PJN
ISSN 1680-5194

PAKISTAN JOURNAL OF NUTRITION

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308 Lasani Town, Sargodha Road, Faisalabad - Pakistan
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OPEN ACCESS Pakistan Journal of Nutrition

ISSN 1680-5194
DOI: 10.3923/pjn.2019.671.676

Research Article
Effect of *Lawsonia inermis* Linn. Extracts on Blood Glucose Level in Normal and Streptozotocin-Induced Diabetic Rats

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Abstract

Background and Objective: *Lawsonia inermis* Linn. is one of the common medicinal plants that are being used widely to treat diabetes in Medan, North Sumatera, Indonesia. Scientific evidence to confirm its efficacy is still lacking. Hence, this study was designed to evaluate the effect of *L. inermis* leaf extracts on glucose level in normal and streptozotocin-induced diabetic rats. **Materials and Methods:** Serial extraction of *Lawsonia inermis* Linn. leaf was conducted to obtain the respective n-hexane (HE), ethyl acetate (EAE), ethanol (EE) and distilled water (A1E) extracts. Another water extract (A2E) was obtained using infusion technique. These extracts were administered orally at the dose of 1 g kg^{-1} b.w. to normal and streptozotocin-induced diabetic rats. An acute hypoglycemic, glucose tolerance and antihyperglycemic effects were assessed. Qualitative phytochemical screenings were conducted prior to the *in vivo* tests. **Results:** The phytochemical screenings revealed the presence of steroid and triterpenoid in HE, alkaloid, tannin, flavonoids, saponin and glycoside in EE and EAE. While in A1E and A2E, tannin, flavonoid, saponin and glycoside were identified. Hypoglycemic test in normal rats showed HE was able to decrease blood glucose level (BGL) significantly ($p<0.05$). A1E and A2E were able to inhibit the rise of BGL after being challenged with glucose intraperitoneally. Furthermore, administration of single dose of extracts showed that only EAE exerted significant antihyperglycemic activity. **Conclusion:** All the extracts of *Lawsonia inermis* Linn. leaf, except for EE, have shown certain degree of antidiabetic activity with possible different mechanisms of antidiabetic action.

Key words: Antidiabetic, glucose level, henna, *Lawsonia inermis* Linn., medicinal plant

Received: April 09, 2019 Accepted: May 09, 2019 Published: June 15, 2019

Citation: T. Widyawati, Y.S. Pane and N.A. Yusoff, 2019. Effect of *Lawsonia inermis* Linn. extracts on blood glucose level in normal and streptozotocin-induced diabetic rats. Pak. J. Nutr., 18: 671-676.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nature has been used as a source of medicinal products, as indicated by a number of modern drugs has been derived from medicinal plants[1]. *Lawsonia inermis* Linn. is a traditional plant with religious associations. The local names of *L. inermis* Linn. include *Henna* (English)[2], *Mehandi* (Hindi), *Aivanam* (Tamil) and *Daun Inai* (Indonesia)[3]. This plant has long been used as a herbal treatment[4]. Based on a survey conducted among the diabetes patients in Medan, North Sumatera, Indonesia, some respondents used its leaves as an alternative medicine to control the blood glucose level[5]. Diabetes is a metabolic disorder happened due to lack of insulin production and/or inadequate insulin action, a hormone that is required to convert glucose into energy[6]. Furthermore, long-term use of oral antidiabetic agents causes many side effects and this is one of the reasons why patients turn to medicinal plants as an alternative medicines.

Syamsudin *et al.*[7] showed that ethanol extract (0.8 g kg^{-1}) of *L. inermis* leaves decreased blood glucose level (BGL) of alloxan-induced mice from 194 to 75 mg dL^{-1} after 14 days administration. The same finding was reported by Chikaraddy *et al.*[8] whom studied the antidiabetic effect of ethanol extract of *L. inermis* Linn. leaf at the doses of 150, 200 dan 400 mg kg^{-1} in alloxan-induced diabetic Albino rats. Singh *et al.*[9] also reported the reduction in blood glucose level (39.08%) after *L. inermis* Linn ethanolic extract administration at a dose of 400 mg kg^{-1} for 21 days. The methanolic leaves extract of the plant also significantly inhibited (60.97%) enzymatic activity of the amylase at the concentration of 10 µg mL^{-1} [10].

