Phytochemical Screening and Antidiabetic Activity Test of Extracts and Fractions of Lactuca Indica (L.) in Streptozotocin-Induced Diabetic Mice

Tamba Rosanto¹*, Nainggolan Marline², Rosidah Noersal³

¹Graduate Student, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.
²Department of Pharmacy Biology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.
³Department of Pharmacology, Faculty of Pharmacy, University of Sumatera Utara, Medan, Indonesia.

A B S T R A C T

Objectives: The purpose of this study was to determine the antidiabetic effect of ethanol extract and Lactuca indica leaf fraction to determine the effective dose of mice induced with streptozotocin compared with metformin.

Design: The design of this study was experimental in which the extraction and fraction of Lactuca indica leaves were tested for the value of decreasing blood glucose levels of mice after induction of streptozotocin. Antidiabetic activity tests were divided into 12 groups. Group I (baseline) group II (negative control) were given CMC 0.5%, Group III (positive control) were given metformin 65 mg/kg BW, while Groups IV to XII were given Lactuca indica leaf extract and fractions at their respective doses -each 100, 150 and 200 mg/kg BW

Interventions: The variable that was intervened in this study was the concentration of extract used.

Main outcome measure: The main measurement results in this study were to determine extracts and fractions that were able to reduce blood glucose levels in mice.

Results: The antidiabetic effect of Lactuca indica leaves shows that EELL, EAFLL and NHFLL have antidiabetic effects, this is supported by the chemical content contained in the extracts, namely flavonoids, tannins, saponins, glycosides and triterpenoids/steroids. The most effective activity to reduce blood glucose levels in streptozotocin-induced mice is EAFLL 100 mg/kg BW

Conclusion: Ethyl acetate fraction of Lactuca indica leaves has an effective antidiabetic activity at a dose of 200 mg/kg BW has given antidiabetic activity in mice induced by STZ, on the 9th day BGL mice have dropped below 120 mg/dl.

Keywords: Antidiabetic activity, Lactuca indica, streptozotocin, mice.

INTRODUCTION

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the pancreatic b-cells with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat, and protein metabolism in diabetes is deficient action of insulin on target tissues¹.

The management of diabetes is a global problem until now and successful treatment is not yet discovered. The modern oral hypoglycemic gents produce undesirable and side effects. Therefore, it needs to find an alternative drug to avoid adverse event from conventional treatment i.e. herbal medicine from plant ². One of the plants that have the potential to be developed is the Lactuca indica leaf.
**MATERIAL AND METHODS**

**Plant and Chemical Material**

Lactuca indica (Compositae) is edible wild lettuce widely distributed in Asia and has been popularly used as folk medicine. The whole plant of Lactuca indica, prepared by using boiling water or an ethanolic solution, has been taken orally or topically administered in various medications for its anti-inflammatory and antibacterial activities and to treat intestinal disorders. However, limited scientifically proven information is available on the bioactivities, pharmacological functions, and specific clinical efficacies of this plant.

Lactuca indica is used in medicine and has pharmacological effects on human health, has anti-inflammatory and antibacterial properties, can also reduce intestinal disorders. Besides being used as a diuretic, Lactuca indica shows a direct secondary effect on epithelial cells that can protect against Escherichia coli infection in urinary tract infections. Lactuca indica leaf extract has potential as an anti-hepatitis virus. Lactuca indica is an edible wild vegetable, used as an anti-inflammatory, antibacterial, free radical antioxidant (DPPH IC50 6.1 ± 0.01 (µg / ml) in the ethyl acetate fraction and DPPH IC50 19.4 ± 0.02 in the water fraction) and leukemia treatment.

Based on the description above, the ethanol extract and n-hexane and ethyl acetate of Lactuca indica leaves were tested as antidiabetic in mice (Mus musculus L.) induced by streptozotocin (STZ) to find the most effective fraction to reduce BGL (blood glucose level), as comparison used metformin.

**Experimental Animals**

Healthy adult mice (Mus musculus L.) (20-35 g BW). Mice were housed in polycarbonate cages in a room with 12 hrs day-night circle. They were fed on a standard pellet diet and water ad libitum. The study was approved by Animal Research Ethics Committees (AREC) of University of Sumatera Utara, and then experiments were conducted according to the ethical norms and AREC guidelines.

**Induction of diabetes**

Experimental diabetes was induced by a single intraperitoneal injection of 55 mg/kg of STZ, freshly dissolved in NaCl, 0.9%. After 3 days of STZ injection, mice with fasting glucose above 200 mg/dl were considered as diabetic and included in the study.

**Experimental design**

The animals used for the experimental design of the antidiabetic test were divided into twelve groups consisting of four animals for each group. The twelve groups consisting of 1 basic group, 2 control groups and 9 test groups consisting of a dose of 100 mg / Kg BW, 150 mg / Kg BW, 200 mg / Kg BW, summarized as follows:

| Group | Group name       | Group Description |
|-------|------------------|-------------------|
| I     | Baseline         | Normal            |
| II    | Negative control | Suspension CMC Sodium 0.5% |
| III   | Positive control | Metformin dose equivalent to 65 mg / kg BW |
| IV-VI | EELL             | Test group, ethanol extract of Lactuca indica leaves (EELL), dosage 100mg/kg; 150mg/kg; 200mg/kg |
| VII-IX| EAFLL            | Test group, ethyl acetate fraction of of Lactuca indica leaves (EAFLL), dosage 100mg/kg, 150mg/kg, 200mg/kg |
| X-XII | NHFLL            | Test group, N-hexane fractionation of Lactuca indica leaves (NHFLL), 100mg/kg; 150mg/kg; 200mg/kg |

The treatment begins after the animal tested positive for diabetes, this is the 1st day of treatment, every two days measurements of blood glucose levels are taken. Testing is carried out for 2 weeks, namely on days 3, 5, 7, 9, 11, 13 and 15.

