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Original Research

The potency of green grass jelly extract (Premna oblongifolia Merr) as antihyperlipidemia towards aorta histopathology representation of rat (Rattus norvegicus) induced with high fatty diet (HFD)

Eka Nora Vitaloka Aprilia P.W[1a*], Anna Roosdiana[2b], Dyah Ayu Oktaviani A.Pratama[3c], Jusak Nugraha[4d], Marijam Purwanta[5e], Muh Husni Rifa'I[3f], Achmad Nur Rendy[3g]

[1] Post-Graduates School of Immunology, Universitas Airlangga, Indonesia
[2] Department of Chemistry, Faculty of Mathematic and Science, Universitas Brawijaya, Indonesia
[3] Department of Veterinery, Faculty of Veteriner, Universitas Brawijaya, Indonesia
[4] Department of Clinical Pathology, Faculty of Medicine, Universitas Airlangga, Indonesia
[5] Department of Pharmacy, Faculty of Pharmacy, Universitas Airlangga, Indonesia

[a] Email address: vitalokaaprilia@gmail.com
[b] Email address: arros@ub.ac.id
[c] Email address: dyahayoe_oap@yahoo.com
[d] Email address: jusak.nugraha@yahoo.com
[e] Email address: marijampurwanta@yahoo.com
[f] Email address: muhrifai@yahoo.com
[g] Email address: ach_rendy@gmail.com

HIGHLIGHTS

Green grass jelly extract (*Premna oblongifolia Merr)* could prevent fatty acid cell infiltration and prevent macrophag infiltration in rat (*Rattus norvegicus*) hyperlipidemia model

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A B S T R A C T

Green grass jelly *(Premna oblongifolia merr)* is a plant containing fiber and chlorophyll which can lower cholesterol and triglyceride levels. This study have an aim to investigate the potency of green grass jelly extract *(Premna oblongifolia Merr)* to prevent hyperlipidemia. The animal mode used for this study is male *Rattus norvegicus*, Wistar strain, the age of 8 weeks, and weight of 200 g which is divided into 5 groups of treatment namely group Kn (negative control), Kp (positive control), Kp1, Kp2, and Kp3 induced with HFD and green grass jelly extract at a dose of 5.27 g/ kg BW/ daily, 8.43 g/ kg BW/ daily, 9.37 g/ kg BW/ daily. The data of infiltrasi fatty cells and makrophag on aorta histopatholgy was analyzed by description. This research showed that treatment of green grass jelly extract *(Premna oblongifolia Merr)* to animal of hyperlipidemia model reduced infiltration fatty cells and makrophag. The conclusion of this study was the green grass jelly extract was able to prevent increase of fatty cells and makropha infiltration of rat *(Rattus noervegicus)* induced with HFD on dose 9,37 g/ kg/ BW/ daily.

Corresponding Author:
Eka Nora Vitaloka Aprilia Putri W
Sekolah Pascasarjana, Universitas Airlangga
Jalan Airlangga No 4-6, Kertajaya, Surabaya, Jawa Timur, Indonesia, 60282
Email: vitalokaaprilia@gmail.com

1. INTRODUCTION

Hyperlipidemia is a condition of increase blood lipid level consist cholesterol, triglyceride, and *LDL level (low-density lipoprotein)* but decreases blood lipid-like *HDL level (high-density lipoprotein)*. HDL level normal on blood plasma Rattus ≥35 mg/dL, the normal LDL level is 7-27.2mg/dl, and the normal cholesterol level is 10-54mg/dl.[1]

Hyperlipidemia could happen on pets (cat and dog). Chronic hyperlipidemia could cause endothelial dysfunction. The endothelial dysfunction caused by blood vessels increased, which make LDL easy to include at the blood vessel. Stress oxidation cause LDL easier to change to be LDL-ox, and than the macrophage scavenger receptor will catch it and change to be a foam cell. The accumulation of LDL on blood vessel walls could be cell foam.[2]

The high fatty diet was the food which compounds fat, and it can make the stress oxidation inside of the body.[2] Stress oxidative caused by free radical which binding with another compound to make a stable compound and could destroy another macromolecule, like the cell membrane of lipid, DNA and protein.[3] It could increase blood lipid, which is cholesterol and triglyceride level, which called hyperlipidemia.[4] Free radical can be caused LDL increased in the blood and also inflammation in the wall of the blood vessel. It's an infiltration of fatty cells and macrophage at tunica adventitia. The inflammation stimulated cell immunocompetent which is lymphocyte, neutrophil, monocyte and macrophage.[5]