The solvent polarity, extraction methods and constituents instability would influence the composition and quality of the extracts[11,12]. Thus, methods of preparation are tailored towards the type of natural material being processed and the strategy for analysis being undertaken[13]. To date, no study on *L. inermis* Linn. leaf using serial extraction was found. The present study was carried out to investigate the effect of different extracting solvents on the antidiabetic activity of *L. inermis* Linn. leaf extract. The extracts were prepared using different solvents according to the polarity starting from the non-polar to the more polar solvents.

MATERIALS AND METHODS

Plant materials: The study was carried out on March-November 2016 at Pharmacology Laboratory, Pharmacy School, Universitas Sumatera Utara, Medan, Indonesia.

The leaves of *Lawsonia inermis* were obtained from Titi Kuning, Medan, Indonesia (Geographical coordinates: 3.522988, 98.682834) and were identified by D. Nurhasara Pasaribu, MSc at Biology Faculty, Universitas Sumatera Utara, Medan, Indonesia (No.672/MEDA/2016). A voucher specimen of this material has been deposited in Herbarium Bogoriense, Indonesian Institute of Science, Research Centre for Biology, Bogor, Indonesia (No.924/IPH.1.01/If.07/III/2017) that verified by Dr. Joeni Setio Rahajoe. The fresh leaves were washed using running water and dried at room temperature. The dried leaves then were grounded into powder using grinding machine.

Chemicals: Metformin HCl BP 500 mg and glibenclamide 10 mg were used as the positive controls. Streptozotocin (STZ) was purchased from Sigma-Aldrich Chemical Company (St. Louis, MO, USA). Blood glucose level (BGL) was determined using EasyTouchÒ glucometer (Chiuan Rwey Enterprise Cp., Ltd., Taiwan).

Preparation of extracts: The fresh leaves were dried under shade and ground into powder[5]. About 1.5 kg of the powdered leaf was extracted serially by maceration in n-hexane, ethyl acetate, ethanol and distilled water to obtain four extracts namely hexane extract (HE), ethyl acetate extract (EAE), ethanol extract (EE) and water extract (A1E). Another water extract (A2E) was obtained by infusion technique. The freeze-dried extracts were stored in the refrigerator (-20°C) until further use for experimentation. Prior to treatment, the extracts were reconstituted using 5% tween 80 in distilled water.

Phytochemical identification: Phytochemical qualitative analyses were performed using the general method for phytochemical screening of alkaloid, steroid, triterpenoids, tannins, flavonoids, saponin and glycosides[14,15].

Animals: Healthy male Wistar rats weighing between 180-250 g (n = 126) were obtained from the Animal House, Universitas Sumatera Utara (USU). These animals were housed in the transit animal room, School of Pharmacy, USU and allowed to acclimatize for a week before the commencement of the experiments. The animals had *ad libitum* access to water and food; a 12 h light-12 h dark cycle and room temperature (25-30°C). The test procedures were approved by the Animal Ethics Committee, FMIPA USU, Medan, Indonesia (Approval number: 184/KEPH-FMIPA/2016).

Hypoglycemic test in normal rats: Normal male Wistar rats were divided into seven groups (n = 6) and fasted overnight (8:00 pm-8:00 am) randomly. The first group was treated with glibenclamide (10 mg kg^{-1}) that served as the positive control group. The second, third, fourth, fifth and six groups were treated with HE, EAE, EE, A1E and A2E (1 g kg^{-1}, respectively). The seventh group served as the negative control and was treated with distilled water (10 mL kg^{-1}). All treatments were administered orally using a 16-G oral needle. BGLs were measured before treatment and at the time intervals of 1, 2, 3, 5 and 7 h after each treatment.

