Antihyperlipidemic Activity of Ethanol Extract Mindi's Leaves (Melia azedarach Linn.) in Male Wistar Rats Induced Propiltiouracil

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Abstract
Mindi's leaves (Melia azedarach Linn.) is an Indonesian medicinal plant that used as traditional medicine. Mindi leaves contains some secondary metabolites which have potency to decreased total cholesterol and LDL level. The study purposed to know antihyperlipidemic effect of ethanol extract of mindi's leaves (Melia azedarach Linn.) seen from total cholesterol, and LDL in male albino rats. Male wistar albino rats were divided into 5 groups, negative control group (sodium CMC 0.5%), positive control group (simvastatin 0.193 mg/200 gBW), group I (ethanol extract mindi's leaves with dosage 300 mg/200 gBW), group II (ethanol extract mindi's leaves with dosage 600 mg/200 gBW), and group III (ethanol extract mindi's leaves with dosage 1200 mg/200 gBW). The rats were given high-fat supplement and propylthiouracil for 15 days to increase cholesterol, and the extract was given for the next 15 days. Average cholesterol level, LDL, and body weight after induction was 90.28 mg/dL, 31.09 mg/dL, and 222.32 g. The result showed ethanol extract mindi's leaves could decreased total cholesterol level and LDL level with % decreased in total cholesterol (%PDTC) and % decreased in LDL (%PDLDL) of group I is 37.78% and 35.57%, group II is 45.99% and 40.39%, and for group III is 56.29% and 52.42%. The result showed that ethanol extract of mindi's leaves has antihyperlipidemic activity and significantly different from negative control (p>0.05). Based on the percentage relation of decreased total cholesterol and LDL levels to dose, then the effective dose 50 (ED50) value of ethanol extract mindi's leave is 869 mg/200 gBW for total cholesterol and for LDL reduction level is 1086.84 mg/200 gBW.

Keywords
Melia azedarach Linn, antihyperlipidemia, total cholesterol, low density lipoprotein (LDL)

1. INTRODUCTION
Hyperlipidemia is the increase in one or more of the total cholesterol, LDL, and triglycerides, or decrease in HDL (Wells et al., 2009). Generally, a person is said to have hyperlipidemia one of the characteristics is if the total cholesterol in the blood exceeds the normal limit (> 200 mg/dL). The data results of Riskesdas (2013) shows the prevalence of hyperlipidemia in Indonesia based on the levels of total cholesterol over 240 mg/dL is of 35.9%. Treatment of hyperlipidemia can be overcome with the use of synthetic drugs (Priyanto, 2009).

The use of synthetic drugs in the long term can cause side effects like myopathy, redness, and itching on the face (Brunton et al., 2008). Many researches on herb plants are done. One of the plants suspected of potentially as antihyperlipidemia is the mindi's leaves (Melia azedarach Linn.).

Mindi leaves (Melia azedarach Linn.) is a plant in the Meliaceae family. The use of mindi leaves traditionally used for malaria, diabetes, coughs, skin diseases, etc (Azam, 2013). The pharmacology activities are related to the content of secondary metabolites in the leaves of mindi.

Mindi leaves are known to contain secondary metabolites including alkaloids, tannins, saponins, phenolics, steroids, terpenoids, and flavonoids (Fitriyani et al., 2016). Flavonoid compounds that act as antioxidants play a role in delaying lipid oxidation processes so as to prevent increases in LDL (Low Density Lipid) and cholesterol total (Davies, 2003). According to Agustina (2009), alkaloid compounds can inhibit lipase enzyme activity that can inhibit the breakdown of fat so that the reduced amount of fat absorbed.

Based on that background, the mindi leaves is thought to have a compounds that can lower blood cholesterol and LDL levels. The aim of this research is to know the potency of mindi leaves ethanol extract in decreasing total blood cholesterol level and LDL level of male white wistar rats induced high fat supplements and propiltiouracil.
2. EXPERIMENTAL SECTION

2.1 Chemicals
The ingredients used of simplicia mindi leaves, ethanol 70%, filter paper, dilute HCl, akuades Mg, powder, the reactant reactant Mayer, Wagner, reactant Dragendorff, Alumuniumm chloride, ethyl acetate, anhidirida acetate acid, concentrated sulfuric acid, FeCl₃, silica gel plate GF254, fat goats, pig oil, butter, yellow duck egg, feed standard, propilitoriasulis, Na CMC, sim-vastatin, reagent cholesterol (CHOD-PAP), and LDL reagent (polyvinyl sulfate).

