Antihyperglycemic and antihyperlipidemic effects of pirdot (Saurauia vulcani Korth.) leaves extract in mice

Salomo Hutahaean¹*, Masitta Tanjung¹, Diah Puspita Sari¹, Vevy Elfia Ningsih¹

¹Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Jl. Bioteknologi No. 1, Kampus USU, Medan 20155

*Email: salomo@usu.ac.id

Abstract. Approximately eighty percent of deaths in diabetic patients result from atherosclerosis, which is related to hyperlipidemia tendencies in diabetes. In North Sumatra, the use of plant-based ingredients as diabetes therapy has long been recognized. One of the local species which traditionally used was the pirdot plant (Saurauia vulcani Korth.). In this paper, we report the antihyperglycemic and antihyperlipidemic effects of the extract of pirdot leaves in model mice. In experiment I, twenty-five alloxan-induced diabetic mice was divided randomly into five groups of 5 mice, namely: control diabetic mice; diabetic mice + metformin; and three groups diabetic mice + pirdot leaves extract of 100, 200, or 300 mg/kg BW respectively. All the treatments were given daily for 21 days by oral gavage. In experiment II, another twenty-five mice were divided randomly into five groups of 5 mice. The treatments were as follows: a control group that did not receive any treatment; hyperlipidemic control (received quail yolk diet) for 30 days; and three groups of hyperlipidemic mice + orally treated pirdot leaves extract at a dose of 100, 200, or 300 mg/kg BW respectively. The result showed the pirdot leaves extract has the potential as antihyperglycemic. The effects obtained are equivalent to the effects of antidiabetic drug metformin. On the other hand, the antihyperlipidemic effect was not conclusive, because the extract lowered total cholesterol significantly, but no significant effect on triglyceride, marked reduced LDL, but significantly decreased the HDL level.

Keywords: Blood glucose level, HDL, LDL, Saurauia vulcani, total Cholesterol, tryglyceride

1. Introduction

Diabetes mellitus is a metabolic disease characterized by high blood sugar level caused by impaired insulin secretion, its action, or both. The predicted prevalence of world diabetes sufferers is 2.3% in 2000 to 4.4% by 2030, with the number of patients estimated to increase from 171 million to 368 million [1]. Therapies for the treatment of diabetes are the use of insulin and other drugs, such as sulfonylurea analogs, alpha-glucosidase inhibitors, and biguanides. These medications have certain side effects, such as causing hypoglycemia at higher doses, liver organ problems, lactic acidosis, and diarrhea [2]. The study of plant-derived antidiabetic agents is thus of great importance, including the scientific testing of plant material used in traditional medicine.

In North Sumatra, the use of plant-based ingredients as a diabetes drug has long been recognized. One of the local species of North Sumatra that has the potential is the pirdot plant (Saurauia vulcani Korth.). The Bataknese community traditionally used the pirdot leaf as a cure for various diseases, such as cancer, wounds, and diabetes [3,4]. Chronic diabetes is associated with long-term damage, dysfunction, and failure of different organs, such as the eyes, kidneys, nerves, heart, and blood vessels. Cardiovascular complications are the leading cause of death in diabetes mellitus. Eighty percent of deaths in diabetic patients result from atherosclerosis [5,6]. These cardiovascular complications are, among other things, related to hyperlipidemic tendencies in diabetes. In this paper, we report the
antihyperglycemic and antihyperlipidemic effects of the extract of pirdot leaves in alloxan-induced diabetic mice.

2. Materials and Methods

2.1. Experimental Animals
The mice (Mus musculus L.), two-month old, weighing about 25-30 gram, were used in the study. They received from Faculty of Pharmacy, University of Sumatera Utara. Before the experiment, mice were kept two weeks in a cage for adjustment to new environment. During the study, animals were kept in 12 hours light / dark cycle at the temperature of 27-30 °C, and fed with standardize laboratory diet. Food and drinking water offered on an ad-libitum basis.

2.2. Preparation of Plant Extract
Pirdot leaves were obtained from the forest of Aek Nauli Parapat, North Sumatra. The leaves were air dried for approximately five days and then powdered with an electric blender. Extraction conducted with ethanol (70%; 40 hours) by maceration method. Macerate obtained was filtered and evaporated by using rotary evaporator at a temperature of 40°C.

