The Effect of the Ethanolic Extract of Asam Jawa Leaf (*Tamarindus Indica* L.) in Total Cholesterol, Triglyceride, LDL and HDL Concentration on Male Sprague Dawley Rats

T Nofianti¹*, S Nurmayasari¹, M Priatna¹, R Ruswanto¹, M Nurfatwa¹

¹Department of Pharmacy, Sekolah Tinggi Ilmu Kesehatan Bakti Tunas Husada, Tasikmalaya, Indonesia

*tnofianti.wamsu@gmail.com

Abstract. The objective of this study is to investigate the effect of ethanolic extract of *Tamarindus indica* L. in total cholesterol, LDL, triglyceride, and HDL level. The rats were grouped into 6 groups: namely normal control, negative, positive, and the test group that divided into doses I (1.748 mg / 200 g BW), dose II (3.497 mg / 200 grams BW) and dose III (6.994 mg / 200 grams BW). The rats come by switched from commercial food to an egg yolk and induced by propylthiouracil for 10 days (exception normal control group). The test compounds are administered one hour after induction for 10 days. The blood was taken in days of 11. The method to determine lipid fractions using Enzymatic Photometric Test. The results of this study showed that three doses of ethanolic extract of tamarind leaves can decrease the total cholesterol, LDL, and increase HDL levels. The percentage of cholesterol total decreasing were 16.70% (test dose I), 18.05% (test dose II), 19.18% (test dose III), LDL decreasing were 31.94% (test dose I), 61.05% (test dose II), 65.19% (test dose III ) and the percentage of HDL increasing were, 17.73% (test dose I) 79.10% (test dose II) 84.76% (test dose III). All the test dose showed significant (p<0.05) than the negative group. The obtained results revealed that ethanolic extract of *Tamarindus indica* exhibited significant effect (p<0.05) of lowering concentration of total cholesterol, LDL, triglyceride, and the increase of HDL concentration.

1. Introduction

Coronary Heart Disease (CHD) is the leads cause of mortality and disability of people in the developed country. It was an estimate there is 1 million death caused by CHD in the United States of America in 1999 and reach 40% from all mortality [1]. The incidence of CHD associated with an increase in total cholesterol, LDL, triglyceride, and the decrease of HDL [2]. The majority of death CHD patient are caused by atherosclerosis and another complication. Hyperlipidemia is the most to contribute to atherosclerosis pathogenesis [1]. Moreover, there is another risk factor that increases the CHD event, namely hypertension, smoking, diabetes mellitus, less activity, heredity, and stress[3]. Therefore, management of hyperlipidemia especially of decrease of total cholesterol, LDL, triglyceride, and the increase of HDL is the main goal of therapy[4]. Effective drugs lowering lipid have been shown to the prevention of CHD, such as statin group. Nevertheless, Indonesian people still believe in medicinal plants to treat hyperlipidemia. Asam Jawa leaf (*Tamarindus indica* L.) is an
example medicinal plant that used to treat hyperlipidemia by Indonesian people. The contents of the secondary metabolite of Tamarindus indica L. is saponin, flavonoid dan tanin [5]. Based on the explanation above, we investigated the effect of the ethanolic extract of Tamarindus indica L. in total cholesterol, triglyceride, LDL and HDL level.

2. Experimental and Method

2.1. Materials

Animal scales, analytic scales, micropipette, mortar and stamper, dissecting set, centrifuge, hotplate, macerator, rotary evaporator, effendrof tube, Photometer TC 3300. Ethanolic extract of Tamarindus indica, egg yolk, propylthiouracil (PT. Indofarma), PGA 1%, simvastatin (PT. Kimia Farma), ammonia, chloroform, Hyperchloric acid, dragendorf reagent, Mayer reagent, Magnesium, FeCl₃, gelatin, Natrium Hydroxide, vanniline 10%, ether, Lieberman Bouchard, Fluitest chol reagent kit.

2.2. Plant Materials

Dried leaf of Tamarindus indica was purchased from Manoko, Lembang, West Java, Indonesia and authenticated by School of Life Science and Technology Institut Teknologi Bandung.

