The lipid profile of rats (Rattus norvegicus L.) induced by high fat ration after exposed to ethanolic neem (Azadirachta indica) leaf extract

S Isdadiyanto, A J Sitasiwi, S M Mardiati
Biology Department. Faculty of Science and Mathematics. Diponegoro University

Corresponding author: isdadiyanto@yahoo.com

Abstract. The objective of this study was to analyze the effect of neem leaf ethanol extract as an herbal antihyperlipidemia agent on white rats induced by high fat ration. The white rat used was male Wistar strain with 2 months of age and average body weight of approximately 200 grams. The rats were divided into 6 groups of 4, namely: Negative control (P0) was given commercial ration, positive control (P1) was given high fat ration and duck egg yolk per oral 2.5 ml / g BW, P2 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 8 mg / g BW simvastatin in 1 ml of distilled water, P3 was given high-fat ration and duck egg yolk orally 2.5 ml / 200 g BW + 75 mg / g BW ethanol extract of neem leaf in 1 ml of distilled water, P4 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 100 mg / g BW ethanol extract of neem leaf in 1 ml of distilled water, and P5 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 125 mg / g BW ethanol extract of neem leaf in 1 ml of distilled water. The variables observed were total cholesterol, HDL, LDL and TG levels. Data were analyzed using ANOVA followed by Least Significant Difference (LSD) test with 95% confidence level using SPSS 10.0 software. The results showed that administration of the ethanol extract of neem leaf (A. indica) can raise levels of HDL, lowering levels of LDL cholesterol and TG in blood serum of white rats (Rattus norvegicus L.).

1. Background
The analysis of Azadirachta indica leaf extract contain 6 compounds namely quercetin 3 obd-glucoside, myricetin-3-0-routineoside, quercetin-3-o-routineoside, Kaempferol-3-o-routineoside, Kaempferol-3-oBD-glucoside, quercetin- 3-oL-rhamnoside [1]. Phytochemical screening of neem leaves with ethanol solvent shows that neem leaves contain bioactive compounds such as cardiac glycosides [2], alkaloids and saponins [3,4]. Other test results also found several compounds such as phenols, flavonoids, and tannins from neem leaf ethanol extracts [5,6,7].

High-fat ration is a food that contains high concentrations of fat. The intake of fat into the human body in large amounts can increase total blood cholesterol and blood LDL [8]. An increase in cholesterol levels due to consumption of high amounts of fat occurs because in the metabolism of fat, the fat consumed in part will be converted to cholesterol. Fat derived from local synthesis and food, will be transported to the liver. Fat derived from local synthesis is released and transported to the liver in the form of free fatty acids, while fat from food is transported in the form of chylomicrons [9]. Sherwood (2001) states that arterial disease can occur with elevated levels of LDL and VLDL cholesterol in the blood (hypercholesterolemia) [10]. According to Linder (1992) the consumption of
saturated fat foods in high amounts continuously is a major factor that can increase cholesterol and triglyceride levels in plasma which causes hyperlipidemia [11].

Disease in arteria can occur with increased levels of LDL and VLDL cholesterol in the blood (hypercholesterolemia). This increase in cholesterol levels can occur when there is a disturbance in the formation of cholesterol in the liver or small intestine [10]. High levels of LDL cholesterol will trigger the accumulation of cholesterol in blood vessel cells, which causes the emergence of atherosclerosis and the formation of plaque in the walls of blood vessels [12].

Nevertheless, according to Barter et al. (2007) HDL cholesterol levels and / or the ratio between total cholesterol and HDL cholesterol are strong predictors for the incidence of cardiovascular disease. The higher the HDL cholesterol level in relation to the total cholesterol level, the smaller the risk of cardiovascular disease. It was further said that HDL levels were risk factors for cardiovascular disease that were stronger than LDL levels [13].

According to Nicholson & Hajjar (1998), atherosclerosis is a multifactorial disease, which involves various factors, including: genetic factors, such as: hypercholesterolemia, hypertension, diabetes mellitus and obesity, and environmental factors, including smoking and stress. At present, most people still recognize atherosclerosis as a degenerative disease that develops slowly and is considered a disease that only attacks elderly individuals. Classic factors, which accompany it include hypercholesterolemia, hypertriglyceridemia or hypertension. But research that runs continuously from year to year proves, that atherosclerosis is a disease that is very complicated, exceeding pre-existing assumptions [14].