2.3. Induction of Diabetes Mellitus
Diabetes mellitus experimentally induced in animals fasted for 18 hours by intraperitoneal injection of 150 mg/kg BW alloxan dissolved in NaCl solution (0.9%). Three days after injection, mice were screened for serum glucose levels. We considered the mice with blood glucose level above 200 mg/dL as diabetic and used for experiment I.

2.4. Induction of Hyperlipidemia
Hyperlipidemia experimentally induced by feeding the mouse with japanese Coturnix yolk. The yolk was separating from albumin and making emulsions by shaking it slowly. The Coturnix yolk diet was given 0.3 ml per day orally by gavage for 30 days. Animals received the food considered hyperlipidemic and used for experiment II.

2.5. Experimental Designs
2.5.1. Experiment I. The experimental research using 5 X 5 completely randomized design (CRD). Twenty-five mice were divided randomly into 5 groups of 5 mice with different treatment for each group, as follows: group K1 consisting of diabetic mice were orally administered an equivalent volume of vehicle (distilled water); group K2 was diabetic mice and orally given a standard antihyperglycemic drug (Metformin); group P1-P3 were diabetic mice and orally treated with extract of pirdot leaves at a dose level of 100, 200, or 300 mg/kg b.w. respectively. All treatments were given daily for 21 days by gastric intubation. Blood glucose level were measured by using the EasyTouch glucometer test kit. The tail of mice was cut and the blood was allowed to drip onto glucose strip test, and after 10 seconds blood glucose level will appear on the screen. Blood glucose level measured at day 1, 7, 14, and 21 after alloxan injection. The body weight also measured on the same day.

2.5.2. Experiment II. The experimental research using 5 X 5 completely randomized design (CRD). Twenty-five mice were divided randomly into 5 groups of 5 mice with different treatment for each group, as follows: group K0 served as control and did not receive any treatment; group K1 served as hyperlipidemic control (received yolk diet) for 30 days; group P1-P3 were hyperlipidemic mice and orally treated with extract of pirdot leaves starting from day 11 to day 30 at a dose of 100, 200, or 300 mg/kg BW respectively. Total cholesterol levels were measured by using the EasyTouch cholesterol test kit in the blood which taken from the tail of the mice. The tail of mice was cut, blood was dripped onto cholesterol
strip test, and after 150 seconds cholesterol levels will appear on the screen. The measurement of total cholesterol performed on days 0, 10, 20 and 30. All animal dissected at the end of the experiment and 1 ml of blood was taken from the heart and placed into an EDTA tube. Blood was centrifuged at 4000 rpm for 15 minutes. Serum was taken and then used for measurement of triglycerides, HDL, and LDL [9]. Serum triglycerides estimated according to Colorimetric Enzymatic Test GPO (Glycerol-3-Phosphate Oxidase) method. HDL-Cholesterol and LDL-Cholesterol estimated according to the CHOD-PAP Enzymatic Colorimetric Test for Cholesterol With Lipid Clearing Factor (LCF) method using Mikrolab 300.

2.6. Statistic Analysis
All data analyzed with ANOVA and Duncan’ test method. The differences between the means of the various groups were considered significant at p<0.05.

3. Results and Discussion
3.1. Experiment I. Antihyperglycemic Effect of Pirdot Leaves Extract
In the diabetic mice (K1), the blood glucose level increased from 226.8 ± 35.2 mg/dl to 292.4 ±74.9 mg/dl from day 4 after alloxan injection to day 24, while in metformin group (K2) BGL decreased from 230.2 ± 23.8 mg/dl to 135 ± 25.1 mg/dl. The extract of pirdot leaves at all dosages significantly lowered blood glucose level starting from day ten until the end of the experiment.

The antihyperglycemic effects of pirdot leaves extract at 100, 200, and 300 mg/kg were examined ten days after injection of the alloxan. As shown in Table 1, pirdot leaves extract tended to decrease BGL, but significant effect started at day 17. Furthermore, when the effect of the extract compared among the different groups of mice, BGLs were significantly lowered in all extract treatment compared to control (K1). No significant differences observed between all the extract treatment compared to the metformin group.

