

Physical Exercise Does Not Improve Colon Inflammation in Mice Induced Lambda Carrageenan

Ana Qonitatillah^{1*}, Kristanti Wanito Wigati², Roedi Irawan³

¹Medical Science Program, ²Physiology Department, ³Child Health Department, Nutrition and Metabolic Disease Division. Dr. Soetomo General Hospital, Faculty of Medicine, Universitas Airlangga, UNAIR A-Campus, Tambaksari, Surabaya, East Java, Indonesia 60132
*Corresponding author: anaqonitatillah2@gmail.com

Abstract

Inflammatory Bowel Disease (IBD) is a well known reported gastrointestinal tract disease, which the prevalence continuous to increase in Southeast Asia and other developing countries. Animal model have already been widely used for gut inflammation study. Lambda (λ) carrageenan is a chemical substance which commonly used to induced inflammation in IBD animal models. Physical exercise is a non-pharmacological therapy recommended for IBD. However, further study is needed to determine the effects of this therapy. This study was aimed to determine the physical exercise effect on colon inflammation in mice induced λ -carrageenan. Thirty-eight mice were divided into 2 groups. Control group (C) which induced with λ -carrageenan and treatment group (T) which induced by λ -carrageenan and treadmill exercise. The degree of colon inflammation was obtained by histopathological examination using Hematoxylin-Eosin (HE) staining and inflammation scoring system, which include inflammatory cell infiltration and intestinal architecture. All mice colon samples in both group were inflamed with varying degrees. The treatment group had a higher Inflammatory degree score than control group ($p < 0.05$). Physical exercise does not improved the degree of colon inflammation in mice induced λ -carrageenan due to uncontrolled stress induction and water intake containing λ -carrageenan.

Key words: IBD, inflammatory cell infiltration, intestinal architecture, λ -carrageenan, physical exercise, treadmill

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INTRODUCTION

Inflammatory Bowel Disease (IBD) is intestinal inflammation whose causative factors of the disease are not fully understood yet. Among all gastroenterology cases, IBD, which consist of Ulcerative Colitis (UC) and Crohn's Disease (CD) is widely reported (Whittle *et al.*, 2010). IBD is associated with immune complex disorders, influenced by genetic and environmental factors (Wehkamp *et al.*, 2016). Physical exercise is constantly associated with an increase in the immune system (Gleeson, 2007). Therefore, IBD patients are encouraged to do physical exercise as a management for the treatment of the disease. Physical exercise recommendations are mainly to reduce symptoms, complications, and extraintestinal manifestations of IBD (Narula and Fedorek, 2008; Packer *et al.*, 2010).

In the last few decades, there have been many experimental studies for examining intestinal inflammation, which is very useful for obtaining new information about the pathogenesis of a disease and preclinical evaluation for a new therapy (Kolios, 2016). Experiments with colitis require induction of chemicals, one of which is λ -carrageenan, because it has potential inflammatory effect in the bowel. Intestinal tissue that has been induced by chemicals such λ -carrageenan, has special characteristics when evaluated through histopathological analysis HE staining using a preexisting scoring system (Ghia *et al.*, 2006).

However, the literature which stated that physical exercise can reduce the symptoms of IBD is weak and inconsistent (Narula and Fedorek, 2008). The effectiveness of physical exercise and its mechanism on disease activity is unclear (Narula and Fedorek, 2008). So that further research is needed to strengthen the

opinion that physical exercise can be done for IBD (Narula and Fedorek, 2008) and experimental and clinical research is also needed to confirm its effectiveness as an inflammatory bowel therapy (Ko and Anyeung, 2014).

This study was aimed to determine the physical exercise effect on colon inflammation in mice (*Mus musculus*) induced λ -carrageenan.

MATERIALS AND METHODS

Ethical Approval

All procedures were approved ethically under supervision of the Health Research Ethic Committee, Universitas Airlangga. Letter number 232/EC/KPEK/FKUA/2019.

Animals

A total of 38 BALB/c mice were obtained from Animal Laboratory, Biochemistry Department in Faculty of Medicine, Universitas Airlangga. During the experiment, all mice were housed in a well-controlled environment and kept free access to mineral water and the same animal diet. After a one-week adaptation period, assigned to control and treatment groups. Both of group were induced by λ -carrageenan supplemented in drinking water at a concentration of 20 mg/L (Shang *et al.*, 2017).

The treatment group was given treadmill exercise, 7 m/minutes for 30 minutes (include 5 minutes warming up, 20 minutes exercise, and 5 minutes cooling down) (Kim *et al.*, 2018), and 3 times a week (Durigan *et al.*, 2009). After six weeks of treatment (Shang *et al.*, 2017), all mice were sacrificed with cervical dislocation technique.