2.2 Plant materials
Samples of mindi leaves were taken in Plaju of South Sumatera, Indonesia. The sample of mindi leaves was made herbarium and determined by UPT Balai Konservasi Plant of Purwodadi Botanical Garden - LIPI. Sampel was thoroughly washed with tap water, sorted while wet, dried in the shade, and grinded into powder.

The 1000 g of leaves of mindi simplicia was macerated in 6 mL of ethanol 70% for 2 days, then filtered. The filtrate was re-macerated in 4 mL of ethanol 70% until the solvent was clear and then re-filtered. The resultant filtrate was then concentrated with a rotary evaporator at 65 °C at a rate of 40 rpm. This process is done until the concentrated extract is obtained, then calculated the value of rendenten extract etanol of simpliciausedinextr action x simpliciausedinedercentageextract calculated by using equation 1.

\[
\text{% yield} = \frac{\text{obtainedthickextract}}{\text{simpliciausedinextraction}} \times 100\% \quad (1)
\]

2.3 Preparation of extract

2.4 Extract Characterization
2.4.1 Phytochemical Test Using Reagents
The alkaloid identification was performed by 0.1 g extracts plus 5 mL of chloroform and 5 mL of 0.05 N ammonia. Filtrate was added 5 mL 2N H₂SO₄ and shaken regularly to form 2 layers. The upper layer is then divided into 3 parts, each added 2 drops of Mayer, Wagner, and Dragendorff reagents. If the precipitate is formed then the sample contains alkaloids (Al-Daihan and Bhat, 2012).

Flavonoid test was performed by of 0.5 g of extracts were again extracted using 5 mL of hot ethanol for 5 minutes in the test tube. The extraction results are filtered and add a few drops of concentrated HCl to the filtrate. Enter about 0.2 mg of magnesium metal. Positive tests of flavonoids are characterized by the appearance of red, yellow, or orange. Another way can be done by adding 2 drops of 10% NaOH to ethanol extracted filtrate (Al-Daihan and Bhat, 2012).

The saponin test used 0.1 g viscous extract plus 10 mL of hot distilled water, then shaken for 10 minutes in a closed state. Extracts containing saponins will form a stable froth for 10 minutes (IndonesianMinistryofHealth, 1977).

2.4.2 Flavonoids test with Thin Layer Chromatography
The flavonoid identification was done by of an ethanol extract solution of 70% of the mindi leaves on the TLC plate measuring 5x1 cm. The TLC plate was then eluted with eluent in a saturated chamber, a mixture of ethanol and ethyl acetate (8:2). TLC was eluted by observation of spots on UV lights 254 and 366 nm, after which sprayed with Aluminum chloride and caused yellow spots after spraying.

2.5 Test Animal Preparation
The test animal used was white male rats Wistar aged 2 – 3 months with 150 – 200 g weight of 24 tails. The treatment group was divided into 6 groups as in Table 1.

2.6 Induction of Fat Supplements and PTU
Test animals in all groups were induced with a high-fat supplement and 0.01% propylthiouracil solution for 14 days orally to obtain hyperlipidemic conditions in mice (>54 mg/dL for total cholesterol and >27.2 for LDL). Blood sampling was performed on day 15 to determine total cholesterol and rat LDL levels.

2.7 Antihiperlipidemia Activity Test
Rats with a total cholesterol of more than 54 mg/dL and LDL over 27.2 mg/dL were used to continue in antihiperlipidemia testing. The division of animal group test as in Table 1 of each group was given treatment in each dose of each test preparation once every 1 day for 14 days orally. The weight of the test animal was weighed during the treatment.

2.8 Measurement of Total Cholesterol and LDL
2 mL Rat blood was taken with a hematocrit pipette in the retroorbital plexus section. Blood was silenced for 15 minutes and centrifuged for 10 minutes at 3000 rpm. Blood serum is piped with a 10 µL micro pipette and inserted in a test tube. Serum is then fed into the bio system analyzer. CHOD-PAP cholesterol reagent solution was added to 1000 µL samples, blanks and standards for total cholesterol measurements while for LDL level measurements added polovinylic sulphate reagents.
Table 1. The design of animal test groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>High fat supplement + Propiltiouracil 0.01% + Sodium CMC 0.5%</td>
</tr>
<tr>
<td>Positive Control</td>
<td>High fat supplement + Propiltiouracil 0.01% + Simvastatin dose 0.193 mg/200 gBW</td>
</tr>
<tr>
<td>Group I</td>
<td>High fat supplement + Propiltiouracil 0.01% + suspension extract of mindi leaves dose 300 mg/200 gBW</td>
</tr>
<tr>
<td>Group II</td>
<td>High fat supplement + Propiltiouracil 0.01% + suspension extract of mindi leaves dose 600 mg/200 gBW</td>
</tr>
<tr>
<td>Group III</td>
<td>High fat supplement + Propiltiouracil 0.01% + suspension extract of mindi leaves dose 600 mg/200 gBW</td>
</tr>
</tbody>
</table>

ED₅₀ was calculated based on the correlation between percentage effect of decreasing total cholesterol and LDL on the dose of extract analyzed using linear regression.