The therapy was already used to patient hyperlipidemia, usually consist of SSRI (selective serotonin reactive inhibitor). But, a therapy used synthetic medicine has a negative effect and cause systemic distribution, vital organs like liver and ren. Green grass jelly *(Premna oblongifolia Merr)* is safe and easy therapy as preventive to hyperlipidemia. It is a plant consists of soluble fiber like pectin.[6] Because it, green grass jelly, called soluble fiber, which could decrease total cholesterol levels and serum LDL. Pectin is soluble fiber at the digestion tract, and it's used to bind bile acid. It's a final result of cholesterol and will be the end of the metabolism process as feces. It's expected could be to decrease the cholesterol level in the body.[5] This study has a purpose in knowing the fatty cell infiltration and the macrophage infiltration on aorta histopathology representation of rat hyperlipidemia model after giving preventive therapy by green grass jelly extract.

2. MATERIALS AND METHOD

Preparation Experimental number 421-KEP-UB Animal

Kn group is rat without giving any treatment (negative control/Kn), Kp group is ratt with hyperlipidemia condition (positive control /Kp), treatment group 1 (P1) is ratt with green grass jelly extract 5.27 gram/kg BW/day as preventive treatment and diet HFD *(high Fatty Diet)*, treatment group 2 (P2) is ratt with green grass jelly extract 8.43 gram/kg BW/day as preventive treatment and diet HFD *(high Fatty Diet)*, treatment group 3 (P3) is ratt with green grass jelly extract 9.37 gram/kg BW/day as preventive treatment and diet HFD. First, we have to adaptation the animal trial with laboratory area for seven days by giving them standard food to all rat. The ingredients of standard food are carbohydrate, protein, fat, mineral, vitamin, and water.

Preparation of Green Grass Jelly Extract

The sample we use is green grass jelly *(Premna oblongifolia merr)* which harvest at April 2018 in the morning in Batu, Malang. The green grass jelly we get, we cleaned it and drained it at the oven with temperature 30˚C. The green grass jelly extract then weighed with three dose different which is 1.35 g then dissolved with 8mL water (aquades), 2.7 g then dissolved with 10 mL water and 5.4 g then dissolved with 18 ml water and then just let stand every extract at bowl one by one, then homogenous it or stir it, then pour it to filter and press it, then collect the filtrate. Take the filtrate use spuit 3 mL until getting the green grass jelly with contains soluble in a small volume.

Preparation Animal Model Hyperlipidemia

Rat (*Rattus norvegicus*) can use be an animal model of obesity is male rat strain Wistar, 8 weeks, 200-gram body weight, with one-week acclimatization in the laboratory.[7] The researcher was chosen ratt (*Rattus norvegicus*) strain Wistar by easy to get, easy to care and have a fast metabolic. That was a benefit in experimental research which involved with metabolism.[8] Rat 6-8 weeks still not effected by growth hormone and sexual hormone.[9]

The male Wistar Rat, 20 rats, which is age 8 weeks, was adaptation one week at the laboratory. *High fatty diet* was compound by 1 gram quail yolk egg: 2 gram margarin: 2 fat cow.[10] A week after food adaptation, we give green grass jelly extract as preventive as the group treatment in

a week, for making sure the animal model not on hyperlipidemia phase we did total check cholesterol. Then we did the next step of treatment like give a high-fat diet an hour after green grass jelly extract treatment as long two weeks.

After that, the rat didn't give food and water (fasting) a day for did a checkup total cholesterol level. After that, to make sure the animal model already on the hyperlipidemia phase, then we need to check the cholesterol level in animal model hyperlipidemia. The result of the cholesterol level will be higher than in normal conditions.

The Green Grass Jelly Extract Treatment

The green grass jelly extract treatment starts giving after adaptation, giving by oral used sonde method to gastric rat as long 21 days. The green grass jelly extract *(Premna oblongifolia Merr)* was giving to rat with 3 concentration different which isKn group (negative control) normal rat, Kp group (positive control)rat with hyperlipidemia not give any treatment, P1 treatment (treatment 1)each rat with average weight 200 gram with 5.27 g/kg BW dose (2.5 mL), groupP2 (treatment 2) the rat with average weight 200 gram giving 8.43 g/kg BW (2.5mL) dose and group P3 (treatment 3) the rat with average weight 200 gram giving 9.37 g/kg BW (2.5mL) dose once a day.