Chemical and Reagents

λ -carrageenan, Tokyo Chemical Industry co., Ltd.[®]

Sample Collection and Procedure

Colon samples were collected in the 6th week of the study. Colon samples were fixed in 10% neutral-buffered formalin for 12-24 hours and embedded in paraffin, cutted and stained with HE. Tissue samples were well-oriented with

longitudinally cut crypts to precisely assess alterations in the overall intestinal tissue architecture.

Histopathological Evaluation

Samples were evaluated by standard light microscope with 100x and 400x magnification. The degree of inflammation was scored based on the criteria for chemically-induced colonic inflammation in the journal by Erben *et al.*, 2014., which shown on Table 1.

Statistical Analysis

Data were collected and analyzed statistically for correlation between groups using statistical software (IBM SPSS Statistics version 20). All results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

All of colon samples in both group showed inflammation (38/38) with varying degrees. The number of samples in each score are shown in Figure 1.

In the control group, there was one sample with score 2, twelve samples with score 3, and six samples with score 4. Score 3 is the dominant score in the control group (Figure 2). In the treatment group, there was one sample with score 3, seventeen samples with score 4, and one sample with score 5. Score 4 is the dominant score in the treatment group (Figure 3). The highest inflammation degree score in this study was 5 (Figure 4). The Inflammation degree score showed significant differences regarding control and treatment group ($p < 0.05$).

Mechanism of Induction λ -carrageenan to gut inflammation is unclear and multifactorial, partly because of the stimulation of pro-inflammatory cytokines, damaging the epithelial barrier, and disrupting the innate intestinal mucosal immune response to microorganisms (Malik *et al.*, 2009; Buffie *et al.*, 2009), reducing the amount of glycoprotein in the colon (Al-Suhail *et al.*, 1984), inhibiting interaction between macrophages and lymphocytes (Chong

Table 1. Inflammation scoring system: Inflammation score is the sum of score A and score B

Inflammatory cell infiltrate		Score A
Severity	Extent	
Mild	Mucosa	1
Moderate	Mucosa and Submucosa	2
Marked	Transmural	3

Intestinal Architecture		Score B
Epithelial changes	Mucosal Architecture	
Focal Erosion		1
Erosion	+/- Focal Ulceration	2
	Extended focal ulceration	3
	+/- granulation tissue	
	+/- pseudopolyps	

