

## EFFECT OF WATER CLOVER (MARSILEA CRENATA) EXTRACT ON TRIGLICERYDES OF THE HYPERTRIGLYCERIDES RATS

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### ABSTRACT

**Background:** High levels of triglyceride (TG) is a risk factor in cardiovascular disease. The flavonoids in water clover contain 10,58 mg of extract. This research aims to prove that flavonoids can decrease triglyceride.

**Materials and Methods:** True experimental study used design pre-post test control group design. The sample was selected based on by completed randomized design sampling to be divided into five groups, the negative control (n=5) were only given normal diet, positive control (n=5) were only given high cholesterol diet and treatment groups with three doses water clover extract (n=15) were given cholesterol diet during the 14 days and water clover extract for 14 days. Statistical analysis used Paired Sample T-Test and MANOVA (Multivariate Analyses of Variance).

**Result:** The results of this research can decreased TG levels as an effect of water clover extract before and after treatment, that there was significant ( $p. < 0.05$ ). Levels of triglycerides in negative control group compared with positive control and third treatment showed a significant difference ( $p.< 0.05$ ).

**Conclusion:** Conclusions of this research is water clover extract has effect to decrease TG levels significantly.

**Keywords:** hypertriglyceridemia, Water Clover, flavonoid

## 1.0 Introduction

Hypertriglicemia is the increasing concentration of triglycerides (TG) levels. Triglycerides levels of 150 mg/dL is associated with a risk of cardiovascular disease in both men and women (Austin, 1998). Triglycerides levels of women were increased in the United States in 2009-2012 by 14,7% at the age of 20-39 years, 23% at the age of 40-59 years and 21,5% at the age over 60 years. While in men were increased by 25,1% at the age of 20-39 years, 34,9% at the age 40-59 years and 28,7% at more than age of 60 years (Carol, 2013). Based of Healthy Basic Research in 2013, the population of Indonesia experienced an increased of TG in high and very high category in men as much as 14,7% and woman 10,2% (Riskesdas, 2013).

Triglycerides are ester compound, compose of glicerol and three fatty acid molecules. Triglycerides are stored in the body lacks glucose supply (Hartono, 2006; Jae-Kwang, 2014). However, TG rich chylomicron and very low density lipoprotein (VLDL) remnants which rapidly penetrate arterial wall and contribute atherosclerotic lesions and associated with APO-C3 containing VLDL particles and their metabolic remnants which is associated with risk of cardiovascular disease (CVD) (Sacks, 2010).

Since the last 20 years, plant-based therapies that been interested, increasing rapidly in worldwide. It because this therapy has a minimal effect and no side effects at all. Plants contains antioxidants such as isoflavones, phytosterols, saponin, fibers, polyphenols, ascorbic acid and flavonoids which have role in fat metabolism (Visavadiya, 2011). Water Clover is alternative of plant-based therapy is a popular food in Surabaya, it called *Pecel Semanggi*. Water clover contains bioactive components are alkaloids, steroids, saponin, carbohydrates, reducing sugar, amino acid, essentials oils and flavonoids (Nurjanah, 2012; Tiyaningsih, 2007). Based on the results of antioxidant screening conducted was found that water clover extract containing flavonoid was 10,58 mg.

Based on this background, then conducted in depth study of flavonoid activity in water clover against triglycerides in rats fed high cholesterol diet. This study is expected to be an effective solution without causing side effects for patients with hypertriglycerides.

## 2.0 Materials and Methods

The type of this research used true experimental, with pretest posttest control group design. Subject of this study is rats strain Wistar. The number of samples used were five per treatment group (normal diet; high cholesterol diet; high cholesterol diet and Water Clover extract with three doses). High cholesterol diet were given to rats are quail egg yolk, cattle brain and pure glucose.

This diet were given by oral gavage for each rats as much as 2 cc in 1,8 grams per day for 14 days. The doses of Water Clover extract were given are 16,63 mg/kg, 33,264 mg/kg and 66,528 mg/kg. Water Clover extract were given by oral gavage for 14 days after the highcholesterol diet is given.

