The Lipid Profile and Aorta Histopathology On Hyperlipidemic Rat by Saurauia vulcani Korth. Leaves Extract

Debi Dinha Octora1, Rosidah2, Jansen Silalahi3, Denny Satria4
1Graduate Student of Faculty of Pharmacy, Universitas Sumatera Utara, Medan
2Department of Pharmacology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan,
3Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Universitas Sumatera Utara, Medan,
4Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Sumatera Utara, Medan; Indonesia
*Corresponding Author: dennysatria@usu.ac.id

ABSTRACT
Dyslipidemia is a pathological condition characterized by increased levels of total cholesterol, triglycerides and LDL and decreased levels of HDL. Dyslipidemia causes cardiovascular disease risk factors by increasing cholesterol levels, increasing triglyceride levels, lowering HDL levels, and increasing LDL levels, as well as decreasing the proliferation of cardiac aortic cells in dyslipidemic rats. The pirdot plant (Saurauia vulcani Korth.) has long been known by the Simalungun, Toba and Karo communities to have the potential to treat various diseases caused by disruption of lipid processes in the body. Pirdot leaves contain bioactive compounds of flavonoids, tannins and saponins that have the potential to improve lipid profiles and prevent cardiovascular disease. This study aims to determine the activity of EEDP on the lipid profile of dyslipidemic rats. Pirdot leaf simplicia powder was extracted by maceration with 96% ethanol solvent and the extract was used on 30 dyslipidemic rats which were divided into 6 groups. Group 1 (Na-CMC), group 2 (Atorvastatin), group 3, 4, 5, 6 EEDP doses of 50, 100, 200, 400 mg/kg BW were administered orally for 21 days. At the end of the treatment, the levels of total cholesterol (TC) and aortic cell proliferation were measured. The results showed that Saurauia vulcani Korth had water content (6.23%), total ash content (7.05%) and acid insoluble ash content (0.48%). From the antidiyslipidemic study it was shown that EEDP 400 mg/kg BW improved blood lipand to increase aortic cell proliferation dyslipidemic rats was 69.84±0.27. Based on the explanation above, it is concluded that EEDP has an antidiyslipidemic effect.

Keyword: Saurauia vulcani Korth, extract, dose, cholesterol, Dyslipidemia, Saurauia vulcani Korth., Lipid profile, Proliferation, Aorta

ABSTRAK
Dislipidemia merupakan suatu keadaan patologis yang ditandai dengan peningkatan kadar kolesterol total, trigliserida serta LDL dan penurunan kadar HDL. Dislipidemia menyebabkan faktor resiko penyakit kardiovaskuler dengan meningkatkan kadar kolesterol, meningkatkan kadar trigliserida, menurunkan kadar HDL, serta menurunkan proliferasi aorta jantung tiku dislipidemia. Tanaman pirdot (Saurauia vulcani Korth.) sejak lama dikenal oleh masyarakat Simalungun, Toba dan Karo memiliki potensi dalam mengobati berbagai macam penyakit yang disebabkan oleh gangguan proses lipid dalam tubuh. Daun pirdot mengandung senyawa bioaktif flavonoid, tannin dan saponin yang berpotensi memperbaiki profil lipid dan mencegah penyakit kardiovaskuler. Penelitian ini bertujuan untuk mengetahui aktivitas EEDP terhadap profil lipid tiku dislipidemia. Serbuk simplicia daun pirdot diekstraksi secara mazerasi dengan pelarut etanol 96% dan ekstrak digunakan pada 30 ekor tiku dislipidemia yang dibagi menjadi 6 kelompok. Kelompok 1 (Na-CMC), kelompok 2 (Atorvastatin), kelompok 3, 4, 5, 6 EEDP doses 50, 100, 200, 400
1. Introduction

The use of natural materials, especially those derived from plant-grown materials used for the prevention and treatment of diseases has been known since ancient times by mankind. These natural ingredients are known as folk remedies because the principles of their use are still traditional. Generally, the efficacy of traditional medicines to date is based solely on empirical experience [1].

Some efforts made to lower cholesterol levels include early lifestyle changes, such as exercising regularly, reducing intake of fatty foods, and treatment with drugs that can lower cholesterol levels that can prevent severe complications from hypercholesteremia [2], [3]. Hypercholesterolemia is closely related to an increase in total cholesterol, an increase in LDL cholesterol, increased levels of triglycerides as well as a decrease in HDL cholesterol [4], [5].