The Analysis Histopathology Aorta Representation use H and E staining

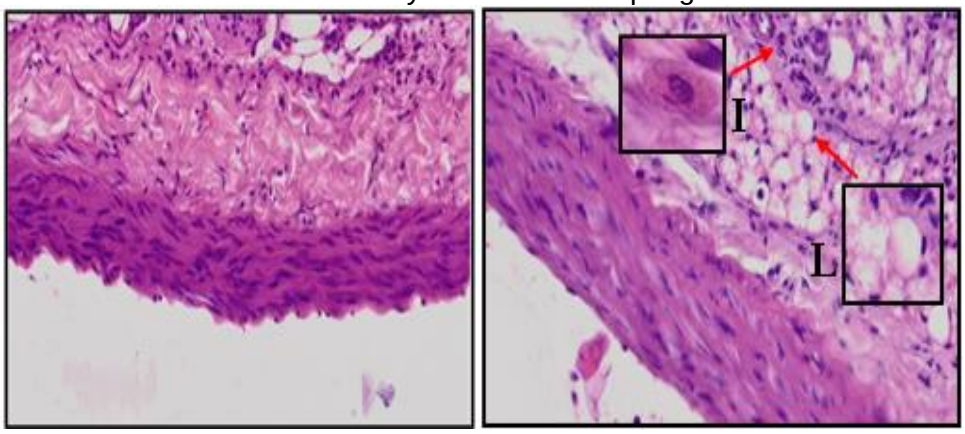
The analysis of histopathology aorta representative uses the H&E staining method by collecting the sample use PFA 4%. Then dehydration the aorta use ethanol, then make it in a paraffin block. The aorta ready to coloring use H&E staining. The next step observed it use a microscope with a 400x scale.

Analysis of Data

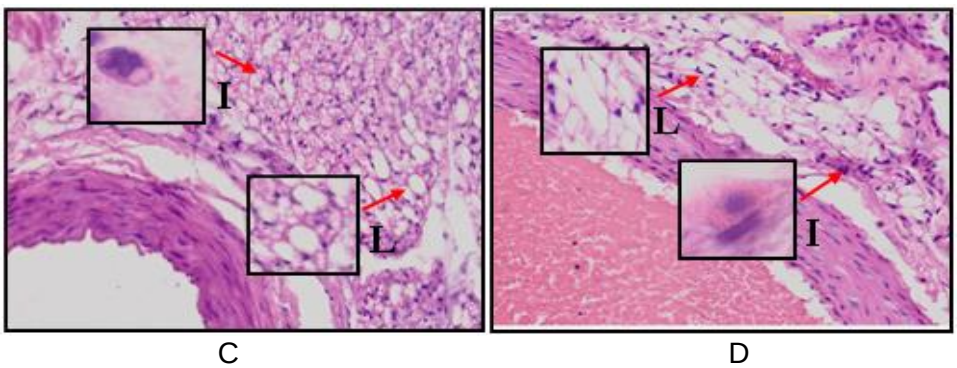
The variable we take in this research is fatty cell infiltration and macrophage infiltration. The analysis of aorta histopathology representation could do use a microscope with a 400x scale for seeing there are or absent fatty cells and macrophage infiltration. The data we get would be written by description.

3. RESULTS AND DISCUSSION

The research result of aorta histopathology representation uses a microscope with a 400x scale for observation of the infiltration of fatty acid and macrophage in the tunica adventitia.



A B



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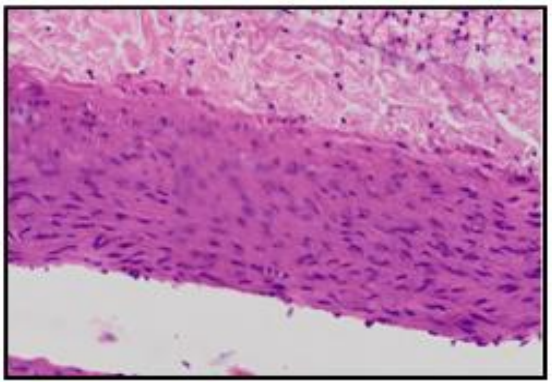

E

Figure 1. Rat *(Rattus norvegicus)* aorta histopathology representation used HE colouring with a 400x scale.
Noted:
Picture A is negative control group
Picture B is positive control group
Picture C is treatment group by green grass jelly extract 5.27 g/kg BW/ day dose
Picture D is treatment group by green grass jelly extract 8.43 g/kg BW/ day dose
Picture E is treatment group by green grass jelly extract 9.37 g/kg BW/ day dose
I is: inflammation (macrophage cell)
L is: Lipid cell

Green grass jelly extract *(Premna oblongifolia Merr)* is a plan to have fiber. The extract from this can be a gel because of the compound by water-soluble fiber. It was like pectin polysaccharide.[11] Pectin is one of some species which water-soluble food and easy to fermented by intestine microflora. Because the compound of pectin inside is a good fiber source.[12]

