The Antioxidant Effect of Noni Fruits Extract on HDL and LDL Level of Wistar Rat Induced by High Fat Diet

Diah Purwaningsari[1], Sihning EJ Tehupuring[2], Nita Pranitasari[1], Suhailah Hayaza[3], Lestari Dewi[1], Dian Ardiana[1]

[1]Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia, [2]Researcher, Faculty of Medicine, Hang Tuah University, Surabaya, Indonesia, [3]Scientist and Data Analyst, Department of Biology, Faculty of Science and Technology, Universitas Airlangga, Surabaya, Indonesia

Abstract

Dyslipidemia is an important risk factor for myocardial infarction, cardiovascular diseases, and Non-alcoholic Fatty Liver (NAFLD) disorders. Inflammatory conditions, oxidative stress, and insulin resistance, as well as several risk factors for cardiovascular disease, are also risk factors for this disease. This study aims to determine the effect of noni (*Morinda citrifolia*) extract on the improvement of dyslipidemia condition, based on the Low Density Lipoprotein (LDL) and High Density Lipoprotein (HDL) level. This research is a laboratory experimental study with a post-test only control group design. Male Wistar rat *(Rattus norvegicus)* were divided into four groups: negative control group with no induction and treatment (K-), positive control group given high-fat diet induction for 14 days (K+), noni extract treatment group given a high-fat diet and a noni extract at a dose of 100 mg/kg BB per day for 7 days (P1), and noni extract at a dose of 200 mg/kg BB per day for seven days (P2). Low Density Lipoprotein and High Density Lipoprotein serum levels were measured using the ELISA method. The results showed that there was a significant increase in LDL and a decrease in HDL in both treatment groups (P1 and P2) compared with K(+) group. Therefore, we conclude that noni extract *(Morinda citrifolia)* played a role as an antioxidant which can increase LDL and decrease HDL serum levels in rats induced by a high fat diet.

Keywords: *Noni extract (Morinda citrifolia), high fat diet, LDL, HDL, antioxidant*

Introduction

Dyslipidemia or atherogenic dyslipidemia is a term, which represents the occurrence of three things in humans, namely an increase in the concentration of Low Density Lipoprotein (LDL), a decrease in High Density Lipoprotein (HDL), and an increase in Triglycerides (TG). Dyslipidemia together with obesity, metabolic syndrome, insulin resistance, and type 2 diabetes mellitus are important risk factors for myocardial infarction and other cardiovascular diseases. Currently, several genes have been associated with this pattern of lipoprotein changes[1]. Apart from cardiovascular disease, dyslipidemia can also lead to non-alcoholic fatty liver (NAFLD) disease. Inflammatory conditions, oxidative stress and insulin resistance, as well as several risk factors for cardiovascular disease, are also risk factors for this disease[2].

According to data, from 2009 to 2012,> 100 million US adults ≥20 years had a total cholesterol level of ≥200 mg / dL; nearly 31 million had levels ≥240 mg / dL. The data also show the presence of insulin resistance - which increases plasma triglyceride (TG) levels, an increase in Low Density Lipoprotein Cholesterol (LDL-C) and a decrease in High Density Lipoprotein Cholesterol (HDL-C) levels - which are important risk factors for vascular disease disorders[3].

Riskesdas 2013 data shows, the prevalence in the population> 15 years of age, there is abnormal total cholesterol of 35.9 %, low HDL 22.9 %, LDL is not optimal with the combined category near optimal-

Corresponding author:
Diah Purwaningsari
email: diah.purwaningsari@hangtuah.ac.id

borderline high 60.3 % and high-very high category 15.9 %, abnormal triglycerides with high borderline category 13.0 % and high-very high category 11.9 %, and abnormal serum creatinine 6.0 %.

The currently established therapy of choice for dyslipidemia is statins. For conditions that are resistant to statins, a combination of statins with ezetimibe, fibrates, and nicotinic acid is used as a second choice. The next option is anti-PCSK9 drugs (evolocumab and alirocumab). In the latest development, to overcome homozygous FH (Familial Hypercholesterolemia), lomitapide and mipomersen therapy are used, but the price is still very expensive[4].

Dyslipidemia requires long-term and repeated treatment, so the use of natural ingredients around us in the treatment of this disease is a very good breakthrough because drug side effects are expected to be minimal and costs incurred for treatment can be more efficient. Noni (*Morinda citrifolia*) or also called Noni, is a natural ingredient in the form of a plant with high antioxidant content, which is strong against free radicals, prevents oxidative damage to major biomolecules and provides significant protection against oxidative damage. This plant is rich in non-enzyme antioxidants such as ascorbic acid, α-tocopherol, carotenoids, flavonoids, phenols, tannins and carbohydrates. The contents of biological compounds in this plant include glycosides, polysaccharides, alkaloids, lignans, fattyacid esters, anthroquinones, scopoletin, morindin, vitamins and minerals, which can be extracted from the fruits, roots and leaves of Noni[5].

