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Research Article

Dietary Fiber of *Glycine max* (L.) Merr Compound as Antihypercholesterolemia to MDA Levels and Hepar Histopatology on Hypercholesterolemic Rats

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ABSTRACT
Black soybean's (*Glycine max (L.) Merr*) is a tropical plant and spread widely in Southeast Asia including Indonesia. It contains of black soybean's are rich in fiber but has not been widely used for the treatment of cholesterol. This paper discusses recent investigation on the benefits of dietary fiber black soybean's decrease the MDA levels on high cholesterol diet induced rats. Twenty wistar male rats (*Rattus norvegicus*) divided into 4 groups. The first group was negative control. The second group was hypercholesterolemic rats induced with hyprcholesterolemic diet consisted of 10% lard, cholic acid, and a quail egg yolk which has been heated (100°C) mixed with corn oil to 2 ml as well as standard feed for 2 weeks. The third and fourth groups of rats were therapied with dietary fiber of black soybean dose of 0.72g/kgWB and 1.44g/kgWB, respectively. The result showed dietary fiber theraphy decreasing of MDA levels of hypercholesterolemic rats to be 38.198% and 48.922% at dose of 0.72g/kgWB and 1.44g/kgWB, respectively. Statistical analysis showed that each treatment group were significantly different ($p<0.05$). It also could repair hepar tissue which showed by decreasing of fat accumulation on hepatocyte. The conclusion from this study was dietary fiber of black soybean had potentialy as an alternative treatment of hypercholesterolemia.

Keywords : Black Soybean, Hypercholesterolemia, Malondialdehyde (MDA), Hepar

INTRODUCTION

Cholesterol is a fat - like substance produced by our bodies and found only in food of animal origin. Our tissues make 75% of the cholesterol in our bodies. Hypercholesterolemia defined as excessively high plasma cholesterol levels. Total cholesterol can be broken down into a diagnostic lipoprotein profile, including high density lipoprotein (HDL), low density lipoprotein (LDL), intermediate density lipoproteins (IDL), very low density lipoprotein (VLDL), chylomicron remnants, and triglycerides[1]. High blood cholesterol is one of the major risk factors for heart disease. If total cholesterol is 200 mg/dL or more or if HDL is less than 40 mg/dL, it lead to hypercholesterolemia condition.

Protein is the major constituent of the black soybean (35.9g/100g), followed by carbohydrates (14.8g/100g), dietary fiber (15.7g/100g) and lipids (2.7g/100g). Dietary fiber intake provides many health benefits. Dietary fiber (DF) decreases the risk for type 2 diabetes mellitus, cardiovascular disease, and colon cancer by reducing the digestion and absorption of macronutrients and decreasing contact time of carcinogens within the intestinal lumen[2,3]. Increasing fiber intake lowers blood pressure, aids in weight loss and serum cholesterol levels[4]. Dietary fiber consists of the structural and storage polysaccharides and lignin in plants that are not digested in the human stomach and small intestine[5]. Consumption of dietary fibers could lower blood cholesterol viscous levels and helps to normalize blood glucose and insulin levels.

Malondialdehyde (MDA) is the principal and most studied product of polyunsaturated fatty acid peroxidation[6]. Malondialdehyde (MDA) is one of several low-molecular-weight end products formed via the decomposition of certain primary and secondary lipid peroxidation products. The TBA test is intrinsically nonspecific for MDA, nonlipid-related materials as well as fatty peroxide-derived decomposition products other than MDA are TBA positive. Since MDA is considered a presumptive biomarker for lipid peroxidation in live organisms, a model for nutritionally induced oxidative stress (hypercholesterolemic rats) was studied in comparison with noncholesterolemic animals[7].

This study aimed to investigated the role of dietary fiber black soybeans as a therapeutic animal model of hypercholesterolemic rats (*Rattus norvegicus*) on hepar as well alteration of hepar histopatology wich induced with hypercholesterolemic diet.

