Hypoglycaemic and Antihyperlipidemic Effects of Henna Leaves Extract (*Lawsonia inermis* Linn) on Alloxan Induced Diabetic Mice

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ABSTRACT

This is a study of the effect of Inai (*Lawsonia inermis* Linn) leaves extract on glucose, total cholesterol and triglyceride of the blood of mice which was induced by alloxan of 70 mg/kgBW. Inai leaves extract was obtained by the percolation of dried inai leaves using 70% ethanol. Sample treatments were done at the 0, 3rd 7th days and 14th days after the mice had underwent the hyperglycaemic condition. The results showed that the feeding of 0.8 g/kgBW of inai extract decreased the glucose concentration from 194 mg/dl to normal condition after the 14th day. A similar result was noticed on total cholesterol concentration as the total cholesterol concentration decreased from 148.9 mg/dl to 55.3 mg/dl, while triglyceride concentration decreased from 225.7 mg/dl to 76.9 mg/dl.

Keywords: *Lawsonia inermis*, diabetes mellitus, total cholesterol, triglyceride.

INTRODUCTION

Diabetes mellitus is a metabolic hereditary disease characterised by hyperglycaemia and glycosuria due to absolute or relative lack of insulin, the fasting plasma glucose cut off level being 7.0 mmol/l.(1) Defects in carbohydrate-metabolizing machinery and in the consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an over-exertion on the endocrine system, which leads to a deterioration in endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycaemia.(2)

Many traditional plant treatments for diabetes mellitus are used throughout the world. Few of the traditional plants treatments for diabetes have received scientific scrutiny as the World Health Organisation has recommended, thus the area warrants attention .(3)

Antihyperglycaemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output, inhibiting the intestinal absorption of glucose, or by facilitating the metabolites in insulin-dependent processes. More than 400 plant species having hypoglycaemic activity have been studied in literature. (4, 5)

This paper describes the study of *Lawsonia inermis* Linn (Lythraceae, commonly known as henna); a common plant in Indonesia which has been widely used in traditional medicine to reduce glucose. This study was thus initiated with the aim of evaluating the effects of *L. inermis* leaves extract on blood glucose level, total cholesterol and triglyceride in alloxan diabetic mice.

GENERAL EXPERIMENTAL

Plant material

The leaves of *L. inermis* were collected from Balitro, Bogor, West Java and authenticated by Dr. Eko Baroto

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Waluyo, Principal and Taxonomist, Research Center for Biology, Indonesian Institute of Sciences. A voucher specimen (IPH 1.02/If.8) was deposited in the same place.

Preparation of extracts

The powdered dried leaves (1 kg) were extracted with 70% aqueous ethyl alcohol by using the percolation process for 24 h. The concentrated aqueous extract (134 g) was suspended in water.

Test animals

Swiss strain male mice (20-25 g body weight) were used in the experiment. The animals were maintained under standard environmental conditions, and were fed with a standard diet (Balivet, Bogor) and water ad libitum. The animals were fasted for 16 h before the experiment but allowed free access to water.

Induction of experimental diabetes

After 16h fasting, mice were made diabetic by an injection of alloxan (60 mg/kg body weight, dissolved in saline) in the tail vein. After 96 h, plasma glucose levels were measured by an auto-analyzer (Basic-plus, Agohuson-gohuson Company, USA) using blood sample taken from the tail vein of mice. Mice with glucose levels higher than 20 mmol/L were considered to be severely diabetic, whereas those with levels between 15-18 mmol/L were considered moderately diabetic.

Experimental procedure

In the experiment, a total of 42 mice consisting of 36 diabetic surviving mice and six normal mice were used. The mice were divided into seven groups of six mice each:

Group 1: Normal untreated mice

Group 2 : Diabetic control mice; which were given 0.1 ml of aqueous solution daily using an intragastric tube for 14 days.

Group 3: Diabetic mice which were given *L. inermis* extract (0.1 g/kg BW) in 0.1 ml of aqueous solution daily using an intragastric tube for 14 days.

Group 4: Diabetic mice which were given *L. inermis* extract (0.2 g/kg BW) in 0.1 ml of aqueous solution daily using an intragastric tube for 14 days.

Group 5: Diabetic mice which were given *L. inermis* extract (0.4 g/kg BW) in 0.1 ml of aqueous solution daily using an intragastric tube for 14 days.

Group 6: Diabetic mice which were given *L. inermis* extract (0.8 g/kg BW) in 0.1 ml of aqueous solution daily using an intragastric tube for 14 days.

Group 7: Diabetic mice which were given chlorpropamide (100 mg/kg BW) in 0.1 ml of aqueous solution daily using an intragastric tube for 14 days.

