INTRODUCTION

The international diabetes federation reports that 415 million people worldwide suffer from diabetes mellitus. A total of 8,554,170 cases of diabetes mellitus were found within the age range of 20–79 years old in 2013, and 172,601 people died from diabetes. The number of diabetes cases increased to 10,021,400 in 2015, and 184,985 adults died from diabetes [1]. The diabetes epidemic does not only threaten individual health but also impedes sustainable socioeconomic development.

How to cure diabetes is very complicated because to overcome the impact of diabetes needs the patient’s big strategy and self-management [2].

A research found that one-third of patients with diabetes exhibit indications of diabetic retinopathy, and one-tenth of these patients lose their eyesight [3]. Patients with diabetes mellitus type 1 and 60% of patients with diabetes mellitus type 2 experience retinopathy [4].

Diabetes, which results in diabetic retinopathy, is the cause of blindness among adults and the third cause of death in the United States. Given the same age, patients with diabetes are at least 2.5 times more likely to experience a heart attack than a person without diabetes. Moreover, 75% of patients with diabetes mellitus die from vascular diseases. Diabetic complications include heart attack, kidney failure, stroke, gangrene, and an increase in intrauterine fetus mortality rate among pregnant women [5].

Pirdot (Saurauia vulcani, Korth.) is well-known among the Karoneese and Tobanese people of North Sumatera as a traditional medicinal plant with eating properties for diabetes and rheumatism. The phytochemical screening results of pirdot leaf show the existence of secondary metabolic compounds, namely alkaloid, flavonoid, saponin, triterpenoid, and tannin, which can decrease blood glucose rate. Thus, research on pirdot leaf as an alternative therapy with low medication cost should be developed.

The current study was conducted to determine the influence of pirdot leaf extract on the blood glucose rate and histologic description of the retina of male mice (Mus musculus strain DDW).

METHODS

Preparation of extract

Pirdot leaf extract was prepared in the Organic Chemistry Laboratory of Natural Materials, Department of Chemistry, Faculty of Mathematics and Natural Sciences (FMIPA), Universitas Sumatera Utara (USU). Pirdot leaves were dried and blended using a blender. Then, pirdot leaf powder was macerated in 70% ethanol solvent to make a solution. The macerate (maceration product) was evaporated and condensed using a vacuum rotary evaporator at 40°C [6].

Preparation of mice

Using these experimental animals has been agreed by Animal Research Ethics Committee from Faculty of Mathematics and Natural Science in Universitas Sumatera Utara. This research used 25 male adult mice, aged±2 months, weighing±25–30 g, and raised in the Animal Structure Laboratory of the Department of Biology, FMIPA, USU in Medan. The mice were placed in a plastic container with an underlayer of rice husk, which was replaced twice a week. The container was covered with wire. The mice were provided with food and drink once a day ad libitum.

Experimental design

This research is entitled “Influence of Pirdot Leaf (Saurauia vulcani, Korth.) extract on the Blood Glucose Rate and Histologic Description of the Retina of Male Mice (M. musculus strain DDW)” It used five
treatments: Two control groups (positive and negative) and three groups with pirdot leaf extract treatment (i.e., groups that were given alloxan and various dosages of pirdot leaf extract). The numbers of treatments and replications in both experiments were in accordance with the formula proposed by Ferede as follows: \( t = 1 \) (1-1 to 15, where \( t \) = treatment group and \( n \) = replication. Each treatment was divided as follows: Treatment 1: Control group (without treatment) for 8 weeks, Treatment 2: Control group with a single injection of alloxan (125 mg/kg body weight [BW] of alloxan was administered as the diabetes trigger) for 8 weeks, Treatment 3: Group provided with alloxan+150 mg/kg BW of pirdot leaf extract for 8 weeks, Treatment 4: Group provided with alloxan+200 mg/kg BW of pirdot leaf extract for 8 weeks, and Treatment 5: Group was provided with alloxan+250 mg/kg BW of pirdot leaf extract for 8 weeks.