MATERIALS AND METHODS

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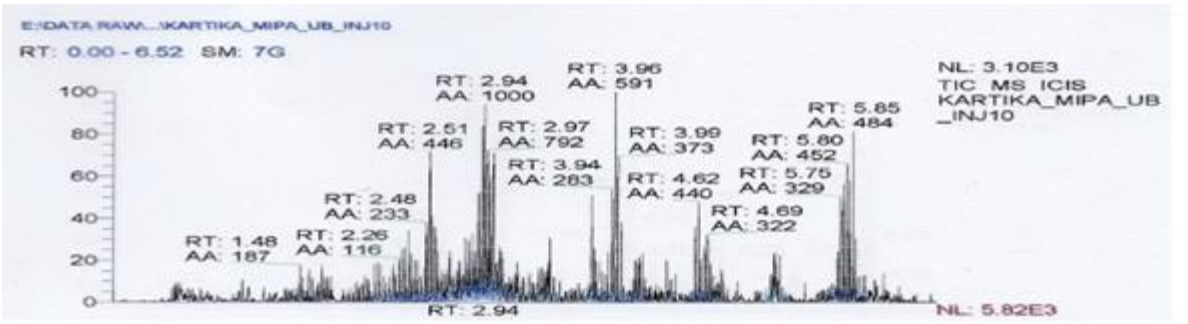

Picture 1 : delphinidin-3-glucoside
Figure 1: LC-MS Analysis

Tabel 1: MDA levels on treatment rats (*Rattus norvegicus*)

Groups	MDA levels (µg/ml)* ($\bar{X}$±SD)	Increasing of MDA levels (%)	Decreasing of MDA levels (%)
Negative Control (A)	1.733±0.068[a]	-	-
Positive Control (B)	5.351±0.074[d]	208.77	-
Therapeutic dose 0.72g/kgBW (C)	3.307±0.110[c]	-	38.198
Therapeutic dose 1.44g/kgBW (D)	2.632±0.037[b]	-	48.922

*Note: superscript showed significantly different (P<0.05)

Chemicals and instruments

The chemicals used in this study were black soybeans powder, 10% lard, 0.1% cholic acid (Kasei Tokyo), standard feed, egg yolk quail, hematoxylin-eosin stains, corn oil (SIGMA), Na-Thio, washing buffer, hydrochloric acid, potassium hydroxide, 0.9% sodium chloride, phosphate buffer saline-azide, distillated water, trichloroethanoic acid, and aluminum foil.

The tools used in this research were glass tools, mortar, UV-Vis spectrophotometer (Thermoscientific Genesys 20), oven, water bath, vortex, gavage, scales, centrifuge (KITMAN-T24), autoclave, scapel, micro-pipette, glass objects, microscope (Olympus BX51), scissors, eppendorf tube, rats cage, animal restrain, Easy Touch GCU to check level cholesterol of experimental animal, freezer -20 ºC, refrigerator 4 ºC, and elisa plate, elisa reader.

Preparation of hypercholesterolemic rats

Preparation of hypercholesterolemic's rat-model based Gloria *et al*. (2010)[4]. Male white rats (*Rattus norvegicus*) of Wistar weigh of 150-200 g divided into 4 groups. In the first group was healthy rats (negative control), second group was hypercholesterolemic rats (positive control) which fed by high cholesterol diet, then the third and fourth group were hypercholesterolemic rats with black soy bean therapy dose of 0.72 g/kg BW and 1.44 g/kg BW orally for fourteen days. The used of animal model in this research was approved by Research Ethics Committee of Brawijaya University (No. 472-KEP-UB).

Hypercholesterolemic diet prepared consist of 10% lard, cholic acid, and a quail egg yolk which has been heated (100℃) mixed with corn oil to 2 ml. Rats were given standard feed as much as 16.78 g/rat for 2 weeks[4]. All rats were sacrificed then serum and hepar were collected.

Preparation black soybean's dietary fiber

Procedure was undertaken based on Yang *et al*., (2014)[8]. The black soybean powder were added with 50 ml of 2 N HCl solution to obtain pH about 1.5-2. The mixture was heated for 2 h at 60 ºC, and further washed with distillated water until neutral pH was achieved. The supernatant was separated after centrifugation at 5000 g. Then, the supernatant was basified with 50 ml of 2 N KOH and further heated for 2 h at 60 ºC. The resulted solid fiber was filtered and washed with distillated waster until neutral pH and following with drying process.

