Conference Paper

# Iron Overload Reduces Cholestrol and Triglyceride Serum of Mice

**Devi Agustin Setiawati[1], Mas Rizky A. A. Syamsunarno[1,2,3], Pandji Irani Fianza[4], Nur Atik[5], Neni Anggraeini[1,2,6], Mohammad Ghozali[2], Ratu Safitri[2,3,7], and Ramdan Panigoro[2]**

[1]Department of Biochemistry and Molecular Biology Faculty of Medicine Universitas Padjadjaran
[2]Central Laboratory, Universitas Padjadjaran
[3]Department of Biology, Faculty of Mathematics and Natural Sciences, Bandung-Sumedang Street KM 21$^{st}$, West Java 45363, Indonesia
[4]Department of Internal Medicine Faculty of Medicine Universitas Padjadjaran, Pasteur Street No. 38, Bandung, West Java, Indonesia
[5]Medical Laboratorium Technologyst of Bakti Asih School of Analyst, Padasuka Atas Street No.233, Padasuka, Cimenyan, Bandung West Java, Indonesia
[6]Department of Anatomy and Cell Biology, Bandung-Sumedang Street KM 21$^{st}$, West Java 45363, Indonesia
[7]Study Program of Biotechnology, Post Graduate School, Universitas Padjadjaran, Dipati Ukur Street no. 35, Bandung West Java, Indonesia

Corresponding Author:
Mas Rizky A.A. Syamsunarno
rizky@unpad.ac.id

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## Abstract

Introduction: Most of the case of patient with hemoglobin disorder genes need routine blood transfusion throughout their life. It leads to increase iron accumulation with liver as the main organ for iron storage. Liver is the main organ for metabolic process, including triglyceride and cholesterol. However, correlation between metabolism of triglyceride and cholesterol, and iron overload condition is remain uncertain. The purpose of this study was to investigate the effect of iron overload to triglyceride and cholesterol level in the serum of mice.

Material & Method: Three groups of mice were divided by the dose of iron dextran (0, 0.1 and 0.3 mg/mouse). Iron dextran was injected intraperitoneally. After 14 days of treatment, liver histology and serum triglyceride and total cholesterol were examined.

Result & Discussion: Liver weight was higher in iron dextran injected mice proportional with dose injection. The liver histology showed normal tissue and slightly inflammation condition with no fibrosis sign. Total cholesterol and triglyceride serum were lower 21,46% and 27,68% respectively in mice injected with 0.3 mg/mouse of iron dextran compare to control group.

Conclusion: Iron dextran injection in 0.3 mg/mice of dose reduces cholesterol and triglyceride serum without alteration liver morphology.

**Keywords:** iron overload; Triglyceride serum; Cholesterol Serum.

# 1. Introduction

There are about 5% of world population carrying hemoglobin disorder genes especially thalassemia.[1] This condition would make individuals need routine blood transfusion throughout their life. patient who receive regular blood transfusion will have iron accumulation much higher than normal range.[2] Iron overoad condition in the body can be defined as increase of iron deposition with no consideration about the presence of tissue destruction.[3] Iron overload can results in organ dysfunction due to high iron concentration which likely generates toxic free radicals and leads to parenchymal cells destruction.[4, 5] The most affected organs caused by that condition are heart, liver, pancreas and endocrine glands.[6–8]

In the iron overload condition, liver would have higher iron deposit leads to hepatic iron overload condition and end up with liver fibrosis.[6, 8–10] Hepatic iron overload would likely to generate free radicals and lipid peroxidation causing progressive hepatic injury result in fibrosis, cirrhosis and hepatocyte carcinoma.[11–13]