| Treatments | blood sugar level (mg/dL) on days- |
|------------|-----------------------------------|
|            | 0              | 3        | 10       | 17         | 24         |
| K1         | 109.4 ± 36.7^a | 226.8 ± 35.2^a | 237.2 ± 43.9^a | 267.8 ± 47^b | 292.4 ±74.9^b |
| K2         | 102.6 ± 22.8^a | 230.2 ± 23.8^a | 189 ± 30.3^a   | 154 ± 36.7^a | 135 ± 25.1^a |
| P1         | 97.2 ± 14.5^a  | 301.4 ± 13^a  | 278.4 ± 97.4^a | 192 ± 76.9^a | 173 ± 74.8^a |
| P2         | 117 ± 38.3^a   | 225.2 ± 24.7^a | 175.4 ± 24.9^a | 134.2 ± 21.5^a | 129.8 ± 23.4^a |
| P3         | 122.4 ± 15^a   | 279.8 ± 58.8^a | 191.2 ± 41.5^a | 156 ± 27.9^a | 134.4 ± 41.7^a |

Different superscripts mean statistically different (P<0.05).

3.2. Experiment II. Anti Hyperlipidemic Effect of Pirdot Leaves Extract
3.2.1. Total Cholesterol Level
The cholesterol level obtained every ten days. The result showed total cholesterol level increased in all groups after yolk diet administration (Figure 1).

The cholesterol level in control (K0) tended to stabilize between day 0 to 30, whereas in control yolk group the level increased starting from day 10 to the end of the experiment (day 30). Quail egg yolk contains high cholesterol content (220-250 mg per yolk) and an important saturated fat that can increase blood cholesterol [10].
In extract treatment groups, total cholesterol increased from day 0 to day 10, but decrease after extract administration. At the day 30, their level was not significantly different in comparison to control group (P>0.05). It seems that several compounds in this plant, especially flavonoids, saponins, and tannins [7], might have the potential to decrease the total cholesterol in mice blood.

Figure 1. Total cholesterol levels in mice after administration of high lipid diet and pirdot leaves extract at doses of 100 mg / kgBW, 200 mg / kgBW, 300 mg / kgBW for 30 days.

3.2.2. Serum Triglycerides
At the end of the experimental period, control yolk mice and all three leaves extract groups demonstrated a marked increase in serum triglycerides (Figure 2). Here, the yolk diet was able to increase serum triglycerides in animal test. According to Gani et al. [11], the rise and fall of blood triglyceride levels are influenced by the amount of fat consumed. In excessive consumption, fat will be stored in adipose tissue in the form of triglycerides. In animals with hypercholesterolemia an elevated triglyceride level occurs, due to visceral fat accumulation and decrease of lipoprotein lipase (LPL) enzyme activity [12].

Figure 2. Serum triglyceride levels in mice after administration of high lipid diet and pirdot leaves extract at doses of 100, 200, or 300 mg/kg BW for 30 days.
We also found that statistically, the level of serum triglycerides of the control group (K0), control yolk group, and all three treatments group was not significantly different (p>0.05). It seemed that pirdot leaves extract did not decrease the level of serum triglycerides.

3.2.3. Low-Density Lipoprotein (LDL) Level
At the end period of the experimental, there was a marked higher LDL in yolk diet group and sharp decline in three extract groups (Figure 3), but statistically, there was no significant difference among all groups (P>0.05).

![Figure 3. The LDL levels in mice after administration of high lipid diet and pirdot leaves extract at doses of 100, 200, or 300 mg/kg BW for 30 days](image)

3.2.4. High-Density Lipoprotein (HDL) Level
Results showed that the highest HDL level found in group K1. Its level was significantly higher (P<0.05) compared to control and also to three extract groups. In Figure 4, the HDL level on the three extract groups is lower than the level in the yolk group. The levels of HDL obtained in three groups of extract treatment was not statistically different from control (P>0.05), and all were within normal range (40-60 mg/dl).
Figure 4. The HDL levels in mice after administration of high lipid diet and pirdot leaves extract at doses of 100 mg / kgBW, 200 mg / kgBW, 300 mg / kgBW for 30 days.

This result means that pirdot leaves extract decreased both LDL and HDL level. The decrease in both LDL and HDL have been discussed in pinto bean experiment [13], which reported that consumption of pinto beans showed decreases in both LDL- and HDL cholesterol which might be due to chemical composition had the effect on both plasma cholesterol.

In conclusion, the results obtained confirm the pirdot leaf extract has potential as antihyperglycemic. The effects obtained are equivalent to the effects of antidiabetic drug metformin. On the other hand, the antihyperlipidemic effect of the extract has not been conclusive, because the pirdot leaf extract lowered total cholesterol significantly, and also marked lowered LDL, but it also lowered the HDL level and no significant effect on triglyceride level.

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