LC-MS Analysis

The flavonoid compounds compose in dietary fiber of black soybean (*Glycine max (L.) Merr*.) were analyzed and identified qualitatively using liquid chromatography mass spectrometry (LC-MS).

The Measurement of Malondialdehyde (MDA) Levels

The MDA measurement was conducted using Tiobarbituric acid reactivity assay (TBAR). The hepar sample was collected : 0.5 g of Hepar was cut into small pieces and crused on cold mortar. Then 1 mL of 0.09% Na-phys was added. Homogenates were sentrifuged at 4000 rpm, 4ºC for 20 min and supernatat were collected. Then 100 µL supernatant was added by 550 µL distilled water, 100 µL of TCA, 250 µL of 1N HCl and 100 µL of Na-Thio then mixed and homogenized using vortex, then it was centrifuged at 500 rpm, 4ºC, for 15 min. Then the supernatant were collected and incubated at a 100℃ for 10 min. Samples were measured at λ max 532 nm.

RESULTS AND DISCUSSIONS

LC-MS is a hyphenated technique, which combines the separating power of High Performance Liquid Chromatography (HPLC), with the detection power of mass spectrometry. Mass Spectrometry is a wide-ranging analytical technique, which involves the production and

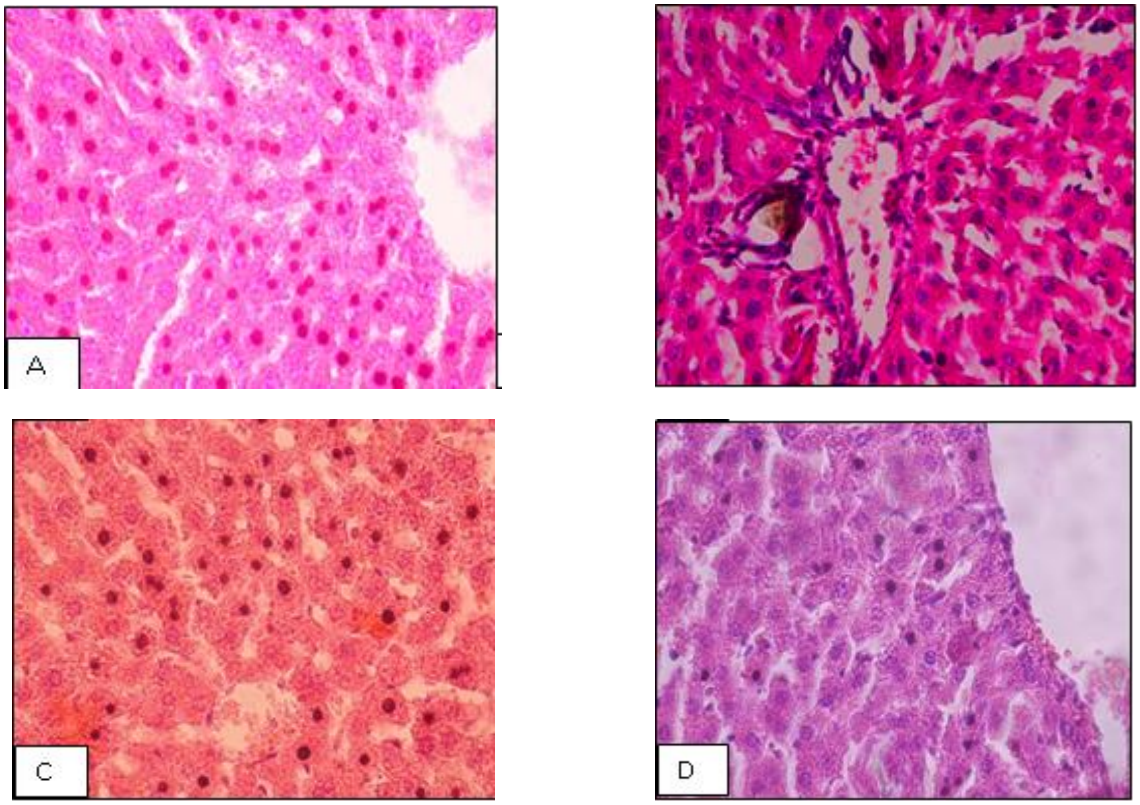

Figure 2: Hepar histopathology on treatment rats
(A)Negative control; (B) Positive control; Dietary black soyben therapy dose of 0.72 g/kgBW (C); and 1.44 g/kgBW

subsequent separation and identification of charged species[9].