Research flow

The male mice were raised in the Animal Structure Laboratory of the Department of Biology, FMIPA, USU in Medan. Three pirdot leaf extract treatments were prepared for the three groups with extract treatment. Diabetes was induced among the mice in four groups through intramuscular injection of 125 mg/kg BW of alloxan. Alloxan compound can cause disfunction of pancreas \( \beta \)-cell [7]. The mice with a blood glucose rate of 200 mg/dl were used in the experiment. Then, 150, 200, and 250 mg/kg BW of pirdot leaf extract were provided in Treatments I, II, and III, respectively. These treatments (0.3 mL, pirdot leaf extract) were given orally to the mice for 8 weeks. The blood glucose rate of each mouse was measured. Then, the mice were killed by dislocating their necks to remove the eyes and prepare blood from the retina using the paraffin method and double coloring with hematoxylin-eosin (HE). The histologic structure of the retina was tested through HE staining [8].

Blood preparation procedure

The right and left eyes of the mice were removed and washed with 0.9% NaCl solution. They were soaked in solutions of buffer formalin 10% (1) and (2) for 1 h each. Then, the eyes were dehydrated gradually with 70%, 80%, 90%, and 96% alcohol for 1 h and 30 min each and with absolute alcohol (1), (2), and (3) for 1 h each. As soon as the dehydration process was finished, treatment was followed by purification using xylene (1), (2), and (3) for 60 min each. The eyeballs were buried in paraffin [9]. Embedding was performed with liquid paraffin (Merek) at 56 °C (1) and (2) for 2 h each. Then, blocking in the cassette was chilled in Paraffin apparatus station at 4°C for a certain period and attached on mikrotom (Leica). The eyes were then sliced into 4 μm width. Then, the mower's albumin was smeared, and distilled water was dripped on the object glass. Several paraffin tapes were placed on the surface of the distilled water and left to stand for a certain period on the surface of the object glass. The object glass was then moved to the heating table until the paraffin dried. Staining was provided using HE.

Research parameter

The research parameter observed was the blood sugar rate of the mice, which was measured using a digital blood sugar rate meter with the brand Easy Touch Digital. The mass of the eyeballs was weighed using a digital scale, and the width of the retina layer was measured based on the width of the retinal ganglion cell (RGC) [3] and the outer plexiform layer (OPL) [9].

The picture of RGC was taken using a digital microscope ZEISS AxioVisionSE64 with 40x objective enlargement with an Axio Camera ERC 5S connected to a computer. The digital pictures were transferred to the Axio Vision 4.8.2 SP3 program by selecting the toolbar, directing it toward the RGC to determine its size.

The picture of OPL was taken using a digital microscope ZEISS AxioVisionSE64 with 40x objective enlargement with an Axio Camera ERC 5S connected to a computer. The digital pictures were transferred to the Axio Vision 4.8.2 SP3 program by selecting the toolbar, directing it toward the OPL, and recording their sizes.

The data were collected and analyzed using SPSS 22 version [8].

RESULTS

The research, which was conducted for 8 weeks, presents the male mice’s blood sugar rate in Table 1.

The width of the RGC of the mice obtained in the research is presented in Table 2.

This research determined the thickness of the OPL of the male mice’s retina as presented in Table 3.

DISCUSSION

Blood sugar rate of the male mice

The results of the statistical testing show that the pirdot leaf extract of P2 (113.40±10.36) was significantly different from that of P1 (160.40±23.33), K(+) (364.80±44.21), whereas the pirdot leaf extract of P2 (113.40±10.36) was not significantly different from that of P3 (128.80±08.04), K(−) (133.40±10.14). The pirdot leaf extract of P3 (128.80±08.04) was significantly different with K(+) and P2.

