly through the *epithelial-mesenchymal transition* (EMT), which is a process in which mature epithelial cells lose their intercellular contact and characteristic pattern of epithelial protein expression, thereby acquiring the phenotypic characteristics of the mesenchymal cells (Glaser, 2009). *Myofibroblastic hepatic stellate cells* (HSC) will be formed as a result of EMT, which is a producer of myofibroblasts, resulting in liver fibrosis (Yu, 2018).

In the aerobic group, there was an average biliary duct proliferation which was higher than the control group. This may be due to the aerobic exercise intervention carried out in the acute phase of inflammation, which causes an increase in ROS produced by mitochondria. This is because a part of the liver falls on ischemia state followed by reperfusion and reoxygenation, which induces excessive production of free radicals (Gonçalves, 2018). Aerobic exercises can trigger lipolysis and cause an elevation in ROS from fat peroxidation by-products (Hashida, 2017). This may lead to a fibrogenic effect with HSC activation. This increase in mitochondrial ROS will also cause hepatocyte steatosis through the *phosphatidylinositol 3-kinase pathway*. (Kohli, 2007). This can be seen from the difference in the degree of hepatic steatosis which was found to be higher in the aerobic group compared to the control group.

5.1 Limitations and Disadvantages of the Study

This study has several limitations. There is a limited number of experimental animals that can be used in this study, besides the use of Masson's trichrome staining has advantages over H&E to assess the degree of fibrosis in this study.

6. Conclusions

Aerobic exercise performed six times a week for 30 minutes every day in this study can increase the degree of liver damage (more bile duct proliferation accompanied by a higher degree of fatness) in *C57BL/6J Mus Muculus* induced by D-galactose and $AlCl_3$

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