Hepatic iron overload is suggested having relation with alteration of hepatic lipid metabolism. High iron concentration in the liver may give influence to increase activity of Acyl-CoA cholesterol Acyltransferase (ACAT) which then improve intrahepatic cholesterol esterification and take effect in secretion of Very Low Density Lipoprotein (VLDL) – cholesterol. Besides, hepatic iron level enhancement may cause upregulation of the transcription of seven enzymes related to cholesterol synthesis, in other words, it increases hepatic cholesterol synthesis.[9] Iron overload also increases intracellular lipid droplets formation through higher expression of 1D cluster (Class 1 inconventional MHC) which related to pathogenesis of non-alcoholic steatohepatitis.[9, 14]

The underlying mechanism of relationship between metabolism alteration due to iron overload condition in the liver is remain uncertain. This study is aimed to investigate the effect of iron overload to triglyceride and cholesterol level in the serum of mice.

# 2. Material & Method

## 2.1. Animals

This study was approved by Health Research Ethics Committee Faculty of Medicine Universitas Padjadjaran The subjects of the study are 18 male mice (*Mus musculus)* aged 10-12 weeks. Mice are purchased from Department of Pharmacology Faculty of Medicine, Universitas Padjadjaran. Mice were housed in room with condition of 12/12 h

light and dark cycle and adequate air circulation and had unrestricted access to water and standard chow. Mice were adapted for 7 days before experiment.

## 2.2. Grouping of Experimental Groups According to Dosage of Iron Administration

The subjects are divided in 3 groups based on the dose of iron injection:

a. Group I (control): given intraperitoneal NaCl physiologic 0.2ml injection

b. Group II: given 0.1mg/mouse intraperitoneal iron dextran injection every day

c. Group III: given 0.3mg/mouse intraperitoneal iron dextran injection every day

Every group was injected with hemadex intraperitonially with different dose for 14 days.

## 2.3. Measurement of Blood Glucose Concentration

On the day 3, 6, 9 and 13 we measure the blood glucose level of the mice. The measurement is conducted by tail vein blood collection continued with shed the blood into the glucometer strip (gluco DR, allmedicus, Gyeonggi-do).[15]

## 2.4. Organ Harvested

After 14 days, blood was collected from the infraorbital vein and kept in eppendorf tube. Mice were sacrified by cervical dislocation technique and liver was harvested. Liver weight was measured and stored in container tube with formaldehyde solution.

## 2.5. Hematoxylin & Eosin (HE) and Masson's Trichrome Staining of Liver Organ

For HE staining, liver organ were deparaffinize and then stained by Hematoxylin solution. After being rinsed by running tap water, it then counterstained by Eosin solution.

The liver were also stained using Masson's Trichrome staining. Firstly, the sample was fixated into Weigert's iron hematoxylin. After that, it stained using three different solutions: Biebrich scarlet, phosphomolybdic acid and methyl blue solutions.

### 2.6. Measurement of Cholesterol and Triglyceride Serum

Blood was incubated in room temperature for 15 minutes. Serum was extracted by centrifuged 1500 rpm for 30 minutes. Total cholesterol (CHOL5 RTU, Akurat Intan Madya, Jakarta) and triglyceride (TG5RTU, Akurat Intan Madya, Jakarta) was measured based on manufacture protocol.[15]

### 2.7. Statistic Analysis

Results were analyzed using one-way ANOVA for samples and Bonferroni's posthoc multiple comparison tests were performed to evaluate the differences between groups A p-value <0.05 was considered as statistically significant.

## 3. Result and Discussion

### 3.1. Liver Weight/Body Weight Rasio

The measurement of liver weight showed that the liver weight of group I were 1.92 ± 0.28 gram, while group II were 2.18 ± 0.13 and group III were 2.20 ± 0.38 gram. Liver weight of the mice was proportional with the iron injection dose.