Blood samples were collected through the tail vein just prior to and on days 0, 3, 7 and 14 after drug administration. At the end of the 14 days, the animals were deprived of food over night and killed by decapitation.

Analytical procedure

Plasma glucose levels were measured by an auto-analyzer (Basic-plus, Agohuson-gohuson Company, USA) using a blood sample taken from the tail vein of mice; total cholesterol and triglyceride were estimated by the method of Zlatkis and Foster [6, 7], respectively.

Statistical analysis

All data were expressed as means ± S.E. Significant differences among the groups were determined by one-way analysis of variance using the SPSS statistical analysis program. Statistical significance was considered at $p<0,05$.

RESULTS AND DISCUSSION

The main characteristics of diabetes mellitus are polydipsya, polyuria and polyphagia, weight loss, muscle a weakness and hyperglycaemia.[8] This work evaluated the effects of extracts of *L inermis* on biochemical parameters such as serum triglyceride, cholesterol and plasma glucose in alloxan-induced diabetic mice. *L inermis*, a well-known traditional Indonesian medicinal herb, possesses diverse biological activities and pharmacological functions. However, the hypoglycaemic and hypolipidaemic activities of the herb have not been reported.

Alloxan is a chemical used conventionally to produce diabetes and hyperglycaemia in experimental animals by selectively destroying β-cells. This chemical induces necrosis to islets β-cells through free radical mediated damage.[9] Intraperitoneal administration of alloxan (70 mg/kg) effectively induced diabetes in normal mice as reflected in glycosuria, hyperglycaemia, polydipsya, polyphagia and body weight loss when compared to normal mice.[10] Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in

alloxan-induced diabetic animals.[11-13]

Body weight

There was a significant decreases in body weight of the mice in diabetic group in comparison to control group. After leaves extract of *L. inermis* supplementation for 7 days, the body weight was recovered significantly but not to the control level (Table 1). After 14 days of this supplementation, the body weight of all the animals was insignificantly different from control level.

Table 1. Effect of leaves extract of *L. inermis* on body weight in alloxan-induced diabetic mice.

Group	Body weight (g)	
	7 days	14 days
Diabetic	24.5 ± 2.4*	26.3 ± 2.5*
Control	36.7 ± 4.3	37.8 ± 2.9
L. inermis extract 0,1g/kg	32.9 ± 1.7	33.9 ± 2.0
L. inermis extract 0,2g/kg	33.3 ± 2.2	34.5 ± 2.3
L. inermis extract 0,4g/kg	33.7 ± 3.2	35.1 ± 2.8
L. inermis extract 0,8g/kg	34.7 ± 3.2	35.9 ± 2.8
Chlorpropamide 100 mg/kg	32.4 ± 2.2	34.7 ± 2.3

ANOVA followed by multiple two-tailed t-test. In each vertical column, mean with asterisk (*) differ significantly from control or diabetic mice ($p<0.05$).

Fasting blood glucose

Fasting blood glucose level of all animals before treatment was within the normal range (Table 2). Fasting blood glucose level was significantly elevated after 24 h of alloxan injection in respect to control level. The administration of *L.* inermis for 7 days for all dosage groups was not able to decrease the blood glucose concentration to reach to the normal level. Administration of *L. inermis* extract and chlorpropamide tends to bring the parameters significantly towards the normal. The effect of *L. inermis* extract at a dose of 0.8 g/kg BW was more highly significant than in the case of the dose of 0.1 g/kg, 0.2 g/kg or 0.4 g/kg BW.

Table 2. Effect of leave extract of *L. inermis* after 7 days and 14 of days treatment on blood sugar level in alloxan-induced diabetic male mice.

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Group	Fasting blood sugar level (mg/dl)			
	At the time of grouping	Days of *L. inermis* supplementation		
		0 day	7 days	14 days
Control	76.2 ± 1.2	76.5 ± 1.4	78 ± 1.7	75.8 ± 1.6*
Diabetic	76.5 ± 1.1	196.3 ± 2.3	178.2 ± 3.1	175.7 ± 2.4
L. inermis extract 0,1 g/kg	76.3 ± 1.5	179.8 ± 3.2	156.7 ± 4.2	111.8 ± 4.7
L. inermis extract 0,2 g/kg	77.0 ± 1.4	191.0 ± 3.3	163.0 ± 4.1	117.0 ± 2.5
L. inermis extract 0,4 g/kg	76.2 ± 1.5	189.5 ± 2.3	134.7 ± 2.6	100.2 ± 2.8
L. inermis extract 0,8 g/kg	77.0 ± 0.9	196.7 ± 3.1	123.7 ± 3.1	74.5 ± 2.6*
Chlorpropamide 100 mg/kg	77.2 ± 1.2	188.9 ± 2.3	134.6 ± 1.9	76.4 ± 2.2*

ANOVA followed by multiple two-tailed t-test. In each vertical column, mean with asterisk (*) differ significantly from control or diabetic mice ($p<0.05$).