According to WHO records about 20,000 plant species are used by the world's population as medicine. One of the medicinal plants is Saurauia vulcani, Korth. known as piridot, Pirdot plant (Saurauia vulcani Korth.) based on previous research containing flavonoids, glycosides, saponins, tannins, and steroids/terpenoids and also several in vivo studies have shown that piridot leaves have activity as an antihyperglycemic and antihyperlipidemic [6], [7].

2. Materials and Method

2.1 Materials

Fresh Saurauia vulcani Korth leaves were collected from Tiga Lingga village, Dairi regency, Sumatera Utara province, Indonesia., Spectrophotometer (Shimadzu), all chemicals and reagents used in this work were of analytical grade unless otherwise stated. Sodium hydroxide, sodium sulfate anhydrous, and sodium carboxymethylcellulose were the products of Merck, Germany. Distilled water was purchased from Bratachem, Indonesia. n-Hexane was obtained from Macron Chemicals, Lard oil and quail egg yolk used to induce dyslipidemia in rats were obtained from the local market. Atorvastatin 20 mg tablets were purchased from the local pharmacy. ethanol 96%.

2.2 Methods

2.2.1 Preparation extract of Saurauia vulcani Korth leaves [8]

Saurauia vulcani Korth leaf powder (500 g) was extracted by maceration using ethanol as solvent 7 days, 5L. The filtrate was collected and evaporated with a rotary evaporator.

2.2.2 Preparation suspension of CMC-Na 0.5% (b/v)

A total of 500 mg of CMC-Na is sprinkled into a mortar containing 10 ml of hot distilled water, closed, and left for 15 minutes until the mass becomes transparent, snarled then diluted with distilled water up to 100 ml.

2.3 Preparation suspension of Saurauia vulcani Korth Leaves

The leaves of piridot ethanol extract are determined based on the orientation of the experimental animals, i.e. a dose of 50 mg/KgBB, 100 mg/KgBB, 200 mg/KgBB, 400 mg/KgBB. Ethanol extract of Saurauia vulcani Korth leaves was inserted into mortar containing a slight Na–CMC suspension of 0.5% homogeneous stirring and then adequacy with Na–CMC suspension 0.5% to 10 ml [12].
2.2.4 Preparation suspension of atorvastatin
A total of 20 mg of atorvastatin is stirring in a mortar, then added Na-CMC suspension 0.5% little by a little while constantly being stirring until homogeneous, then adequacy with Na-CMC suspension 0.5% to 100 ml. [10]

2.2.5 Preparation of animal studies
The experimental animals used in the study were male white rat weight 180 – 200 g. Before testing, mice were acclimatized for 7-14 days, as many as 30 mice were divided into 6 groups of 5% to 625 ml. Each cage is given bedding (chaff) and fed regularly. The experimental animals were grouped into 6 groups, each consisting of 4 rats. After acclimatization period, rats were fasted for 18 h and blood TC was measured using strip test. Then, rats were given with 2 ml/kg BW quail egg yolk and lard oil twice a day. After 30 days of induction, blood TC of each rats was measured and rats with TC higher than 200 mg/dl were used for the test. Group 1 (CMC suspension), group 2 (atorvastatin), group 3 (50 mg/KgBB), group 4 (100 mg/KgBB), group 5 (200 mg/KgBB), group 6 (400 mg/KgBB) [7]

2.2.6 Cholesterol, HDL, LDL, triglyceride, SGOT, SGPT, SOD and LDH levels testing
Cholesterol level testing, HDL, LDL and triglycerides Saurauia vulcani Korth leaves extract doses of 50 mg/KgBB, 100 mg/KgBB, 200 mg/kgBB, 400 mg/KgBB with atorvastatin suspension comparison dose 1.8 mg/KgBB in white rats and 0.5% CMC-Na suspension control for 21 days.

2.2.7 Measurement of cholesterol, HDL, LDL and triglycerides levels [15], [18]
After the end of treatment, rats were fasted for 18 h and anesthetized with 70 mg/kg BW of ketamine intraperitoneally. Blood was collected directly from the heart and centrifuged for 10 min at 3000 rpm to obtain serum. Serum collected was then analyzed for lipid profile. [10]

2.2.8 Statistical analysis
The results are presented as mean ± SD of five animals per group and statistical levels of significance were determined using the one – way ANOVA (p < 0.05 was considered significant).