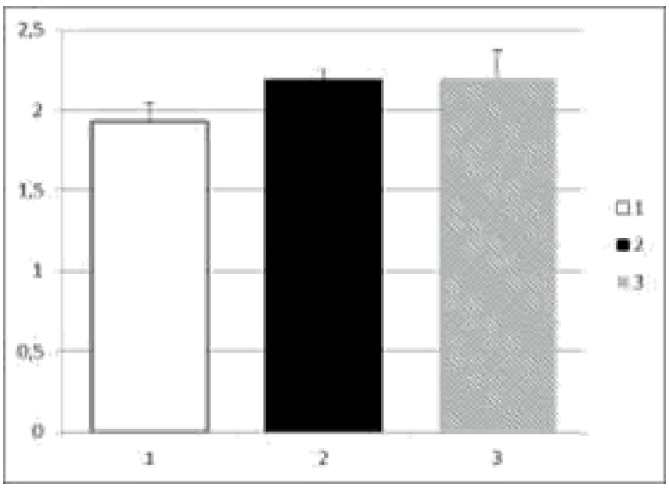
This finding is indicated as the manifestation of liver enlargement due to increase iron accumulation in the body, especially in the liver. When iron uptake if exceed the capacity for export, hepatocytes will be the major site of iron reservoir. Iron accumulation in the liver can be stored in non-parenchymal hepatocellular, exclusively in hepatocytes or mixed hepatic iron deposition in both parenchymal and non-parenchymal hepatocellular.[13, 16]

The graphics show higher liver weight in 0.3 mg iron injection group compared to others. However, the difference is less significant.

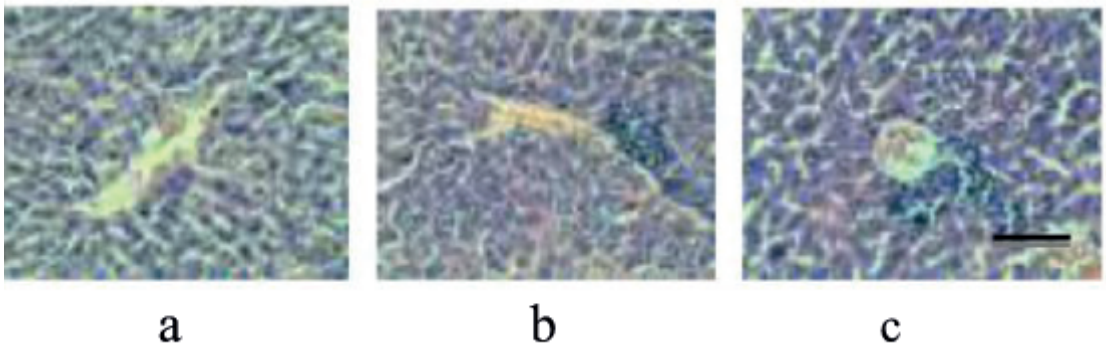
The ratio of liver weight/body weight showed similar trend. The ratio in group I is 55.53 ± 8.24 (mg/gr), while in group II is 53.72 ± 9.90 (mg/gr), and group III showed 48.91 ± 6.26 (mg/gr).

### 3.2. Liver Histology of Mice

Table 1: Liver weight of each groups (gram)




Note: 1: control group, 2: 0.1 mg iron injection group, 3: 0.3 mg iron injection group