Stress oxidation caused by the increase of ROS as effected from HFD. Free radical incline to binding another compound to make a new compound which more stable and could destroy macromolecule, which is cell membrane of lipid, DNA, and protein, which could be stress oxidative.[13] It caused lipid peroxidation.[14] The peroxidation lipid on cell membrane-like free radical reaction with PUFA.[15] It can produce malondialdehyde (MDA).[16]

The increase of ROS caused by synthesis bile duct acid, which increased by high cholesterol and it could increase free radicals.[17] It because adipocyte and preadipocyte have identification as a source of inflammation cytokine, which is TNF-α, IL-1, dan IL-6. Cytokine is a stimulator for produce ROS and nitrogen by makrophage and monochyte. Stress oxidative is not a balance condition of oxidant and antioxidant in the body, so it could induced lipid peroxidation. Lipid peroxidation is a sequence reaction which has a signal by adding a hydrogen atom process by oxygen radical or reaction by free radical with PUFA in the cell membrane.[18] Stress oxidative was sign by MDA level, so MDA level used by the biology mark or measure of oxidative stress in the body.[19] It appropriate with the increased of infiltration lipid in the aorta which shows oxidative damage in lipid (LDL oxidation to LDLox).[20]

The aorta histopathology or rat *(Rattus norvegicus)* on figure 1.A (negative group) showed normal conditions in tunica intima. It's a layer of the aorta which direct contact with blood. This layer build by endothelial cells, the layer which closed with this layer is tunica media. Tunica media was building by smooth muscles and elastic tissue. The outer layer is tunica adventitia which is compound by connective tissue.[7]

There is three-step of lipid oxidation, which are initiation, propagation, and termination. Initiation is free lipid radical, and it is the mean compound of fatty acid, which not stabile and very reactive caused by loose one hydrogen atom. Propagation, loosed of the hydrogen atom, involved binding rearranges to stabilized with establishment Deina conjugation. Termination step showed that same radical could be fuse make a new molecule which not reactive or make a reaction with another antioxidant.[21] On that condition could make an accumulation of LDL in the blood vessel and being dysfunction endothelial. The dysfunction endothelial could make monocyte in the blood come into tunica adventitia and being lipid depotition.[22] It could be inflammation in the blood vessels and in tunica adventitia full of inflammation cells. The inflammation reaction could be caused by the initiation of immunocompetent cells like macrophage.[5] From figure 1.B could see that aorta histopathology representation clearly is infiltration macrophage and lipid cell. The trigger of influx and migration cells

didn't know clearly, and tunica adventitia looks like a point place of fatty acid cell infiltration and other inflammation cells.

The damage of endothelial cells was stimulated by ROS, which could be binding with LDL in the blood and be an LDL oxidation. That condition makes leucocyte inside of blood vessels or in the endothelial site, which damages.[23] On the other side, endothelial damage was caused by endothelial permeability to increase and be a cavity between the cells and be a lipid infiltration and inflammation cells in the tunica adventitia. It could be dysfunction endothelial or vasodilatation, which caused damage to endothelial cells.[24] But, at that histopathology, representation didn't show endothelial dysfunction clearly.

The infiltration decreased by green grass jelly extract which compound by water-soluble fiber like pectin which caused fermented easily by intestine microflora which produced acetic acid, propionate and butyrate which could inhibit cholesterol synthetic that showed on figure 1.C and 1.D.[25] The highest decreased cholesterol and triglyceride was shown on figure 1.E, it because the soluble water fiber was done by increased free radical and cholesterol with bile duct acid which in by *hepatic duct* over by pancreas, then into proximal duodenum which near with pylorus in gastritis tract and out by the last product of secretion.[20] Except for compound by green grass, fiber is also compound by antioxidants, which is chlorophyll. The high chlorophyll level could decrease cholesterol level, and triglyceride to increased, caused by chlorophyll can give oxygen which can be neutralized fee radical and inhibit free radical activation, so didn't be a stress oxidative and membrane cell damage which mark by decreased of lipid infiltraion.[15]

4. CONCLUSION

Giving green grass jelly extract (*Premna oblongifolia Merr)* with 5.27 g/kg BW, 8.43 g/kg BW dan 9.37 g/kg BW dose in rat (*Rattus norvegicus*) hyperlipidemia model could prevent fatty acid cell infiltration and prevent macrophage infiltration by giving the best dose 9,37 g/kg BW.

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CONFLICT OF INTEREST

We declared in this work, not any conflict of interest.

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