2.9 Data Analysis
The data obtained were statistically analyzed by Shapiro-Wilk normality test to find out whether the data obtained had a normal distribution (p > 0.05). Normally distributed data is continued with paired t-test and one way ANOVA using SPSS 16.0 For Windows program with provisions if p < 0.05 then there is a significant difference, but if the data is not normally distributed then proceed with Kruskal-Wallis non parametric test.

3. RESULTS AND DISCUSSION
3.1 Preparation Of Extract
Mindi leaf extract was obtained through a process of maceration using 70% ethanol solvent. The choice of the ethanol as solvent because ethanol (70%) is very effective in generating the optimal amount of the active ingredient. Maceration was chosen because of the extracted target compounds are compounds of flavonoids not resistant to warming and easily oxidized at high temperature (> 90°C) (Francis et al., 2002).

Maceration results concentrated with a rotary evaporator with a rotation speed of 40 rpm at a temperature of 65°C to obtain viscous extract. The extraction process is done using powder simplisia mindi leaves as many as 1000 grams and produce a thick extract of 238.96 grams with the percentage yield of 23.896%. The yield of rendement can also indicate the possible amount of chemical compounds contained in the extract.

3.2 Phytochemical Screening Extracts
Based on phytochemical screening tests in Table 2, a positive reaction to the testing of flavonoids, alkaloids, tannins, phenolics, saponins, and steroids, but gave a negative reaction to triterpenoids.

3.3 Identification of Flavonoids using TLC
The identification of flavonoid compounds on mindi leaves ethanol extract was performed by qualitative test using thin layer chromatography (TLC). The stationary phase used is a silica gel plate measuring 5 cm x 1 cm GF254 (Merck), while the mobile phase used is ethanol:ethyl acetate (8:2) capable of providing the best separation. Flavonoid compound group examinations were detected under 366 nm and 254 nm UV light. TLC results are reacted with aluminum chloride spray reagent to detect flavonoids. Spray reagents used to identify flavonoid compounds include AlCl₃. Based on the results of extract chromatogram with aluminum chloride spray reagent showed yellow color. The flavonoid compounds in the ethanol extract of the mindi leaves will bind to aluminum (Al) to form a yellow stable complex (Grundy, 1991)).

Table 2. Phytochemical test of extract by using reagent

<table>
<thead>
<tr>
<th>Secondary Metabolite</th>
<th>Extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>Phenolic</td>
<td>+</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>-</td>
</tr>
</tbody>
</table>

Description: (+) positive and (-) negative

Figure 1. TLC results of ethanol extract of mindi leaves (a) UV 254 (b) UV 366 (c) AlCl₃ stain appearance
3.4 Provision of Dosage and Induction of Test Animal

Wistar male white rats used had a 2 - 3 month age criterion and weighed ± 200 g. Male white rats were selected as test animals because they had better hormonal stability compared with female white rats. Female white rats have a period of estrus, pregnancy, and estrogen hormones that can influence the results of observation (Pratiwi, 2010).

The process of acclimatization is done during one week aims to adopt rats on laboratory conditions so that it does not appear the stress in rat. The next test animals were given induction of high-fat supplements are per oral for 15 days to increase the total and LDL cholesterol levels in rat. In addition to induction with high-fat supplements, animal tests are also given propiltiourasil (PTU) per oral. Propiltiourasil worked as an antitiroid that inhibits thyroid cells in rat so that the production of thyroid hormone is inhibited and leads to hypothyroidism (Rahayuningi and Tita, 2015).

3.5 Measurement of Weighted Animal Test

Weight observations were performed to see the differences in body weight between treatment groups and to see if there was any effect of high-fat supplementation on body weight of the test animals. The results showed that the weight of all test animals increased after the administration of high-fat supplements for 14 days and there were significant differences (p <0.05). That is, the provision of high-fat supplements can increase body weight test animals.