The analysis results LCMS from dietary fiber of black soybean (*Glycine max (L.) Merr*.) indicated isoflavone compounds genestein, daidzein, and glycitein[9] and anthocyanin compounds (delphinidin-3-glucoside, cyanidin-3-glucoside and petunidin-3-glucoside).

Delphinidin-3-glucoside (m/z 302.50), cyanidin-3-glucoside (m/z 286.50) and petunidin-3-glucoside (m/z 316.50) can be found in all green plants. Delphinidin-3-glucoside is an anthocyanin. Anthocyanins belong to flavonoids, and widely spread in flowers, fruits and vegetables[10]. Naturally occurring anthocyanins are pelargonidin, cyanidin, delphinidin, peonidin, petunidin and malvidin (delphinidin-3-glucoside). Anthocyanins act as potent antioxidants by donating hydrogen atoms to highly reactive free radicals. It also plays a role to break the free radical chain reaction.

The TBAR assay for lipid peroxidation was modified to minimize artifactual oxidative degradation of lipids during the assay. MDA levels on hypercholesterolemic rats showed significantly different ($P<0.05$) to healthy rats. Dietary fiber of black soybean (*Glycine max (L.) Merr*) significantly ($P<0.05$) lowered MDA levels. The best therapuetic dose of dietary fiber of black soybean was 1.44 g/BW, even it showed significantly ($P>0.05$) to negative control group (Table. 1)High levels of MDA in hypercholesterolemic rats caused by lipid peroxidation process, which directly indicate by the production of free radicals. MDA may be generated during hydrolysis by the oxidation of polyunsaturated fatty acids (PUFA) in the sample and by the degradation of pre existing oxidation products. Hepar MDA level on hypercholesterolemia condition showed the highest compare with others, and it was increase to be 208.77% from normal (healthy rats) condition. Dietary fiber of black soybean could reduce MDA levels significantly. The more doses administered, the lower of MDA level were obtained. The highest dose of dietary black soybean of 1.44g/kgBW was the best theurapeutic used in this research. It could reduce MDA levels to be 48.922%. Reducing of MDA levels was related with reducing of free radicals and lead to improvement on rat's tissue. Dietary fiber of black soybean could reduce cholesterol level by decreasing of fat adsorption in intestines which lead to inhibit cholesterol aggression to hepar tissue.

In normal condition, showed normal structure of hepatocyte which locate around of central vein. In positive control of hypercholesterolemic rat showed fat accumulation in the central vein. In therapeutic groups of dietary fiber of black soybeans with dose 0.72g/kgBW and 1.44g/kgBW showed reduction of fat accumulation in hepatocyte. The accumulation of fat on hypercholesterolemia condition due to decreased activity of lipoprotein to hydrolyze VLDL so that TG accumulated in hepatocyte. The formation of free radicals cause cells are unable to remove TG causing fatty degeneration. Increased levels of free radicals further will cause necrosis[11]. The alteration of liver histopathology by dietary fiber of black soybean cause by

antihypercholesterolemia and antioxidants activities. Antioxidants activity inhibit lipid peroxidation by capturing free radicals so it reduce excessive free radicals resulted from TG accumulation. This findings showed the ability of flavonoid containing from black soybean could reduce MDA levels and repair hepar histopathology on hypercholesterolemic rats. Dose of 1.44g/kgBW showed the best result obtained to reduce MDA levels and reduce fat accumulation on hepar tissue.

CONCLUSION

Dietary fiber of black soy bean *Glycine max (L.) Merr*) has potentially used as hypercholesterolemia therapy by reducing MDA level as well as repairing hepar histopathology on hypercholesterolemic rat.

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