2.3. Preparation of Extract

Powdered crude of Tamarindus indica leaves was extracted with ethanol 70% in the macerator apparatus. It was attended by evaporation using rotary evaporator until the viscous extract was obtained. This extract was referred to as an ethanolic extract of Tamarindus indica. It was kept in refrigerator 4 ºC until it would be used for pharmacological studies.

2.4. Antihyperlipidemic activity preparation

The male rats were used and acclimatized for 7 days. They were kept under standard laboratory conditions with free access to food and water. The rats were grouped into 6 groups where each group consisting of five animals. Each group namely normal control (PGA 1%), negative (egg yolk 10 ml/Kg BW and PTU 0.02%), positive (egg yolk 10 ml/Kg BW, PTU 0.02% and simvastatin (2.45 mg/200 g BW of rats), and test group (Ethanolic extract of Tamarindus indica) that divided in dose I (1.748 mg / 200 g BW), dose II (3.497 mg / 200 grams BW) and dose III (6.994 mg / 200 grams BW). The rats are switched from commercial food to an egg yolk and induced by propylthiouracil for 10 days (exception normal control group). The test compounds are administered one hour after induction for 10 days. The blood was taken on vena jugularis in days 11.

2.5. Cholesterol Preparation

The determination of cholesterol level using CHOD-PAP methods (Enzymatic Photometric Test.) As much 10 µl blood serum were added into 1000 µl reagents and then incubated for 10 minutes at 20 – 25 ºC. The absorbance read at 546nm (Kit Reagen, Fluitest Chol®).

2.6. Tryglyceride Preparation

The determination of triglyceride levels using Colorimetric Enzymatic Test GPO (Glycerol-3-Phosphate Oxidase). As much 10 µl blood serum were taken with a micropipette, then input into the tube reaction and added 1000 µl reagents. Afterward incubated for 10 minutes at 37ºC. The absorbance read at 550nm with Photometer TC-3300.

2.7. HDL Preparation

The determination of HDL level using CHOD-PAP methods (Enzymatic Photometric Test.) As much 1000 µl blood serum were added into 500 µl reagent HDL and then incubated for 10 minutes on 20 – 25 ºC. The 10µl obtained supernatant added into 1000 µl cholesterol reagents and incubated for 10 minutes at 20 – 25 ºC. The absorbance read at 546nm (Kit Reagen, Fluitest Chol®).

2.8. LDL Determination

The Determination of the LDL level, it can be indirectly measured using the Friedewald equation below (Fischbach, 1999). LDL cholesterol = total cholesterol – HDL cholesterol.
2.9 Analysis Data
Statistical analysis was by one-way ANOVA followed by Least Significant Difference (LSD) post-hoc test by SPSS 13. The value of <0.05 was taken as a significant point.

3. Result and Discussion
3.1 Phytochemical screening

| Compound      | Simplisia | Extract |
|---------------|-----------|---------|
| Flavonoid     | +         |         |
| Alkaloid      | +         |         |
| Tanin         | -         | -       |
| Polyphenol    | +         | +       |
| Triterpenoid  | +         | +       |
| Monoterpenoid | +         |         |
| Sesquiterpenoid|          |         |
| Kuinon        | -         | -       |
| Saponin       | -         | -       |
| Steroid       | +         | -       |

The results of organoleptic of the extract are viscous, chocolate color, and specific odor. Moreover, the phytochemical screening obtained show above in table 1.

3.2 Cholesterol Total Determination

| No | Group   | Lipid Parameter | Cholesterol  |
|----|---------|-----------------|--------------|
| 1  | Normal  |                 | 86±1.15      |
| 2  | Negative|                 | 110.75±2.06  |
| 3  | Positive|                 | 79.75±4.78*  |
| 4  | Dose 1  |                 | 92.25±3.31*  |
| 5  | Dose 2  |                 | 90.75±2.21*  |
| 6  | Dose 3  |                 | 89.5±3.11*   |

Values are expressed as Mean ± SD, * = significantly different from negative group (p < 0.05)

![Figure 1](image_url)