After being treated according to Table 1 all the test animals lost weight and there were significant differences (p <0.05), which showed that the extract of mindi leaves ethanol was able to decrease the weight of the test animals. Weight loss is caused by the content of the compounds that exist in the extract including flavonoid compounds. Flavonoids can inhibit FAS (Fatty Acid Synthase) by blocking Acetil-CoA and Malonil-CoA so as to inhibit genes that play a role in adipogenesis thereby decreasing the amount of adipocytes (Jeyakumar et al., 2005).

The positive control group experienced considerable weight loss, this is due to the provision of simvastatin in positive control which is the enzyme inhibitor HMG-CoA reductase which can disrupt the cholestrol synthesis in the liver so that it can lower the triglyceride level. Decreased triglyceride levels cause storage in adipose tissue is also decreased (Kasim et al., 1992).

3.6 Measurement of Total Cholesterol and LDL

Measurement of total cholestrol and LDL in this study was done 3 times after acclimation, after the induction of high-fat supplements, and after the provision of the test material. Blood is taken in the retroorbital part of the rat’s eye because the blood collection is relatively fast and smooth so as to minimize the occurrence of hemolysis. The results of measurement of total cholesterol and test animals can be seen in Table 3 and Table 4.

The normal range for total cholesterol in mice was 10-54 mg/dL (Harini, 2009), whereas in LDL was 7 -27.2 mg/dL (Herwiyarirasanta, 2010). The results showed that there was an increase in total cholesterol levels and after the induction of high-fat supplements and PTU significantly (p <0.05). This proves that the induction of high-fat supplements for 14 days was able to increase total cholesterol and LDL levels significantly.

This is in line with the statement of Grundy (1991) that the consumption of foods rich in fatty acids and cholesterol can inhibit the formation of LDL receptors, so that cholesterol accumulates in the blood. While the role of PTU is as antithyroid substances that can damage the thyroid gland and cause hypothyroid conditions. Under these conditions, there is a decrease in synthesis and expression of LDL receptors in the tissues. Therefore, LDL circulates much in the blood and causes hyperlipidemia. The results of the total cholesterol and LDL test after treatment showed that the test material at three doses and positive controls decreased total cholesterol and LDL levels significantly (p <0.05) when compared with the negative controls (Figures 2 and 3).

The results showed the highest decrease in total and LDL cholesterol occurred in the positive control group. The positive control group experienced a decrease in total cholesterol levels due to the presence of simvastatin which has a structure similar to HMG-CoA reductase, works by inhibiting HMG-CoA reductase competitively in the cholesterol synthesis process in the liver (Suyatna, 2007).

The negative control group experienced only a slight decrease in total cholesterol and LDL levels. This is because the
Table 3. Results of Measurement Total Cholesterol of Animal Test

<table>
<thead>
<tr>
<th>The treatment groups</th>
<th>Average levels of Total cholesterol (mg/dL) ± SD</th>
<th>Average Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After Acclimatization</td>
<td>After induction of high-fat*</td>
</tr>
<tr>
<td>Negatif Controls</td>
<td>47.49 ± 6.29</td>
<td>87.85 ± 2.08</td>
</tr>
<tr>
<td>Positive Controls</td>
<td>47.4 ± 5.86</td>
<td>97.69 ± 3.87</td>
</tr>
<tr>
<td>Group I</td>
<td>49.84 ± 3.34</td>
<td>86.37 ± 6.57</td>
</tr>
<tr>
<td>Group II</td>
<td>50.25 ± 5.54</td>
<td>91.91 ± 3.46</td>
</tr>
<tr>
<td>Group III</td>
<td>46.79 ± 2.05</td>
<td>87.57 ± 5.82</td>
</tr>
</tbody>
</table>

Description: *a: p <0.05 data differ significantly to the value after acclimation *b: p <0.05 data differ significantly to the value after induction

Table 4. Results of Measurement LDL of Animal Test

<table>
<thead>
<tr>
<th>The treatment groups</th>
<th>Average levels of Total cholesterol (mg/dL) ± SD</th>
<th>Average Decline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After Acclimatization</td>
<td>After induction of high-fat*</td>
</tr>
<tr>
<td>Negatif Controls</td>
<td>9.84 ± 1.56</td>
<td>29.83 ± 2.02</td>
</tr>
<tr>
<td>Positive Controls</td>
<td>9.80 ± 2.20</td>
<td>32.77 ± 3.65</td>
</tr>
<tr>
<td>Group I</td>
<td>8.79 ± 2.17</td>
<td>28.82 ± 1.15</td>
</tr>
<tr>
<td>Group II</td>
<td>8.02 ± 0.22</td>
<td>29.23 ± 1.01</td>
</tr>
<tr>
<td>Group III</td>
<td>8.63 ± 0.81</td>
<td>34.83 ± 4.13</td>
</tr>
</tbody>
</table>