At the last treatment (29<sup>th</sup> days), the rats blood were taken to measure triglyceride level after treatment. Statistical analysis used Paired Sample T-Test to compare triglyceride levels before and after given high cholesterol diet and Water Clover extract. The difference of triglyceride levels in each group used MANOVA (Multivariate Analyses of Variance).

### 3.0 Result

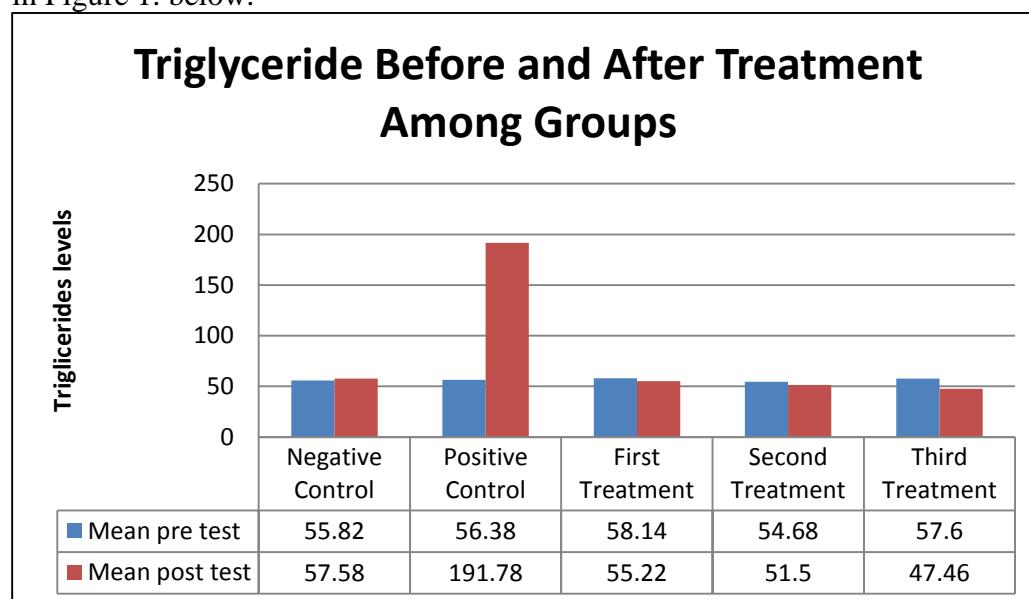
#### 3.1 Analysis of Triglyceride levels before and after treatment

The mean of triglyceride levels among groups before and after treatment in the negative control group were 55.82 (pretest) and 57.58 (posttest), the positive control (high cholesterol diet) were 56.38 (pretest) and 191.78 (posttest), the first treatment (16,63 mg) were 58.14 (pretest) and 55.22 (posttest), the second treatment (33,264 mg) were 54,68 (pretest) and 51,50 (posttest), and the third treatment (66,528 mg) were 57,60 (pretest) and 47,46 (posttest).

**Table 1.** The Mean Difference of Triglyceride Levels Before and After Treatment

Group	n	Pretest	Posttest	p.
		Mean $\pm$ SD (mg/dl)	Mean $\pm$ SD (mg/dl)	
Negative Control (NC)	5	55.82 $\pm$ 4.16	57.58 $\pm$ 3.95	0.632
Positive Control (PC)	5	56.38 $\pm$ 5.30	191.78 $\pm$ 3.24	0.000
First Treatment (T1)	5	58.14 $\pm$ 3.71	55.22 $\pm$ 3.81	0.000
Second Treatment (T2)	5	54.68 $\pm$ 1,56	51.50 $\pm$ 1.77	0.047
Third Treatment (T3)	5	57.60 $\pm$ 1.28	47.46 $\pm$ 1.87	0.001

The mean difference of triglyceride levels in five group before and after treatment can be seen in Figure 1. below.



**Figure 1.** Graph of Triglyceride Levels Before and After Treatment Between Groups

Based on statistical analysis, showed that there were differences of triglyceride levels in the positive control (high cholesterol diet group), first treatment, second treatment, and third treatment before and after treatment significant ( $p < 0.05$ ). However, in the positive control (normal diet group), there was no significant difference ( $p > 0.05$ ).