Azadirachta indica at a dose of 500mg/kg BW can reduce cholesterol levels in diabetic-induced mice [15]. One of the potentials of various compound contents of neem leaves is to reduce cholesterol levels which can trigger heart and blood vessel disease [16]. Repeated administration of A. indica leaf ethanol extract in diabetic rats can prevent an increase in total cholesterol (TC), triglyceride (TG), Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) compared to diabetic rats that are not given neem leaf extract , while High Density Lipoprotein (HDL) cholesterol levels increased significantly [17].

Based on that fact, this study was performed to find out the effect of ethanolic Neem leaf extract on rats serum which characterized by lipid profile i.e. cholesterol, LDL, HDL and TG. The outcome of this study is offering the advantages of Neem leaf extract as an effective herb in antihyperlipidemia.

2. Material and method

2.1. Preparation of the ethanolic leaf extract of Neem
The neem leaf were collected from Diponegoro University campuss area. Then the leaf was rinsed and dried at 40-50 °C for ten days. The leaf ethanolic extract were made by maseration method using 70% ethanol as explained by Sitasiwi et al.[18]. The extracts were kept in 4 °C in a dark closed bottle until antihyperlipidemia testing.

2.2. Making high fat rations
High-fat ration consists of commercial ration and reused cooking oil. Used cooking oil used in this study was obtained from one liter packaged cooking oil used to fry tofu weighing 450 g for 10 minutes at a temperature of 150-165°C with deep fat frying techniques for nine times the frying pan [19,20].

2.3. Laboratory animals
Twenty four male Rattus norvegicus L. rats with 2 months of age and ± 200g in body weight were used as testing animals. The rats were obtained from the Department of Biology Laboratory of Semarang State University, Indonesia. They were acclimatized for seven days in the laboratory condition before being used for experiments. The animals were handled in a well-controlled room, with the temperature of 26 ± 1°C. The rations and water were given ad libitum.
2.4. Experimental design and treatment of animals procedures
Research conducted at the Laboratory of Biological Structure and Function of Animals Department of Biology, Faculty of Science and Mathematics University of Diponegoro, in May and July 2019. High-fat ration is given every morning for 45 days as much as ± 30 g, then given drinking water every day along with 75 ml of ration. Giving duck egg yolk is given once every two days (morning), while simvastatin and ethanol extract of neem leaf are given every evening orally for 44 days by syringe injection berkanul tip. The tools used are 24 individual cages equipped with drinking and rationing, measuring cups, digital scales, gives a chance to set. This study uses a completely randomized design with 6 treatments and 4 replicates ie: Negative control (P0) was given commercial ration, positive control (P1) was given high fat ration and duck egg yolk per oral 2.5 ml / g BW, P2 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 8 mg / g BW simvastatin in 1 ml of distilled water, P3 given high-fat ration and duck egg yolk orally 2.5 ml / 200 g BW + 75 mg / g BW ethanol extract of neem leaf (A. indica) in 1 ml of distilled water, P4 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 125 mg / g BW ethanol extract of neem leaf (A. indica) in 1 ml of distilled water, and P5 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 100 mg / g BW ethanol extract of neem leaf (A. indica) in 1 ml of distilled water.

2.5. Statistical analysis
The cholesterol, HDL, LDL and TG were expressed as the mean ± standard deviation, each with four replicates. The collected data were analyzed by One-way Analysis of Variance (ANOVA) based on completely randomized design (CRD) at the level of 95% (α = 0.05) and continued by Least Significant Difference Test (LSDT). P-values differences at p<0.05 were considered significant. SPSS was applied to analysed the data.

3. Results and discussion
The result shows that the ethanolic Neem leaf extract interferes the profile lipid of testing animals (Table 1.). The cholesterol, HDL, LDL and TG of treated animals shows a significant difference (p<0.05) with the group was given high fat ration.