In our present study we have observed that extract of *L. inermis* leaves can reverse these effects. The possible mechanism by which *L. inermis* leaves bring about its antihyperglycaemic action may be by potentiation of pancreatic secretion of insulin from β-cell of islets or due to enhanced transport of blood glucose to peripheral tissue. In

this context, a number of other plants have also been reported to have antihyperglycaemic and insulin-release stimulatory effects.[14, 15] Phytochemical investigation on *L. inermis* leaves has shown the presence of compounds such as p-coumaric acid, lawsone, apigenin and luteolin.[16] However, so far, the hypoglycaemic effect of these plant has not been reported.

Total cholesterol and triglyceride levels

To identify the effect of *L. inermis* extract on the total cholesterol and triglyceride in the mouse blood, the mice were killed after the 14th day and the blood was taken from the cardiac puncture to determine the total cholesterol and triglyceride concentration. Hypercholesterolaemia and hypertriglycerideaemia are common complications of diabetes mellitus. The effects of *L. inermis* on these biochemical parameters in alloxan-induced diabetic mice are shown in Table (3). Compared to the normal level, the cholesterol and triglyceride concentration levels tended to increase in the untreated-diabetic mice after alloxan was injected for 14 days (P<0,05).

Table 3. Effect of leave extract of *L. inermis* after 14 days of treatment on total cholesterol and triglyceride level in alloxan-induced diabetic male mice.

Group	Total cholesterol Triglyceride
Control	57.8 ± 2.3* 78.9 ± 3.5*
Diabetes	148.9 ± 1.9 225.7 ± 2.5
L. inermis extract 0,1 g/kg	98.5 ± 4.8 108.5 ± 3.8
L. inermis extract 0,2 g/kg	92.5 ± 4.9 106.3 ± 2.2
L. inermis extract 0,4 g/kg	83.7 ± 1.9 91.9 ± 2.6
L. inermis extract 0,8 g/kg	55.3 ± 3.9* 76.9 ± 3.2*
Chlorpropamide 100 mg/kg	61.3 ± 2.5* 75.8 ± 2.8*

ANOVA followed by multiple two-tailed t-test. In each vertical column, mean with asterisk (*) differ significantly from control or diabetic mice (p<0.05).

The abnormal high concentration of total cholesterol and triglyceride in the diabetic subjects is mainly due to an increase in the mobilization of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolaemia and hypertriglycerideaemia have been reported to occur in alloxan diabetic mice (4). After the treatment with *L. inermis* 0.8 g/kg for 14 days, there was a significant decrease (p<0.05) of serum triglyceride compared to normal and diabetic control mice (p<0.05).

In conclusion, *L. inermis* leaves extract at dose 0.8 g/kg BW and chlorpropamide showed significant hypoglycaemic and hypolipidaemic activities in diabetic mice after oral administration. Thus, the claim made by the traditional Indonesian system of medicine regarding the use of this plant in the treatment of diabetes is confirmed. Present efforts are directed to isolate the active constituents from extracts of *L. inermis* leaves for the elucidation of mechanism of action.

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فاعلية خلاصة أوراق الحنا (*Lawsonia inermis* Linn) في خفض السكر والدهنيات عند الفئران المصابة بالسكري بواسطة الوكسان

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ملخـص

هذه دراسة لفاعلية خلاصة أوراق الحنا (*Lawsonia inermis* Linn) على الغلكوز، والكلوستيرول والجليساريدات الثلاثية على دم الفئران بعد حقنها بالوكسان 70 ملغم/كغم من وزن الجسم . تم الحصول على خلاصة اوراق الحنا بواسطة نقع الأوراق الجافة باستعمال 70% ايثانول. تمت المعالجة بالخلاصة في الأيام 0، ،3، 7 و14 بعد حالة ارتفاع السكر عند الفئران.
أظهرت النتائج بأن اعطاء0.8 غم/كغم من وزن الجسم من خلاصة أوراق الحنا عن طريق الفم تخفض نسبة الغلوكوز من 194 ملغم/دل إلى الوضع الطبيعي بعد 14 يوما. وتم ملاحظة نتائج مشابهه على تركيز الكلوستيرول الكلي حيث انخفض تركيز الكلوستيرول الكلي من 148.9 ملغم/دل إلى 55.3 ملغم/دل، بينما انخفض تركيز الجليساريدات الثلاثية من 225.6 ملغم/د ل إلى 76.9 ملغم/دل.

الكلمـات الدالـة: *Lawsonia inermis* ، مرض السكري، كلوستيرول كامل، جليساريدات ثلاثية.

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