Description: *a: p <0.05 data differ significantly to the value after acclimation *b: p <0.05 data differ significantly to the value after induction

test animals on negative controls are only given a solution of sodium CMC that serves as a suspending agent and not given anything that could potentially reduce total cholesterol levels so that the cholesterol levels of test animals only slightly decreased. The occurrence of decreased total cholesterol levels in the negative control one of them can be caused by the diet of cholesterol, fatty acids, and calories. Naturally, the body can degrade excess cholesterol to bile acids (Murray et al., 2006).

The results showed that the extract of mindi ethanol leaves can lower total cholesterol and LDL of test animals. The dose of mindi leaf extract is best in lowering cholesterol and LDL levels in group III treatment (1200 mg/200 gBB). This is due to the presence of metabolite compounds contained in the extract of ethanol leaves mindi. The result of statistical test with t-pair showed significant difference of total cholesterol and LDL of test animal before and after treatment (p <0.05).

The results of phytochemical screening showed that the extract of mindi ethanol contains flavonoid compounds, alkaloids, tannins, phenolics, saponins and steroids. The possibility of such a decrease effect is due to the compounds of the group. Flavonoids work by reducing cholesterol synthesis by inhibiting the activity of 3-hydroxy-3-methyl-glutaril-CoA enzyme causing inhibition of cholesterol synthesis (Arief et al. 2012). Flavonoids can affect the metabolic process of LDL cholesterol by increasing the ability of LDL to bind to its receptors (Wilcox et al., 2001). The mechanism of tannin compounds in the extract of ethanol leaves mindi to decrease LDL levels is by inhibiting the work of the enzyme HMG-CoA reductase, the enzyme that play a role in the formation of cholesterol. Research Francis et al (2002) states that saponins can lower serum sodiumol levels with the possibility of binding saponins with cholesterol.

Data analysis was performed using SPSS® application, it is known that normality test of total cholesterol and LDL data is normally distributed with significance value > 0.05. Test of total cholesterol and LDL using one way ANOVA obtained significance value p <0.05 which states that data have relationship and significant. One way ANOVA was performed to observe differences in data in one test group, so that based on these data, there were significant differences in cholesterol and LDL levels between groups. Result of post hoc test analysis, it is known that there is no significant difference of data of decrease of cholesterol and LDL level between positive control group with group I, II, and III (p> 0.05). The positive control group and groups I, II, and III showed a significant difference with the negative control group (p <0.05).

3.7 Effective Dose (ED₅₀)

According to the Department of Pharmacology and Therapeutic FKUI (2011), a 50% effective dose (ED₅₀) is a dose that has a therapeutic effect on 50% of individuals (median dose of therapy). ED₅₀ reduction in total cholesterol levels was calculated by linear regression between doses with percent decrease in total cholesterol (% PDTC) (Fig. 4). Linear regression equation obtained is \( y = 0.020x + 32.62 \) with value \( R^2 = 0.984 \). (ED₅₀) results show that (ED₅₀) from mindi leaves ethanol extract is...
869 mg/200 gBW.

ED₅₀ reduction in total cholesterol levels was calculated by linear regression between doses with percent decrease in LDL (%PDLDL) (Figure 5). Linear regression equation obtained is $y = 0.019x + 29.35$ with value $R^2 = 0.998$. The result of ED₅₀ calculation shows that ED₅₀ from mindi leaves ethanol extract is 1086.84 mg/200 gBW.

4. CONCLUSIONS

Based on the result and discussion it can be concluded that the dosage of ethanol extract of mindi leaf has an effect on decreasing total cholesterol level of white male rat of Wistar strain with % PDTC at dose 300 mg/200 gBW equal to 45.99 %, and at a dose of 1200 mg/200 gBW of 56.29%. The extract of ethanol leaves mindi influences in decreasing LDL level of Wistar male white rat with % PDLDL dose 300 mg/200 gBW equal to 40.39%, and dose 1200 mg/200 gBW equal to 52.42%. The ED₅₀ value of ethanol extract of mindi leaves to lower total cholesterol was 869 mg/200 gBW, whereas to decrease LDL was 1086.84 mg/200 gBW.

5. ACKNOWLEDGEMENT

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