Table 1. Cholesterol, HDL, LDL and TG of rats induced high fat ration after exposed to ethanolic Neem leaf extract for 45 days

|          | P0                      | P1                      | P2                      | P3                      | P4                      | P5                      |
|----------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|-------------------------|
| Cholesterol | 106,18±2,25a | 105,90±2,37a | 133,51±2,96c | 114,49±2,06b | 117,07±0,61b | 114,80±1,99b |
| LDL      | 75,47±0,73d | 85,64±0,72d | 105,17±2,18e | 83,50±0,65c | 82,19±0,49c | 79,80±0,94b |
| HDL      | 25,99±1,10d | 21,42±0,58ab | 20,31±0,44a | 21,59±1,16b | 22,43±0,82bc | 23,48±1,01c |
| TG       | 105,64±1,94ab | 112,62±1,56d | 109,35±1,31c | 109,59±2,38c | 108,09±1,33bc | 108,09±1,33bc |

Description: Numbers with different superscripts in the same row show the real difference among the treatments. Negative control (P0) was given commercial ration, positive control (P1) was given high fat ration and duck egg yolk per oral 2.5 ml / g BW, P2 was given high fat ration and duck egg yolk per oral 2.5 ml/200 g BW + 8 mg / g BW simvastatin in 1 ml of distilled water, P3 was given high-fat ration and duck egg yolk orally 2.5 ml / 200 g BW + 75 mg / g BW ethanol extract of neem leaf (A. indica) in 1 ml of distilled water, P4 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 100 mg / g BW ethanol extract of neem leaf (A. indica) in 1 ml of distilled water.
ml of distilled water, and P5 was given high fat ration and duck egg yolk per oral 2.5 ml / 200 g BW + 125 mg / g BW ethanol extract of neem leaf (A. indica) in 1 ml of distilled water

Results of the analysis of the provision of 75, 100, 125 mg / g BW ethanol extract of neem leaf (A. indica) showed significantly different results (P <0.05). The ethanol extract of neem leaf (A. indica) are provided, decreasing cholesterol levels in rats (Rattus norvegicus L.) were given high fat ration. Results of the analysis of the levels of LDL in the treatment giving 75, 100, 125 mg/g BW ethanol extract of neem leaf showed significant differences to the rats were given high fat ration. Results of the analysis of the levels of HDL in the treatment giving 75, 100, 125 mg/g BW ethanol extract of neem leaf showed significant differences to the rats were given high fat ration. Results of the analysis of the levels of TG in the treatment giving 75, 100, 125 mg/g BW ethanol extract of neem leaf showed significant differences to the rats were given high fat ration. Results of the analysis as shown in table 1.

The results showed that the relationship with total cholesterol LDL is directly proportional. This happens because the 45% is in the form of LDL cholesterol [18]. That is if your total cholesterol LDL down then also fell. This happens because the inhibition or disruption of the process of absorption of cholesterol in the small intestine and increasing bile acid excretion in the feces. Bile acids are end products of metabolism of cholesterol. With the high excretion of bile acids, the more cholesterol is converted into bile acids to emulsification of fat, so that the serum total and LDL cholesterol decreased. Mechanism of increased levels of HDL because of the influence of ethanol extract of neem leaf likely caused by the binding of bile acids by metabolites (contained in ethanol extract of neem leaf) in the small intestine that causes increased excretion of bile acid fecal, resulting in decreased absorption of fat and cholesterol, it causes cholesterol in the liver low so cholesterol to produce bile acids less. This condition stimulates the synthesis of HDL in the liver to meet the shortage of cholesterol. As a result, lower serum LDL than HDL serum. Along with a decrease in serum cholesterol levels in the blood of the results of the analysis of HDL levels in treatment giving ethanol extract of neem leaf showed significant differences to the rats were given high fat ration.