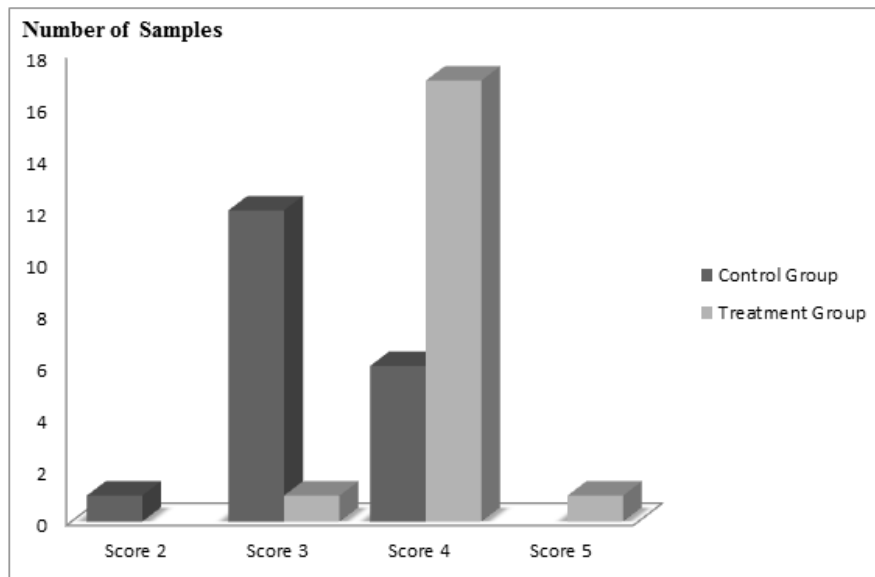


Figure 1. The distribution of the number of samples for each inflammation score

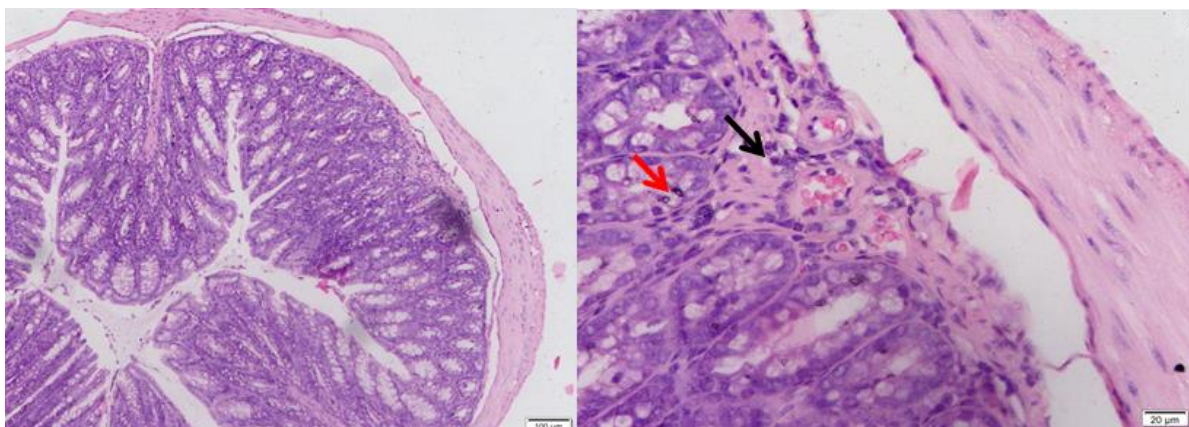


Figure 2. Representing histomorphology of colon tissue with sum scores 3 referred to Table 1, submucosal infiltrates (score A:2) with focal erosion (score B:1); Original magnification x100 and x400; black arrows-inflammatory cell infiltration within submucosa; red arrows-focal erosion; HE tissue stained

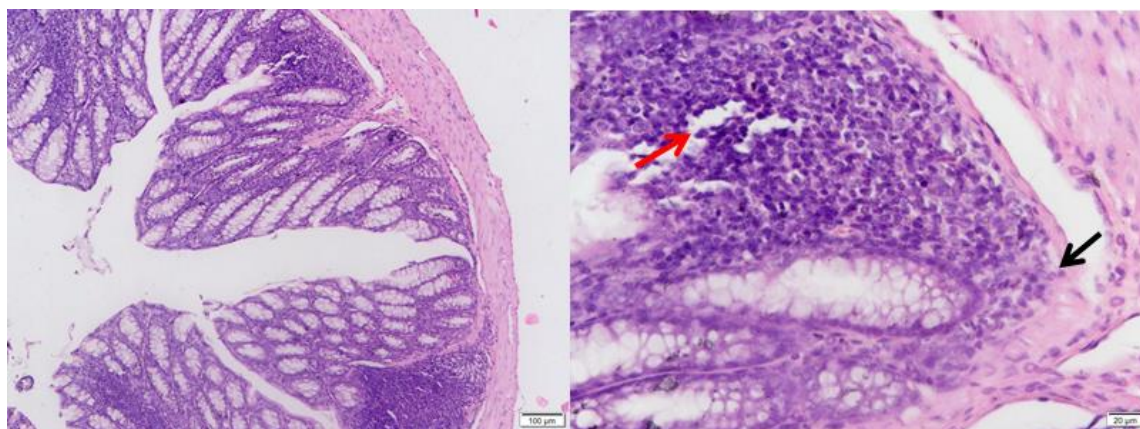


Figure 3. Representing histomorphology of colon tissue with sum scores 4 referred to Table 1. submucosal infiltrates (score A:2) with focal ulceration (score B:2); Original magnification $\times 100$ and $\times 400$; black arrows-inflammatory cell infiltration within submucosa; red arrows-focal ulceration; HE tissue stained