2.2.9 Preparation of Microscopic Preparations (Histopathology)
Mice were dissected and the aorta was taken to observe the proliferating cells. Observation of cell proliferation was carried out using the immunohistochemistry (IHC) method, which began with the manufacture of paraffin blocks, preparation of HE preparations, and continued with immunohistochemistry (IHC) using the Ki-67 protein.

3. Results and Discussion

3.1 Result Of Treatment Lipid Profile
After 21 days of treatment, all rats were sacrificed for blood from the heart vein. This test is carried out to see total cholesterol levels in the blood in mg/dl units. The average value of total cholesterol levels after induction was higher than before induction. This shows that the induction can increase total cholesterol levels in the blood of rats.

<table>
<thead>
<tr>
<th>Group</th>
<th>Day 7 Average cholesterol levels (mg/dl)</th>
<th>Day 14 Average cholesterol levels (mg/dl)</th>
<th>Day 21 Average cholesterol levels (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMC Natrium</td>
<td>300.20 ± 8.136</td>
<td>312.20 ± 3.633</td>
<td>346.60 ± 4.561</td>
</tr>
<tr>
<td>Atorvastatin</td>
<td>223.8 ± 4.919</td>
<td>190.60 ± 6.877</td>
<td>172.40 ± 6.542</td>
</tr>
<tr>
<td>EEDP 50 mg/KgBB</td>
<td>288.8 ± 6.380</td>
<td>263.60 ± 4.775</td>
<td>238.20 ± 5.119</td>
</tr>
<tr>
<td>EEDP 100 mg/KgBB</td>
<td>255.2 ± 2.950</td>
<td>227.00 ± 4.359</td>
<td>200.00 ± 3.162</td>
</tr>
<tr>
<td>EEDP 200 mg/KgBB</td>
<td>220.6 ± 4.450</td>
<td>182.60 ± 6.066</td>
<td>163.20 ± 6.058</td>
</tr>
<tr>
<td>EEDP 400 mg/KgBB</td>
<td>225.8 ± 5.933</td>
<td>189.60 ± 3.782</td>
<td>171.60 ± 3.847</td>
</tr>
</tbody>
</table>

Table 1. Results of decreased total cholesterol levels every week
Based on Table. 1 and Table. 2 above obtained the results of the average lipid profile value shows the administration of Saurauia vulcana Korth ethanol extract has the effect of decreased total cholesterol levels, LDL, triglyceride, as well as increased HDL. Saurauia vulcana Korth leaves have some activated content such as flavonoids, tannins, saponins. Flavonoids can lower total cholesterol levels, triglyceride levels, and LDL levels. Flavonoids have known to inhibit the increase in total cholesterol levels by inhibiting the activity of the HMG KoA reductase enzyme which plays an important role in cholesterol biosynthesis [14], [15]. Flavonoids play a role in the prevention of coronary heart disease based on the effects of biological processes that include the process of inhibition of lipid peroxidation and platelet aggregation. Flavonoids have believed to lower atherosclerosis and inhibit LDL oxidation, by inhibiting the formation of free radicals and protecting α-tocopherol in LDL from oxidation [17], [18].

The saponin content of Saurauia vulcana Korth leaves has a mechanism of hypolipidemia through increased excretion of bile acids due to the increasing conversion of cholesterol into bile acids, saponins are also able to inhibit the absorption of cholesterol and bile acids in the intestines by forming micelle formation, so cholesterol cannot be absorbed [19].