**Figure 1.** Effectivity Percentage of ethanolic extract of *Tamarindus indica* on total cholesterol concentration

Based on table 1, the total cholesterol level of the normal group is lower than negative control (p<0.05), (86 ± 1.15) and (110.75 ± 2.06), respectively. This indicated if induction of egg yolk and
propylthiouracil is succeeded. Propylthiouracil had a role to increase lipid level in plasma blood serum through the block of thyroid hormone synthesis and it causes lowering the rate of metabolism of lipid. The positive control that we used simvastatin as a comparison drug showed a significant effect of lowering cholesterol total than negative control (p<0.05). The exact mechanism of action of simvastatin to lower total cholesterol through inhibition of HMG-CoA reductase. This HMG-CoA reductase had a role in the synthesis of cholesterol.

Moreover, the test group of the dose I, II, and III exhibited significantly different than negative control (p<0.05). It showed if the ethanolic extract of *Tamarindus indica* had an effect to lower total cholesterol level, although the mechanism of action still unknown. Nevertheless, the test group of the dose I, II, and III did not show significant effect each other (p>0.05). It was meant, there was no association between dose and effect.

### 3.3 Triglyceride Determination

#### Table 3. Trygliceride Concentration

| No | Group     | Lipid Parameter |
|----|-----------|-----------------|
|    |           | Triglyceride    |
| 1  | Normal    | 53.2±3.34       |
| 2  | Negative  | 120.4±6.97      |
| 3  | Positive  | 62.2±15.97*     |
| 4  | Dose 1    | 97.6±17.57*     |
| 5  | Dose 2    | 82±4.63*        |
| 6  | Dose 3    | 73.8±15.31*     |

Values are expressed as Mean ± SD, * = significantly different from negative group (p < 0.05)

Based on figure 2. The triglyceride concentration on the normal group is 53.2±3.34 lower than negative control (120.4±6.91). Increasing of triglyceride concentration on negative control exhibited in the treatment of egg yolk and PTU is succeeded. Afterward for positive control is 62.2±15.97 is lower than the negative control. In addition, the test group of the dose I, II, and III exhibited lowering triglyceride concentration 97.6±17.57, 82±4.63, 73.8 ± 15.31, respectively. The test group (*Tamarindus indica*) exhibited concentration-dependent of lowering triglyceride concentration. The percentage of Lowering triglyceride concentration of dose I, dose II, and does III is 18.94%, 31.89%, 38.70 %, respectively. Based on ANOVA calculation exhibited significantly different (p<0.05). The positive control, the dose I, dose II, and dose III of the test group exhibited significantly different (p<0.05) than the negative control.
3.4 Determination of HDL Concentration

| No | Group     | Lipid Parameter | HDL (mg/dl) |
|----|-----------|-----------------|-------------|
| 1  | Normal    |                 | 56.3±2.23   |
| 2  | Negative  |                 | 33.97±0.37  |
| 3  | Positive  |                 | 63.57±1.21* |
| 4  | Dose 1    |                 | 40±1.95*    |
| 5  | Dose 2    |                 | 60.85±5.64* |
| 6  | Dose 3    |                 | 62.77±5.04* |

Values are expressed as Mean ± SD, * = significantly different from negative group (p < 0.05)

**Figure 3.** Percentage effectivity of ethanolic extract of *Tamarindus indica* leaf on HDL concentration

Based on figure 3, HDL concentration of the normal group is 56.3 ± 2.23 higher than negative control 33.97±0.37. This result indicated if the treatment of egg yolk and PTU to decrease HDL level is succeed. Afterward, HDL concentration of positive control (63.57±1.21) is higher than the normal group and negative control. In addition, the dose I, dose II, and dose III of the test group exhibited increasing of HDL concentration, 40 ± 1.95, 60.85±5.64, 62.77 ± 5.04, respectively. Based on ANOVA calculation exhibited significantly different (p<0.05). The positive control, dose I, dose II, and dose III of the test group exhibited significantly different (p<0.05) than the negative control. The test group (*Tamarindus indica*) exhibited concentration-dependent of increasing HDL concentration.