**Statistical analysis**

All the data were expressed as mean±standard deviation. The significant difference of data between different groups was compared by ANOVA followed by Duncan’s test.

**RESULT AND DISCUSSION**

**Phytochemical screening**

The results of Lactuca indica leaf fractionation screening showed different chemical compounds in different extracts in Table 2.
Extraction and Fractionation

The extraction process by the way of maceration of 1000 g Lactuca indica leaves simplicial powder produced 140.8 g of Lactuca indica leaves ethanol extract. Then the 90 g ethanol extract was fractionated using a separating funnel to obtain 12.7 g n-hexane fraction, 61.32 g ethyl acetate fraction and 15.55 g fraction remaining.

Anti-Diabetic test

Blood glucose levels are measured enzymatically with a glucometer. Gluco test strips are inserted into the glucometer so that the glucometer will turn on automatically. Blood glucose level testing using healthy mice that have been acclimated fast for 18 hours then weighed and their blood glucose levels measured.

The treatment begins after the animal tested positive for diabetes (day 1) Every day given test material, BW is weighed every two days intervals, then measurements of blood glucose levels for two weeks are on days 1, 3, 5, 7, 9, 11, 13 and 15. The results of observations on average BGL (Table 3) and (Figure 1).

On the 3rd day, there was a decrease in BGL compared to the 1st day. Based on a statistical analysis of BGL reduction on day 3, mice given EELL, EAFLL and NHFLL decreased BGL, this is because the pancreatic damage of mice took place little by little and the dose of streptozotocin needed to induce diabetes depends on animal species, the route of administration and nutritional status. There are significant differences between treatments, to find out significant differences between treatments, Duncan’s average difference test was performed. The test results showed that from all fractions of a dose of 100 mg/kg, 150 mg/kg, 200 mg/kg did not...
provide a significant difference to positive control. This can be due to the efficacious substances of EELL, EAFLL, NHFLL at all doses have the same effect as metformin in reducing BGL of mice.

The result of the decrease in BGL on the 5th day compared to the 3rd day there was a change in the average BGL. Based on a statistical analysis of the reduction in BGL on the 5th day there were significant differences between treatments. To find out the significant differences between treatments, Duncan's average test was performed. The test results showed only EAFLL dose of 150 mg/kg BW showed a decrease in BGL that was significantly different from positive controls while other fractions were not significantly different from positive controls.

The smallest decrease in BGL of 7th-day mice occurred in the administration of EAFLL at a dose of 150 mg/kg BW and the biggest decrease in EAFLL administration at a dose of 200 mg/kg BW. Duncan's average test results showed only EELL dose of 150 mg/kg BW and EAFLL dose of 150 mg/kg BW showed a significant difference to positive control while others were not significantly different from positive control.

The average duncan test results on days 9 to 15 only giving NHFLL 100 mg/kg BW showed a significant difference to positive control, while other fractions were not significantly different from positive controls.

Giving EELL suspension, EAFLL suspension and NHFLL suspension can reduce BGL of mice equal to the effect produced by metformin suspension dose of 65 mg/kg BW. EAFLL 150 mg / kg BW, EELL 150 mg / kg BW on day 9 showed a fairly large decrease in BGL of mice, whereas on day 11 EAFLL 150 mg / kg BW on the graph appeared to have leveled off so the reduction in BGL was very small; EELL 150 mg / kg BW, EELL 200 mg / kg BW, EAFLL 100 mg / kg BW, EAFLL 150 mg / kg BW, EAFLL 200 mg / kg BW, NHFLL 150 mg / kg BW, NHFLL 200 mg / kg BW and suspension metformin 65 mg / kg BW fell at almost the same point.

After calculating the reduction in BGL from the nine measurement times. The biggest decrease in BGL of mice was the administration of EAFLL dose of 200 mg / kg BW where on the 3rd day, 5th day, 7th day, 9th day, 11th day, 13th day and 13th day 15 sequentially showed a decrease in mouse BGL of 290.25 mg / dl; 245.75 mg / dl; 159 mg / dl; 111 mg / dl; 95.50 mg / dl; 90.50 mg / dl and 86 mg / dl.

The results obtained that EELL, EAFLL and NHFLL have antidiabetic effects, this is supported by the chemical content contained in the extracts, namely flavonoids, tannins, saponins, glycosides and triterpenoids/steroids. The hypoglycemic mechanism is thought to be caused by flavonoids which can inhibit glucose reabsorption from the kidneys and can increase blood glucose solubility so that it is easily excreted in urine. So it is suspected that the flavonoid group in the leaves of Lactuca indica can reduce blood glucose levels.

CONCLUSIONS

Lactuca indica leaves can provide effects especially EAFLL and NHFLL both provide effective antidiabetic activity. EAFLL with a dose of 100 mg/kg BW has given antidiabetic activity in mice induced by STZ, on the 9th day BGL mice have dropped below 120 mg/dl.

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