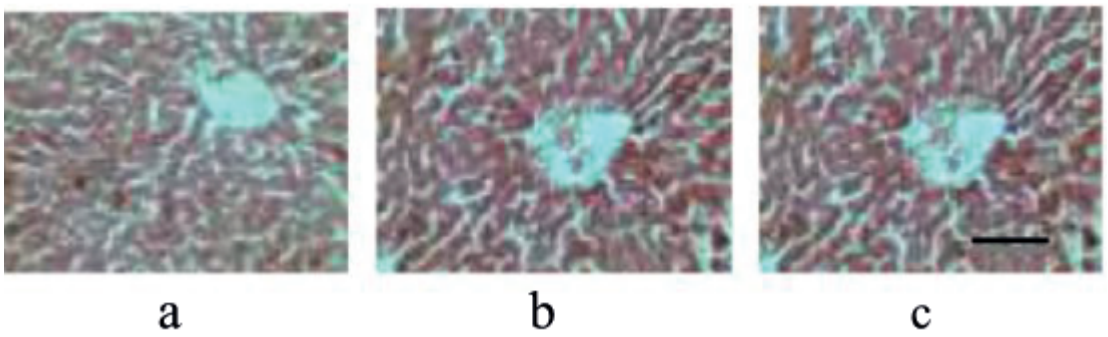

Note: HE staining of liver in (a) control group (b) 0.1mg iron injection group and (c) 0.3mg iron injection group. Scale bar: 100μm

**Figure** 1: HE staining of Mice Liver.

## 3.3. Total Cholesterol serum

Cholesterol serum of the group I was higher among other groups. Total cholesterol serum of group I was 211.28± 63.04 mg/dL, while total cholesterol of group II was 186,40 ± 41,12 mg/dL and the group III was 165.94± 53.04 mg/dL. Cholesterol serum in Mice injected with 0.3 mg/mouse of iron dextran was 20% lower compare to control, Although the data was not statistically significant, our data showed that cholestrol serum concentration tend to decrease after iron overload. this finding suggest that excessive iron might diruspt genes corresponding to hepatic cholesterol synthesis.

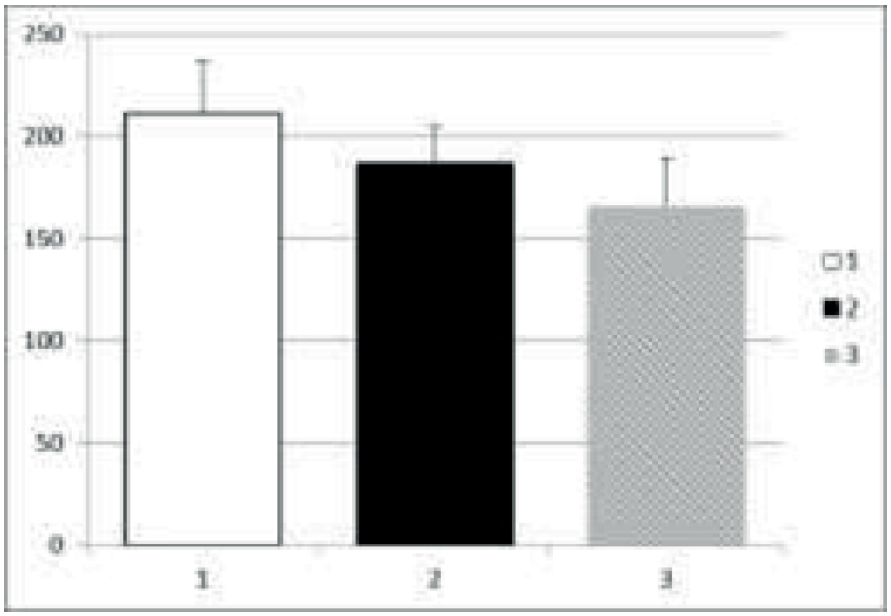
Generally, the liver showed no inflammation state condition in all groups. The previous study showed that there was a close connection between increase iron intake and activation of inflammatory genes. There was increase of liver expression f genes such as TNF-α, IL-6 IL-1β and CRP which indicate the activation of inflammatory




Note: MT staining of liver in (a) control group (b) 0.1mg iron injection group and (c) 0.3mg iron injection group. Scale bar: 100μm

**Figure** 2: Masson's Trichrome staining of Mice Liver.

Table 2: Total cholesterol serum in each groups (mg/dL).