3.5 Determination of LDL concentration

| No | Group     | Lipid Parameter | LDL   |
|----|-----------|-----------------|-------|
| 1  | Normal    |                 | 29.7±1.34 |
| 2  | Negative  |                 | 76.77±1.85 |
| 3  | Positive  |                 | 16.17±4.05* |
| 4  | Test 1    |                 | 52.25±3.24* |
| 5  | Test 2    |                 | 29.9±4.56* |
| 6  | Test 3    |                 | 26.72±4.71* |

Values are expressed as Mean ± SD, * = significantly different from negative group (p < 0.05)
Figure 4. Percentage effectivity of ethanolic extract of *Tamarindus indica* leaf on LDL concentration

Based on figure 7, the HDL concentration of the normal group is $29.77 \pm 1.84$. This result indicated if the treatment of egg yolk and PTU to decrease LDL level is succeed. Afterward, LDL concentration of positive control $16.17 \pm 4.05$ is lower than the normal group and negative control. In addition, the dose I, dose II, and dose III of the test group exhibited lowering of LDL concentration, $40 \pm 1.95$, $60.85 \pm 5.64$, $62.77 \pm 5.04$, respectively. Based on ANOVA calculation exhibited significantly different ($p<0.05$). The positive control, dose I, dose II, and dose III of the test group exhibited significantly different ($p<0.05$) than the negative control. The test group (*Tamarindus indica*) exhibited concentration-dependent of increasing HDL concentration $52.25 \pm 3.23$, $29.9 \pm 4.55$, $26.72 \pm 4.70$, respectively. Lowering the percentage of LDL concentration for the dose I, II, and III of the test group is $31.94\%$, $61.05\%$, $65.19\%$, respectively. The antihyperlipidemic activity of the ethanolic extract of *Tamarindus indica* through the antioxidant effect that inhibited lipid oxidation. The mechanism of action of antioxidant has two functions. The first function is a donor of the hydrogen atom and the second a slower rate of \textit{in vitro}. In addition, the antihyperlipidemic activity of ethanolic extract of *Tamarindus indica* through another mechanism, such as, an alkaloid that contained in this extract has an inhibitory activity of lipase enzyme, therefore it can inhibit the breakdown of lipid. In addition, flavonoid and polyphenol through reducing free radicals and inhibit peroxidation of lipid in microsomal and liposomal implicated of reducing lipoprotein secretion in liver and intestine, besides that this compounds can reduce the risk of forming atherosclerosis. Moreover, this flavonoid can increase the secretion of bile acid that increases the rate of lipid excretion [6,7].

4. Conclusion

The obtained results revealed that the ethanolic extract of *Tamarindus indica* leaf exhibited a lowering of total cholesterol, triglyceride, and LDL. Moreover, this ethanolic extract exhibited increasing of HDL level. The antihyperlipidemic activity of the extract exhibited concentration dependent.

5. References

[1] Lim, Hadyanto 2009 *Farmakologi Kardiovaskular* (Jakarta: PT. Sofimedia)
[2] Harvey, Richard A., Champe Pamela C. 2013 *Farmakologi Ulasan Bergambar edisi 4*. Ailh bahasa Ramadhan, Dian et. al. (Jakarta: EGC)
[3] Suyatna, FD. 2009. *Farmakologi Dan Terapi edisi 5*. (Jakarta: FKUI)
[4] Harvey, Richard A., Champe Pamela C. 2013 *Farmakologi Ulasan Bergambar edisi 4*. Ailh bahasa Ramadhan, Dian [et. al]. (Jakarta: EGC, 293)
[5] Deviana 2010 *Kolesterol, solusi tepat Mengelola Kolesterol*. (Yogyakarta. Cemerlang Publishing)
[6] Carjavall-zarrabal. 2005 The Consumption Of Hibiscus Sabdariffa Dried Calyx Ethanolic Extract Reduced Lipid Profile In Rat. *Plant Foods for Human Nutrition*. 60: 153-159.
[7] Soemardji, A. Andreanus. 2007 *Tamarindus Indica L. Or Asam Jawa The Sour But Sweet and Useful.*