Morinda citrifolia, or noni and locally known as noni, is a member of the madder family, Rubiaceae. This plant is native to tropical Asia and Australia and is distributed throughout the Pacific region. This plant is an evergreen shrub or small tree that grows to about 4–8 m. The branches are elongated short circular shape, thick, shiny ovoid leaves, dark green and muscular. The flowers, about 75–90 in number, are ovate with a round head. The length of the handle is 10-30 mm and the calyx is, in the form of a cut edge. White corolla, five lobes, with a greenish white tube, 7-9 mm[6]. This fruit is used by people in all tropical regions of the world. The shiny green leaves are used by Caribs as a poultice for wounds, rheumatic joints, fevers and headaches. The leaves are applied directly to the affected area for pain relief. Mashed fruit can also be applied directly to the affected area, including deep wounds and broken bones. It is also reported to cure various diseases ranging from arthritis, rheumatism, wounds, ulcers, and even to get rid of head lice[6].

Antioxidants are substances that play an important role in inhibiting and scavenging free radicals, thus providing protection for humans against infections, degenerative diseases, and metabolic diseases. Much research has been focused on natural antioxidants derived from plants because they can produce safe therapies. Natural antioxidants can protect the human body from free radicals by slowing the progress of many chronic diseases as well as inhibiting dietary lipid oxidation. Many diseases related to oxidative stress are a result of the accumulation of free radicals in the body. Free radicals are involved in more than 80 diseases including Diabetes Mellitus, Arthritis, Cancer, Aging. Metabolic disease etc. In the treatment of this disease, antioxidant therapy is suspected to be the most important. In plants, there are free radical scavenger molecules that are rich in antioxidant activity. Free radicals are involved in more than 80 diseases including diabetes mellitus, arthritis, cancer, aging, and metabolic disease[3]. In the treatment of this disease, antioxidant therapy is suspected to be the most important. Therefore, this study was done to determine the effect of noni (*Morinda citrifolia*) extract on HDL and LDL serum levels in Wistar white rats (*Rattus norvegicus*) induced by a high-fat diet.

Materials and Methods

Experimental design

This study was a laboratory experimental research and used a post-test only control group design. There were four groups: two control groups and two treatment groups. Rats were randomly selected. This study was conducted in the Faculty of Medicine, Hang Tua University, Surabaya, Indonesia in 2019. The sample used in this study were 28 male white rats (*Rattus norvegicus*), aged 3-4 months with a bodyweight of ± 150-200 grams, obtained from the Biochemistry Laboratory of the Medical Faculty of Hang Tuah University, Surabaya. The white rats were acclimatized for two weeks. They were maintained under a controlled temperature of 25±2°C, humidity of 50±10%, and a

12 h light-dark cycle and had free access to food and water. Animals were randomly selected and divided into 4 groups consisting negative control group with no induction and treatment (K-), a positive control group was given high-fat diet induction for 14 days (K+), noni extract treatment group given a high-fat diet and a noni extract at a dose of 100 mg/kg BB per day for 7 days (P1), and noni extract treatment group was given a high-fat diet and noni extract at a dose of 200 mg/kg BB per day for 7 days (P2).

Preparation of noni fruits crude extract

The noni fruits (*Morinda citrifolia*) were peeled off the outer skin, then split into several parts. After being chopped, the fruits were dried out using an oven at 60^0C. The dried fruits were then mashed until it became powder. The powder was macerated with 70% ethanol for 24 hours and the filtrate was taken. Then, the filtrate was evaporated on the water heater[7]. Noni extract was obtained.

Preparation of Pulvis Gummi Arabicum solution

The 3% PGA solution was used in the negative control group during the treatment stage. Hot water was poured into the mortar. Around three grams of PGA were sprinkled and inflated for 15 minutes. It was stirred until homogeneous. Then, 100 mL of distilled water was added.

Blood serum analysis

On the 29th day, the rat's blood was collected. The rats were previously was fasten for ± 12 hours and were only given water to drink. Then the rats were anesthetized and blood was drawn from the rats' hearts (intracardiac) using a syringe. Each rat had approximately 3 ml of blood. Then the blood was collected in a serological container without anticoagulants and closed with a rubber cap. The sample proceeded for HDL and LDL levels analysis.