Saurauia vulcana Korth leaves also contain tannins that have the same activity as flavonoids in lowering total cholesterol by inhibiting the HMG KoA reductase enzyme which plays a role in synthesizing cholesterol. The inhibited activity of HMG KoA reductase will decrease the synthesis of apo B 100 and increase LDL receptors on the surface of the liver. Thus blood LDL cholesterol will be attracted to the liver and lower LDL and VLDL [16], [21]. The decrease in total cholesterol levels in the blood after the administration of Saurauia vulcana Korth leaves was due to the flavonoid content in the extract. This can be seen in Table 4.2 results of phytochemical screening of Saurauia vulcana Korth leaves, indicating that Saurauia vulcana Korth leaves contains a number of flavonoids, glycosides, saponins, tannins and steroids/terpenoids. In an in vitro study [23], flavonoids have been shown to reduce lipid peroxides by inhibiting the HMG-CoA reductase enzyme which causes a decrease in cholesterol synthesis. Flavonoids as natural polyphenolic antioxidant compounds can lower cholesterol levels in the blood, protect arteries from damage, and reduce the amount of cholesterol accumulation on the surface of the arterial endothelium. The presence of saponins in Saurauia vulcana Korth leaves can form complex bonds with bile acids and form micelles so that cholesterol cannot be absorbed by the intestine. The tannin content in Saurauia vulcana Korth leaves can also inhibit the absorption of cholesterol in the intestine by reacting with mucosal and intestinal epithelial proteins.

3.2 Result of Cardiac Aortic Cell Proliferation Was Performed Using Ki-67

Assay on cardiac aortic cell proliferation was performed using Ki-67 as an antibody to clarify the slide staining process in viewing the level of cell multiplication in cardiac aortic tissue. The higher the proliferation of cells, the less the formation of foam cells in the heart aorta. The formation of foam cells in the heart aorta occurs as a result of LDL oxidation which will increase fat deposits inside and outside the cells. A microscopic picture of cardiac aortic cells stained with Ki-67 antibody can be seen in Figure 4.4.
Observation of the histopathological picture of rat aorta induced by pork oil and quail egg yolk and given CMC Sodium suspension, showed the presence of a lot of foam cells in the tunica intima (figure I). In mice treated with atorvastatin, there were still few foam cells in the tunica intima (figure II). Meanwhile, mice treated with EEDP showed a small amount of foam cells in the tunica intima indicating cell repair (Figure V and Figure VI). [11], [12].

Table 2. Average Value of Allred Score Calculation on Rat Aortic Cell Proliferation (value in mean±SEM)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Proliferation (Mean±SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>41.35 ± 0.20</td>
</tr>
<tr>
<td>II</td>
<td>71.07 ± 0.34</td>
</tr>
<tr>
<td>III</td>
<td>42.60 ± 0.16</td>
</tr>
<tr>
<td>IV</td>
<td>56.81 ± 0.12</td>
</tr>
<tr>
<td>V</td>
<td>67.07 ± 0.14</td>
</tr>
<tr>
<td>VI</td>
<td>69.84 ± 0.27</td>
</tr>
</tbody>
</table>

Based on Table 2, it is known that the administration of Atorvastatin and EEDP at a dose of 50 mg/kg BW, 100 mg/kg BW, 200 mg/kg BW and 400 mg/kg BW can increase the proliferation of rat aortic cells. The best increase in rat aortic cell proliferation was in group VI with the average value of Allred's calculation on rat aortic cell proliferation, which was 69.89 ± 0.12. The mean value of Allred's calculations on aortic cell proliferation of rats group VI and group II (71.09±0.12) was significantly different (p<0.05) compared to group I as a negative control, this proves that the effectiveness of EEDP dose 400 mg/kg BW was comparable to atorvastatin in increasing the proliferation of aortic cells in rats induced by lard oil and quail egg yolk. [13].

4. Conclusion
Based on the results obtained, it can be concluded that the ethanol extract of Saurauia vulcani Korth leaves at a dose of 50 mg/KgBB, 100 mg/KgBB, 200 mg/KgBB, 400 mg/KgBB with atorvastatin suspension comparison dose 0.80 mg/KgBB and 0.5% CMC-Na suspension control can lower total cholesterol levels dan increase the value of rat aortic proliferation but the best dosage is 400 mg/KgBB.

5. Acknowledgements
We appreciate everyone who helped with this study by participating and/or providing assistance.

6. Conflict of Interest
Competing interests: No relevant disclosures.

References
[22] Morakinyo AO, Iranloye BO, Oyelowo OT, Nnaji J. The mechanism of increasing the enzymes SGOT and SGPT are caused by the inclusion of excess toxic substances into the body that will be metabolized by the enzyme cytochrome P450 in the liver into free radicals. Biology and Medicine, 2012; 4 (3): 134 – 140.