### 3.2 Analysis of Triglyceride levels changes after treatment

The significance value obtained was 0.000 ( $p < 0.05$ ) on MANOVA test. Accordingly, there are differences in triglyceride levels in among groups (negative control, positive control, first treatment, second treatment, and third treatment). Then, to know the different groups significantly based on  $p$  value can be seen in the following table 2.

**Table 2.** The Comparison of Triglyceride Levels Among Groups

Group	NC	PC	T1	T2	T3
Negative Control (NC)	-	0.000	0.476	0.068	0.040
Positive Control (PC)	0.476	-	0.000	0.000	0.000
First Treatment (T1)	0.000	0.000	-	0.117	0.029
Second Treatment (T2)	0.068	0.000	0.117	-	0.610
Third Treatment (T3)	0.040	0.000	0.029	0.610	-

Triglycerides level in the negative control group compared with positive control group and third treatment showed a difference of significant ( $p < 0.05$ ). Also, triglycerides level in the positive control group compared with first treatment, second treatment and third treatment group showed a difference of significant ( $p < 0.05$ ). Between of comparison first treatment and third showed significant difference ( $p < 0.05$ ).

## 4.0 Discussion

Triglyceride levels before and after treatment is significant ( $p < 0.05$ ), except on normal diet group because its just fed standard food for rats. The difference mean of five groups are increased. Triglyceride increased in high cholesterol diet group because the rats consume hypercholesterolemia diet everyday without treatment. In this research, hypercholesterolemia diet use quail egg yolk, cattle brain and pure glucose. Water clover extract can decreased triglyceride levels with flavonoids. Flavonoids are known to increase the activation and inhibition of PPAR (Peroxisome Proliferator Activated Enzyme) which is a subregister of the receptor hormone) PPAR is aided by PPRE (Proliferator response hormon promoter element) and assisted by DNA to achieve domain bindings. Flavonoid compounds have the potential as an antioxidant that can release hydrogen atoms in the hydroxyl group which causes free radical activity to be reduced also has the function of increasing LPL cofactor by increasing PPAR- $\gamma$  which can increase its enzyme activity (Fan, *et.al.*, 2006).

LPL is an enzyme that contains fats, triglycerides, and monogliferids that can be absorbed by muscles and adipose. LPL has an enzymatic reaction to hydrolyze triglycerides and phospholipids. LPL enzyme required to catabolize VLDL to low density lipoprotein (LDL). VLDL and chylomicron appear to compete to interact with LPL, since VLDL increases above

5 µg/ml, then enzyme saturation occurs in decreased VLDL and chylomicron catabolism (Brunzell, 1973). VLDL transported TG can be hydrolyzed to glycerol fatty acid (lipolysis) which is absorbed by muscles and other tissues into energy, and that energy reserves stored by adipose tissue. Flavonoids also have role to inhibit fatty acid synthase (FAS) which is a importance enzyme in fat metabolism that can be reduced the formation of TG (Tian, 2011). Thus, triglyceride-rich lipoprotein has competition to hydrolyze between lipolysis and partial liver removal lipoprotein. LPL will hydrolyze triglycerides and phospholipids in other circulating lipoproteins, such as LDL and high density lipoprotein (HDL). Therefore, it will convert the LDL-rich triglycerides into a smaller, more dense LDL (Goldberg and Merkel, 2002). Based on research conducted by Roslizawaty, *et al.* (2016, p. 79), the provision of ants nest containing flavonoidance significantly ( $p. < 0.01$ ) on the serum LPL activity of male white rats fed high cholesterol diet.

## 5.0 Conclusion and recommendation

Water Clover extract can decreased triglyceride levels after treatment, because it contains flavonoid that can effect activity of lipoprotein lipase enzymes relation with hypotriglyceridemic.

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## Declaration

Author(s) declare that there's no conflict of interest in this research.

## Authors contribution

Author 1: Concept, idea, data collection, statistical analysis, and manuscript writing

Author 2: Concept, idea, literature review and manuscript writing

Author 3: Concept, idea, literature review, and manuscript writing

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