Table 1: Blood sugar rate of male mice

| Group | n | Blood sugar rate (mg/dl) | Notation |
|-------|---|--------------------------|---------|
| K(−)  | 5 | 133.40±10.14             | ab      |
| K(+)  | 5 | 364.80±44.21             | c       |
| P1    | 5 | 160.40±23.33             | b       |
| P2    | 5 | 113.40±10.36             | a       |
| P3    | 5 | 128.80±08.04             | ab      |

K(−): Control group (without treatment) for 8 weeks, K(+): Control group with a single injection of alloxan (125 mg/kg body weight [BW] of alloxan was administered as the diabetes trigger) for 8 weeks, P1: Group provided with alloxan+150 mg/kg BW of pirdot leaf extract for 8 weeks, P2: Group provided with alloxan+200 mg/kg BW of pirdot leaf extract for 8 weeks, P3: Group provided with alloxan+250 mg/kg BW of pirdot leaf extract for 8 weeks, n: Replication

Table 2: Width of the RGC of the male mice

| Group | n | RGC Layer (μm) | Notation |
|-------|---|----------------|---------|
| K(−)  | 5 | 6.95±0.80      | ab      |
| K(+)  | 5 | 3.67±0.17      | a       |
| P1    | 5 | 6.95±1.13      | ab      |
| P2    | 5 | 6.82±2.99      | ab      |
| P3    | 5 | 7.59±2.28      | b       |

K(−): Control group (without treatment) for 8 weeks, K(+): Control group with a single injection of alloxan (125 mg/kg body weight [BW] of alloxan was administered as the diabetes trigger) for 8 weeks, P1: Group provided with alloxan+150 mg/kg BW of pirdot leaf extract for 8 weeks, P2: Group provided with alloxan+200 mg/kg BW of pirdot leaf extract for 8 weeks, P3: Group provided with alloxan+250 mg/kg BW of pirdot leaf extract for 8 weeks, n: Replication, RGC: Retinal Ganglion Cell

Table 3: Thickness of the OPL of the male mice

| Group | n | OPL (μm) | Notation |
|-------|---|----------|---------|
| K(−)  | 5 | 14.28±0.21 | bc      |
| K(+)  | 5 | 15.71±4.16 | bc      |
| P1    | 5 | 17.88±0.57 | c       |
| P2    | 5 | 08.10±2.37 | a       |
| P3    | 5 | 11.26±4.51 | ab      |

K(−): Control group (without treatment) for 8 weeks, K(+): Control group with a single injection of alloxan (125 mg/kg body weight [BW] of alloxan was administered as the diabetes trigger) for 8 weeks, P1: Group provided with alloxan+150 mg/kg BW of pirdot leaf extract for 8 weeks, P2: Group provided with alloxan+200 mg/kg BW of pirdot leaf extract for 8 weeks, P3: Group provided with alloxan+250 mg/kg BW of pirdot leaf extract for 8 weeks, n: Replication, OPL: Outer plexiform layer
of alloxan was given as the diabetes trigger) decreased RGC to 3.67 µm. Leaf extract in P3 significantly increased RGC to 7.59 µm with K(+), (6.95±0.80) and K(+) (3.67±0.17) from P2 (6.82±2.99) and P3 (7.59 ± 0.17), as shown in Table 3. The results of the statistical test demonstrate that the pirdot leaf extracts decreased the blood sugar rate of male mice by 113.4 mg/dl. This research concluded that administering 200 mg/kg BW (P2) pirdot leaf extract significantly (p<0.05) decreased the blood sugar rate of male mice by 113.4 mg/dl. Moreover, administering 250 mg/kg BW (P3) pirdot leaf extract significantly increased RGC to 7.59 µm (p<0.05). Finally, administering 150 mg/kg BW (P1) pirdot leaf extract significantly (p<0.05) increased OPL by 17.88 µm.

CONCLUSION
This research concluded that administering 200 mg/kg BW (P2) pirdot leaf extract significantly (p<0.05) decreased the blood sugar rate of male mice by 113.4 mg/dl. Moreover, administering 250 mg/kg BW (P3) pirdot leaf extract significantly increased RGC to 7.59 µm (p<0.05). Finally, administering 150 mg/kg BW (P1) pirdot leaf extract significantly (p<0.05) increased OPL by 17.88 µm.

AUTHOR’S CONTRIBUTIONS
Adiluddin Hutapea has contributed to collecting the manuscript data and preparing the manuscript. Salomo Hutahaen as a contributor author who suggested the title of the manuscript and Syafrudin Illyas as contributor author who has guided and reviewed the content of the English Grammar.

CONFLICTS OF INTEREST
The authors have declared no conflicts of interest.

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