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The Effect of Inai (*Lawsonia inermis* Linn) Leaves Extract on Blood Sugar Level: An Experimental Study

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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 blood of mice which was induced by alloxan of 70 mg kg^{-1} BW. Inai leaves extract was obtained by the percolation of dried inai leaves using 70% ethanol. Sample treatments were done at day of 0, 3, 7 and 14th after the mice underwent the hyperglycaemic condition. The results showed that the feeding of 0.8 g kg^{-1} BW of inai extract decreased the glucose concentration from 194 mg dL^{-1} to normal condition after the 14th day. A similar result occured on total cholesterol concentration in which the total cholesterol concentration decreased from 148.9-55.3 mg dL^{-1} and triglyceride concentration decreased from 225.7-76.9 mg dL^{-1}.

Key words: *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} (WHO, 1999). Defects in carbohydrate metabolizing machinery and consistent efforts of the physiological systems to correct the imbalance in carbohydrate metabolism place an over exertion on the endocrine system, which leads to the deterioration of endocrine control. Continuing deterioration of endocrine control exacerbates the metabolic disturbances and leads primarily to hyperglycaemic (Bailey, 2000).

Many traditional plant treatments for diabetes mellitus are used throughout the world. Few of traditional plants treatments for diabetes have received scientific scrutiny and the World Health Organisation has recommended that, thus the area warrants attention (WHO, 1981).

Antihyperglycaemic effects of these plants are attributed to their ability to restore the function of pancreatic tissues by causing an increase in insulin output or inhibit the intestinal absorption of glucose or the facilitation of metabolites in insulin dependent processes. More than 400 plant species having hypoglycaemic activity have been available in literature (Kim *et al.*, 2006; Rai, 1995).

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

MATERIALS AND METHODS

Plant materials: The leaves of *L. inermis* were collected from BALITRO, Bogor, West Java and authenticated by Dr. Eko Baroto 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 dried leaves powder (1 kg) was extracted with 70% aqueous ethyl alcohol by percolation process for 24 h. The concentrated aqueous extract (134 g) was suspended in water.

Test animals: Male mice (20-25 g) were used in the experiment. The animals maintained under standard Environmental conditions, 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.

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Experimental procedure: In the experiment a total of 42 mice consisting of 36 diabetic surviving mice and 6 normal mice were used. The mice were divided into 7 groups of 6 mice each

Group1: Normal untreated mice.

Group 2: Diabetic control mice where given 0.1 mL of aqueous solution daily using an intragastric tube for 14 days.

Group 3: Diabetic mice where given *L. inermis* extract (0.1 g Kg^{-1} BW) in 0.1 mL of aqueous solution daily using an intragastric tube for 14 days.

Group 4: Diabetic mice where given *L. inermis* extract (0.2 g kg^{-1} BW) in 0.1 mL of aqueous solution daily using an intragastric tube for 14 days.

Group 5: Diabetic mice where given *L. inermis* extract (0.4 g kg^{-1} BW) in 0.1 mL of aqueous solution daily using an intragastric tube for 14 days.

Group 6: Diabetic mice where given *L. inermis* extract (0.8 g kg^{-1} BW) in 0.1 mL of aqueous solution daily using an intragastric tube for 14 days.

Group 7: Diabetic mice where given chlorpropamide (100 mg kg^{-1} 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 day 0, 3, 7 and 14th after the drug administration. At the end of the 14 days, the animals were deprived of food over night and killed by decapitation.

Analytical procedure: Fasting blood glucose was estimated by o-toluidine method (Sasaki *et al.,* 1972), total cholesterol and triglyceride were estimated by the method of Zlatkis *et al.* (1953) and Foster and Dunn (1973), 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 weakness and hyperglycaemic (Shan *et al.,* 2006). 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 function. 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 (Tiwari and Rao, 2002). Intraperitoneal administration of alloxan (70 mg kg^{-1}) effectively induced diabetes in normal mice as reflected in glycosuria, hyperglycaemia, polydipsya, polyphagia and body weight loss when compared to normal mice (Calabresi and Chabner, 2002). Numerous studies demonstrated that a variety of plant extracts effectively lowered the glucose level in alloxan-induced diabetic animals (Maroo *et al.,* 2002; Nammi *et al*., 2003; Vijayuargia *et al*., 2000).

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 were insignificantly different from control level.

Fasting blood glucose: Fasting blood glucose level of all animals before treatment were 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 the normal level. Only the dosage at 0.8 g kg^{-1} administration for 14 days could decrease the blood glucose concentration to the same level as the normal group and chlorpropamide ($p<0.05$).

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

	Body weight (g)	
Group	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^{-1}	32.9±1.7	33.9±2.0
L. inermis extract 0,2g kg^{-1}	33.3±2.2	34.5±2.3
L. inermis extract 0,4g kg^{-1}	33.7±3.2	35.1±2.8
L. inermis extract 0,8g kg^{-1}	34.7±3.2	35.9±2.8
Chlorpropamide 100 mg kg^{-1}	32.4±2.2	34.7±2.3

ANOVA followed by multiple 2-tailed t-test. In each vertical column mean with asterisk (*) differ significantly from control or diabetic mice ($p<0.05$)

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

	Fasting blood sugar level (mg dL^{-1})			
		Days of *L. inermis* supplementation		
Group	At the time of grouping	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^{-1}	76.3±1.5	179.8±3.2	156.7±4.2	111.8±4.7
L. inermis extract 0.2 g kg^{-1}	77.0±1.4	191.0±3.3	163.0±4.1	117.0±2.5
L. inermis extract 0.4 g kg^{-1}	76.2±1.5	189.5±2.3	134.7±2.6	100.2±2.8
L. inermis extract 0.8 g kg^{-1}	77.0±0.9	196.7±3.1	123.7±3.1	74.5±2.6*
Chlorpropamide 100 mg kg^{-1}	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 ($p<0.05$)

Table 3: Effect of leave extract of *L. inermis* after 14 days 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^{-1}	98.5±4.8	108.5±3.8
L. inermis extract 0.2 g kg^{-1}	92.5±4.9	106.3±2.2
L. inermis extract 0.4 g kg^{-1}	83.7±1.9	91.9±2.6
L. inermis extract 0.8 g kg^{-1}	55.3±3.9*	76.9±3.2*
Chlorpropamide 100 mg kg^{-1}	61.3±2.5*	75.8±2.8*

ANOVA followed by multiple 2-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 effect (Prince *et al*., 1998; Pari and Maheswari, 1999).

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 hypertriglyceridaemia 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 to levels tended to increase in untreated-diabetic mice after alloxan was injected for 14 days ($p<0.05$).

The abnormal high concentration of total cholesterol and triglyceride in the diabetic subject is due mainly to an increase in the mobilisation of free fatty acids from peripheral fat depots, since insulin inhibits the hormone sensitive lipase. Hypercholesterolaemia and hypertriglyceridaemia have been reported to occur in alloxan diabetic mice (Kim *et al*., 2006).

After the treatment of *L. inermis* dose 0.8 g kg^{-1} for 14 days, there was a significant decrease ($p<0.05$) of inserum triglyceride compared to normal and diabetic control mice ($p<0.05$). The antihyperlipidaemic effect of *L. inermis* may be due to the down regulation of NADPH and NADH, a cofactor in the fat metabolism. Higher activity of glucose-6-phosphatase provides H^+ which binds with $NADP^+$ in the form of NADPH and is helpfull in the synthesis of fats from carbohydrates (Retnam *et al*., 1983).

CONCLUSION

In conclusion *L. inermis* leaves extract 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 and elucidation of action mechanism.

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