Diseases of the arteries can occur with elevated levels of LDL and TG in the blood (hypercholesterolemia). The increase in cholesterol levels can occur when there is an interruption formation of cholesterol in the liver or intestine [10]. High LDL levels will trigger the accumulation of cholesterol in the blood vessel cells, which led to the emergence of atherosclerosis and plaque formation in the blood vessel walls12. Atherosclerosis is associated with increased LDL. Effect of elevated levels of LDL will be followed by the accumulation of cholesterol esters in macrophages which then referred to as a macrophage foam cells. High LDL levels lead to high levels of LDL intimal. Furthermore, intimal will oxidize LDL and attracts monocytes from the blood circulation as well as phenotypic change into macrophages. Increased oxidized LDL in the arterial wall is accompanied by the formation of foam cells, will develop into a plate of fat [21].

4. Conclusions
The results showed that administration of the ethanol extract of neem leaf (A. indica) can raise levels of HDL, lowering levels of LDL cholesterol and TG in blood serum of white rats (Rattus norvegicus). It concluded that ethanol extract of neem leaf (A. indica) could potentially be used as a supplement for the prevention of risk to vascular disease and coronary heart disease.

Acknowledgements
This work was financially supported by PNBP Diponegoro University Semarang Indonesia.

References
[1] Chattopadhyay R R 1999 J of Ethnopharmacol. 67 373-376
[2] Sahrawat A, Sharma J, Rahul S N, Tiwari S, Joshi M D, Pundhir A, Kumar R, Akansha R, Bhavya S, Sharib N, Govind and Akash 2018 Journal of Pharmacognosy and Phytochemistry 7(4) 1368-1371

[3] Keta J N, Suberu H A, Shehu K, Yahayya U, Mohammad N K and Gudu G B 2019 Science World Journal 14(1) 98-102

[4] Aathira E P and Suganthi A 2019 World Journal of Pharmaceutical Research 8(5) 1370-1380

[5] Pandey G, Verma K K and Singh M 2014 International Journal of Pharmacy and Pharmaceutical Sciences 6(2) 444-447

[6] Itelima J U, Nwokedi V C, Ogbonna A I and Nyam M A 2016 World Journal of Microbiology 3(1) 56-60

[7] Prasanna R 2017 International Journal of Advance Research, Ideas and Innovations in Technology 3(1) 543-558

[8] Setiawati T, Atmomarsono U and Dwiloka B 2016 Jurnal Teknologi Hasil Pertanian 9(2) 1-8

[9] Mayes P A and Botham K M 2003b. Cholesterol Synthesis, Transport, and Excretion. Harper’s Illustrated Biochemistry, 26th edition. (Mc.Graw Hill) 219-227

[10] Sherwood L 2001 Human Phisiology: From Cells to Systems (A Division of Internasional Thomson Publishing Inc.)

[11] Linder M C 1992 Biochemical Nutrition and Metabolism, with clinical usage. Aminuddin Parakkasi translator. Prints to I. (Jakarta: University of Indonesia Press)

[12] Libby P and Theroux P 2005 Circulation 111 3481-3488

[13] Barter P, Gotto A M, Larosa J C, Maroni J, Szarek M, Grundy S M, Kastelein J J P, Bittner V and Fruchart J C 2007 N.E.I.M. 357 1301-1310

[14] Nicholson A C and Hajjar D P 1998 J. Arterio. Trombo. Vas. Biol. 18 339-348

[15] Chattopadhyay R R and Bandyopadhyay M 2005 African Journal of Biomedical Research 8 101-104

[16] Tembe-Fokunang E A T, Charles F, Kaba N, Donatien G, Michael A and Bonaventure N 2019 Journal of Complementary and Alternative Medical Research 7(1) 1-18

[17] Bisht S and Sisodia S S 2010 Journal of Pharmaceutical Sciences and Research 2(10) 622-627

[18] Sitasiwi A J, Isdadiyanto S and Mardiati S M 2017 AIP Conference Proceeding. ISBN.978-0-7354-1516-4

[19] Muhartono, Yudistira P A, Putri N T, Sari T N and Oktafany 2018 Jurnal Kedokteran Unila 2(2) 129-135

[20] Hanung A, Saktini F and Gumay A R 2019 Jurnal Kedokteran Diponegoro 8(1) 26-37

[21] Mathews K C and Van holde K E 1991 Biochemistry (New York: The Benjamin/Cummings Co.Inc.)