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Research Article

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

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ABSTRACT

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 we're 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.*

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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 tissues[1].

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.

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[3].

Lactuca indica is used in medicine and has pharmacological effects on human health, has anti-inflammatory and antibacterial properties, can also reduce intestinal disorders[3]. 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[4]. Lactuca indica leaf extract has potential as an anti-hepatitis virus[4]. Lactuca indica is an edible wild vegetable, used as an anti-inflammatory, antibacterial, free radical antidote (DPPH IC¬50) 12.2 ± 0.02 in hot water extract, 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[3].

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.

MATERIAL AND METHODS

Plant and Chemical Material

Lactuca indica sample were collected from North Tapanuli, North Sumatera, Indonesia. The part of the plant being used is the leaves. Lactuca indica leaf has been determined by the Herbarium Bogoriense Indonesian Institute of Science, it is known that the species is *Lactuca indica* (L.) with the Family Compositae.

Extraction and fraction

The dried Lactuca indica leaves (1000 g) were crushed using a blender. After that, using the maceration method the leaf powder is soaked with 80% ethanol. The solvent was evaporated at low pressure and the temperature not exceed 60^0 C using a rotary evaporator, then dried using a freeze dryer. Next, the crude extract is taken (90 g) for liquid-liquid extraction to obtain nonpolar (using n-hexane) and semi-polar (using ethyl acetate)[5,6].

Phytochemical screening

Phytochemical screening is carried out on the fraction of Lactuca indica leaf including secondary metabolite examination of alkaloids, flavonoids, glycosides, tannins, triterpenoids/steroids, and saponins[7–9].

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:

Table: 1 Distribution of test groups

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.

Table: 2 Phytochemical screening results

No.	Pemeriksaan	N- Hexane	Ethyl acetate	Water
1.	Triterpenoids/Steroids	Positive	Negative	Negative
2.	Alkaloids	Negative	Negative	Negative
3.	Glycosides	Negative	Positive	Negative
4.	Flavonoids	Negative	Positive	Negative
5.	Tannins	Negative	Positive	Negative
6.	Saponins	Negative	Positive	Negative

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).

Table: 3 Observation of the average blood glucose level (BGL)

Treatment	Dosage (mg/ kg BW)	Initial BGL	BGL After Induction	Day observation (mg / dl)						
				3	5	7	9	11	13	15
Baseline	-	92,25	97,5	99,25	96	98	98,5	101,25	100	100,25
Control (-)	**CMC 0,5 %**	91,75	407,75	399,75	428,5	464,5	412,25	396	373	373,5
Control	**Metformin 65**	94,75	379,5	291	239	157	113,25	109	96,75	92
EELL	**100**	93	385	297,5	206,75	194,75	135,5	129,75	107,25	101,25
	150	91,75	404,5	328,25	297,75	255,75	109,25	108,25	105	98,5
	200	89	332	287,25	239,25	198,5	112,25	108,25	100,75	90,5
EAFLL	**100**	91,5	314,75	275,75	201,5	186,75	116,25	105	96,5	92,75
	150	91,75	430,75	383,25	355,25	268,5	110,75	103,5	98	92
	200	96	339,75	290,25	245,75	159	111	95,5	90,5	86
NHFLL	**100**	94,25	365,25	302,75	239,25	179,5	164,5	140	129	118,5
	150	94,75	359,5	302,5	214,25	168,5	138,25	112,75	95,5	90,25
	200	94	336	284,25	239,25	200	129,5	110,25	90,75	86,75

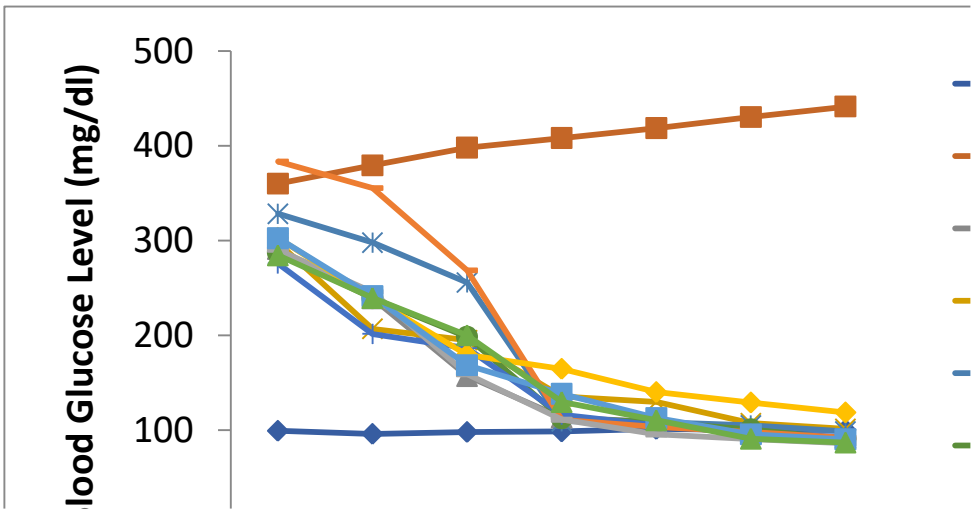

Figure: 1 Graph of BGL measurement with STZ induction treatment

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 EALL 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 samples starting from day 1 to day 7 it was found that EELL 100 mg / kg BW decreased by 320 mg / kg BW, EELL 150 mg / kg BW decreased by 257 mg / kg BW, EELL 200 mg / kg BW decreased by 241.75 mg / kg BW, EAFLL 100 mg / kg BW decreased by 233 mg / kg BW, EAFLL 150 mg / kg BW decreased by 265.75 mg / kg BW, EAFLL 200 mg / kg BW decreased by 275.25 mg / kg BW, NHFLL 150 mg / kg BW decreased by 303.75 mg / kg BW, NHFLL 200 mg / kg BW decreased by 246.75 mg / kg BW and Metformin suspension 65 mg / kg BW decreased in the amount of 331.5 mg / kg BW; compared to the large dose of use, the strength of BGL reduction as follows, metformin 65 mg / kg BW> NHFLL 150 mg / kg BW> EAFLL 200 mg / kg BW> EAFLL 150 mg / kg BW> EELL 150 mg / kg BW> NHFLL 200 mg / kg BW> EELL 200 mg / kg BW> EAFLL 100 mg / kg BW; EAFLL 150 mg / kg BW which is closest to the strength of the effect of metformin 65 mg / kg BW seen on the 15th day. All of the above data are used to determine the most powerful dose and determine the fastest time to decrease BGL of mice, then a comparison of BGL reduction is performed for each mouse BGL measurement time. 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 [10–12]. 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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