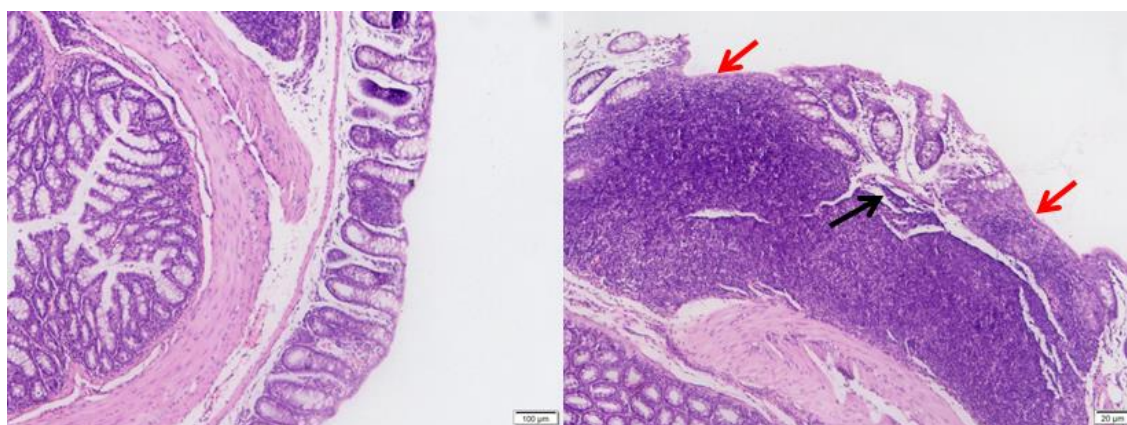


Figure 4. Representing histomorphology of colon tissue with sum scores 5 referred to Table 1. submucosal infiltrates (score A:2) with extensive ulceration (score B:3); Original magnification $\times 100$ and $\times 400$; black arrows-inflammatory cell infiltration within submucosa; red arrows-extensive ulceration; HE tissue stained

and Parish, 1985), induces inflammation in the presence of microbiota because studies in guinea pigs induction carrageenan produces caecum ulceration on days 21-30 (Onderdonk *et al.*, 1977), whereas germ-free guinea pigs do not produce any intestine lesions for up to 6 months of induction (Onderdonk *et al.*, 1978), stimulates the inflammatory cascade in the colon epithelial cell by increasing IL-8 production (Borthakur *et al.*, 2007) which is a key pro-inflammatory cytokines (Martino *et al.*, 2017) and so on. Although the mechanism has not been ascertained, the findings of the effect of carrageenan on intestinal inflammation can already be proven. Increasing IL-6 and TNF- α

expression and reduced IL-10 (Wei *et al.*, 2016) and intestinal inflammation (Wu *et al.*, 2016).

The effect of physical exercise on inflammation has been proven from previous studies, namely reducing the production of inflammatory cytokines in vitro (Flynn *et al.*, 2003), as another example of physical exercise reducing TNF- α , IL-6, and IL-1 β from skeletal muscle in adults (Gielen *et al.*, 2003). However, this study found that physical exercise did not reduce inflammation. There are several studies that have found that physical exercise does not alleviate inflammation, especially not significantly reducing inflammatory biomarkers (Nicklas *et al.*, 2004; Marcell *et al.*, 2005; Hammett *et al.*, 2006; Nassis *et al.*, 2005).

However, previous research only proves that physical exercise does not provide anti-inflammatory effects, there is no research that says physical exercise exacerbate the presence of inflammation.

In the results of this study, physical exercise exacerbated the degree of inflammation, and was significantly different in the treatment group which treated with physical exercise compared to the control group without physical exercise. This can be due to several factors, which includes the inappropriate method of physical exercise and the poorly conditioned environment that give more stress on mice, thus exacerbating the presence of inflammation.

The inappropriate physical exercise may give the same appearance with forced physical exercise. Voluntarily and forcefully physical exercise would affect the intestinal microbiota in mice, as proved by differences in both diversity and structure, and could affect many sites in the intestinal tract, such as the caecum and colon (Jacob *et al.*, 2015), where changes in the microbiota could cause IBD (Allen *et al.*, 2015). Dysbiosis can also cause the release of endotoxins and flagellins from Gram-negative bacteria that bind TLR-5 which will induce inflammation and damage from intestinal epithelium (Vijay-Kumar *et al.*, 2010; Cani *et al.*, 2008).

In another study conducted by Svensson *et al.* in 2016, it was found that forced physical exercise can induce stress responses by increasing anxiety and corticosterone levels, ischemia causes neuronal damage in the hippocampus and results in increase cytokines in brain tissue and blood flow (Svensson *et al.*, 2016). Thus it can be concluded that forced physical exercise will increase stress, ischemia and neuronal damage indirectly, and can induce inflammation and epithelial damage directly. And in this study, stress was not only obtained from the inappropriate method of physical exercise, the other two reasons are likely to cause mice stress, the first is the treatment group which given physical exercise and had to be moved three times a week, while the control group was almost never moved except when

cage was being cleaned. Cage-switch induces stress on rats has been proven by Morimoto *et al.* study in 1993 (Morimoto *et al.*, 1993), and the second in the treatment group mice, exercise was conducted during the day so that it interferes mice circadian rhythms, that resulted in mood disturbance (Vadnie *et al.*, 2017).

Moreover, mice given physical exercise will need more water intake (Goodrick, 1978), therefore in this study, the treatment group will drink more water, and in this study induction of colon inflammation using λ -carrageenan diluted in drinking water, thus it can cause more severe inflammation in treatment group.

CONCLUSION

Physical exercise in mice induced λ -carrageenan resulted in worsening of inflammation both in terms of inflammatory cell infiltration and intestinal architecture.

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