HDL and LDL level analysis

Blood samples were collected in tubes and then centrifuged at 3000× *g* for 20 min. The serum was obtained. The levels of HDL and LDL in the serum were performed using an ELISA kit (Bioassay Technology, Shanghai, China) according to the manufacturer's protocol. After adding 40µl blood serum to wells, around 10µl of anti-HDL and anti-LDL, and 50µl streptavidin-HRP was added. Plates were incubated for 60 minutes at 37°C and washed 5 times with wash buffer. Then, 50µl substrate solution A and B were added to each well and incubated for 10 minutes at 37°C in the dark. Lastly, a 50µl stop solution was added to each well. The optical density was measured using a microplate reader (λ=450 nm).

Statistical Analysis

Data from experiments were expressed as mean ± standard deviation (SD). Statistical analyses were performed by one-way analysis of variance (ANOVA) followed by Duncan's post hoc test to compare the groups using SPSS version 21 (SPSS Inc., Chicago, IL). The significance level for analyses was set at $p < 0.05$.

Result and Discussion

Noni extract contains several active ingredients, namely phenolic compounds and flavonoids which function as antioxidants and reduce levels of lipid peroxidation and increase catalase activity. The main flavonoids that have been detected in *Morinda citrifolia* are catechins and epicatechins[8]. Inhibition of active ingredients of phenolic and flavonoid compounds will cause inhibition of Reactive Oxygent Species, which are produced from modified lipoprotein protein molecules due to oxidation, glycosylation and glycosidation processes[9].

In this study, 5% induction of a high-fat diet for 14 days was proven to increase the LDL blood serum levels of male Wistar white rats (*Rattus norvegicus*), and administration of noni extract (*Morinda citrifolia*) at a dose of 100 mg/kg BW for 7 days was proven to be effective in lowering LDL levels (Table 1). Rats given a high-fat diet will experience an increase in LDL levels, this is due to the intake of saturated fatty acids contained in goat fat, lard, egg yolks and cholic acid[10].

Table 1. Effect of noni fruits on HDL and LDL level in rats induced by a high-fat diet. *p< 0.05 compared to the positive control group. **p< 0,05 compared to both control groups.

Groups	LDL level (µg/mL)	HDL Level (µg/mL)
Negative control	6,86 ± 1,86	31 ± 2,16
Positive control	10,72 ± 1,38	25,86 ± 1,22
P1	5,86 ± 1,35*	30,29 ± 7,72*
P2	5,29 ± 0,95*	33,29 ± 7,50*

Other studies that are in line with this study are research conducted in vitro on animals and active smokers, and the protective effect of noni (*Morinda citrifolia*) / Noni Fruit juice on superoxide anion radicals and lipid peroxide. The effect of this noni fruit can contribute to lowering lipid levels. A double-blind placebo controlled study conducted in a small population showed a decrease in total cholesterol and triglyceride levels in Noni Fruit drinkers[11].

The study stated that the treatment group, namely the group given Noni fruit juice at a dose of 50 and 100 mg/kg BW showed a decrease in total cholesterol, triglycerides and VLDL-cholesterol at both doses when compared to control the disease. However, the reduction in total cholesterol and LDL-C in the administration of noni fruit juice at a dose of 50 mg/kg BW failed to show a statistically significant reduction compared to atorvastatin, a cholesterol-lowering drug that has been established. However, this study has sufficiently proven the hypolipidemic activity of Noni fruit juice in a high-fat diet induced hyperlipidemia in rats[11].

The study, which was conducted on 20 mice, used 40% sucrose induction for 10 weeks to make a metabolic syndrome model, then gave *Morinda citrophilia* extract at a dose of 200 mg/kg BW for 2 weeks in the treatment group compared to the standard diet. Observations were made on rat body weight, serum glucose levels, total cholesterol levels, HDL, TG, uric acid, total protein (PTOT), albumin and globulin. The results showed that the consumption of water extract from dried M. citrifolia leaves reduced abdominal fat and triacylglycerols which are associated with increased adiponectin levels and changes in adipose tissue cell dynamics[12].

Many diseases related to oxidative stress are a result of the accumulation of free radicals in the body[13]. The main mechanism of antioxidants maintained in mammals is through the neutralization of ROS with glutathione (GSH) and three main scavenging antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px). Besides, matrix metalloproteinases (MMPs) promote the degradation of the extracellular matrix (ECM) molecules that play a role in the development of liver damage or even fibrosis[14,15].

Morinda citrifolia can prevent free radical-induced oxidative-pathological events in the liver by inhibiting the inflammatory response and suppressing increased liver enzyme activity; thus preventing damage due to cell membranes. The addition of *Morinda citrifolia* extract shows hepatoprotection against alcohol-induced injury due to regulation of lipid homeostasis, antioxidant status, and alcohol metabolism[15].

Conclusion

In sum, the administration of noni fruit extracts have successfully increased LDL serum level and lowered HDL serum levels in dyslipidemia Wistar white rats induced by a high-fat diet.

Conflict of Interest : The authors declare that they have no conflict of interest.

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