1: control group, 2: 0.1 mg iron injection group, 3: 0.3 mg iron injection group

pathways.[19] However, we did not find fibrosis sign after the histological analysis. It might be due to low dose and short period of iron injection

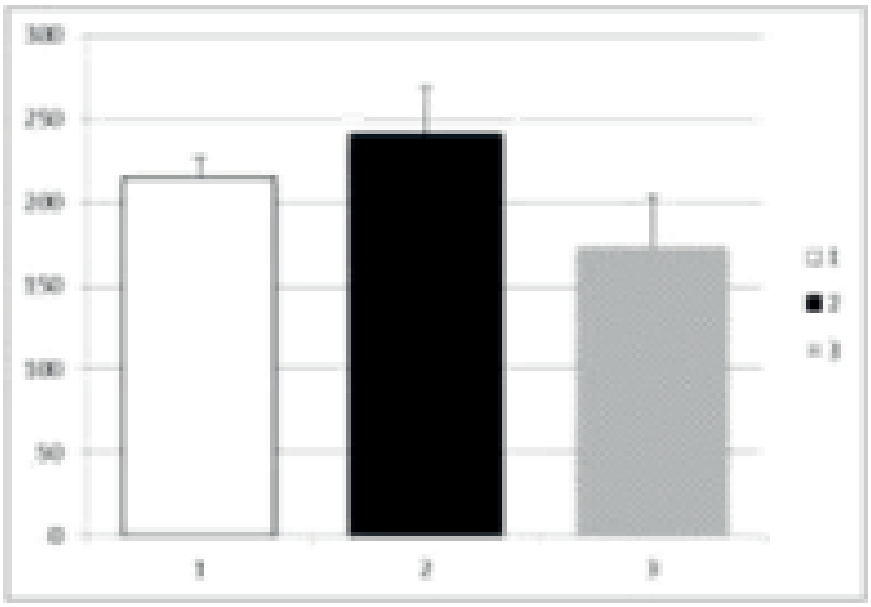
Liver is the main site of iron storage in the body and it also the important site of lipid metabolism. Thus, iron accumulation in the liver may interact and alter hepatic lipid metabolism.[9, 15] Previous study about alteration of cholesterol synthesis in iron overload condition showed that from various genes related to cholesterol synthesis, some genes showed unsignificant increase of expression while a gene showed negative correlation with increase of cholesterol synthesis.[14]

The other study also revealed decline of both forms of 3-hydroxy 3-methylglutaryl CoA synthase (HMGCSA1, HMGCSA2) which considered as enzymes involved in de novo synthesis of cholesterol.[17]

## 3.4. Total Triglyceride serum

Total triglycerida serum of the subjects showed similar result. Total triglyceride serum of group I was 215.11 ± 27.25 mg/dL, while group II was 240.45 ± 63.48 mg/dL and group III was 165.94 ± 53.04 mg/dL.

Table 3: Total triglyceride serum of each groups (mg/dL).



Note: 1: control group, 2: 0.1 mg iron injection group, 3: 0.3 mg iron injection group

Iron overload has corelation with lipid metabolism, however the conclusion is still debatable. Previous study showed that iron overload might alter lipid metabolism in liver through increase in the activity of acyl-CoA cholesterol acyltransferase (ACAT) coupled with reduced activities of HMG CoA reductase and 7α-hydoxylase. Since ACAT was related to VLDL-cholesterol secretion, this condition indicated upregulation of secretory athway of lipid. Meanwhile, other study about hepatic iron overload using carbonyl iron in the methionine-choline deficient rat model of NAFLD is associated with decreased hepatic triglyceride.[9]

Another study of iron overload in humans homozygous for the Cys282-Tyr (C282Y) mutation in HFE, which causes hemochromatosis, plasma low-density lipoprotein (LDL) cholesterol has been found to be reduced. Besides, there was also negative correlation between iron overloas and upregulation of transcriptions of Abca1 and Abcb4 which related to cholesterol export to the plasma.[14] Therefore, reduction of triglyceride secretion might be caused by condition mentioned above which mainly related to decrease of VLDL secretion.

## 4. Conclusion

Iron dextran injection in 0.3 mg/mice of dose reduces cholesterol and triglyceride serum without alteration of liver morphology.

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