Intraperitoneal glucose tolerance test (IPGTT) in normal rats: As many as 42 Wistar rats were divided into seven groups. Each group was consisted of 6 male rats. The treatments were given to the fasted rats as follows: Group 1, the normal control received normal saline (10 mL kg^{-1}.); group 2, the positive control was treated with metformin (500 mg kg^{-1}) and groups 3-6 were treated with 1 g kg^{-1} of HE, EAE, EE, A1E and A2E respectively. Exactly one hour after the treatments were given, the rats were loaded with 1 g kg^{-1} of glucose intraperitoneally. Afterwards, BGL of the rats were measured at 0, 15, 30, 45, 60, 90 and 120 min after glucose loading.

Acute anti-hyperglycaemic test in stz-induced diabetic rats (SDR): Diabetes was induced in normal male Wistar rats using streptozotocin (STZ) at a dose of 55 mg kg^{-1}. STZ, intraperitoneally to the 16 hrs fasted rats. In the first 24 h of STZ injection, rats were given 10 % dextrose to prevent STZ induced fatal hypoglycemia. Diabetes was confirmed 72 h after induction by measuring the fasting BGL. Animals with fasting BGL $\geq$126 mg dL^{-1} were considered diabetic and used in the study.

Forty two diabetic rats were divided into seven groups (n = 6) and treated as follows: Group 1, the positive control received metformin (500 mg kg^{-1}); Group 2-6 were treated with 1 g kg^{-1} each of HE, EAE, EE, A1E and A2E, respectively; Group 7 served as the negative control received distilled water (10 mL kg^{-1}). Fasting BGLs were measured from the tail vein prior to treatment and at 1, 2, 3, 5 and 7 h after treatment.

Statistical analysis: The data was expressed as Mean±standard error of the mean (SEM). Statistical significance was determined by IBM-SPSS statistical program version 21 (IBM Corp., Armonk, NY). One-way ANOVA was used followed by Dunnett's as the *post hoc* test. Kruskal-Wallis was used followed by Mann Whitney as the *post hoc* test[15]. Differences were considered significant with the p-value less than 0.05.

RESULTS

The percentage yield of powder leaf extracts of *L. inermis* in different solvents are as follows; HE 2.6%, EAE 4.7%, EE 18%, A1E 18% and A2E 16%.

Phytochemical screening: As shown in Table 1, each compound was traced as follows alkaloid in EAE and EE; steroid and triterpenoid in HE only, while tannin, flavonoid, saponin and glycoside were traced in EAE, EE, A1E and A2E.

Hypoglycemic test of *Lawsonia inermis* leaf extracts in normal wistar rats: Effect of *L. inermis* leaf extracts on BGL of normal rats in Fig. 1. HE significantly decreased BGL at 3 h after treatment ($p<0.05$) with the percentage reduction of 27.8±1.5% as compared to the control group. Meanwhile,

Table 1: Phytochemical screening of *Lawsonia inermis* Linn. leaf

	Extracts				
Phytochemical screening	HE	EAE	EE	A1E	A2E
Alkaloid	(-)	(+)a	(+)a	(-)	(-)
Steroid	(+)b	(-)	(-)	(-)	(-)
Triterpenoid	(+)c	(-)	(-)	(-)	(-)
Tannin	(-)	(+)d	(+)d	(+)d	(+)d
Flavonoid	(-)	(+)e	(+)e	(+)e	(+)e
Saponin	(-)	(+)f	(+)f	(+)f	(+)f
Glycoside	(-)	(+)	(+)	(+)	(+)

HE: n-hexane extract, EAE: Ethyl acetate extract, EE: Ethanol extract, A1E: Water extract1, A2E: Water extract2, a: Dragendor, f: Red, Mayer: White sediment, b: blue-green, c: Pink or violet color, d: Blue-black, green or blue-green precipitate, e: Green-blue or violet color, f: Formed foam

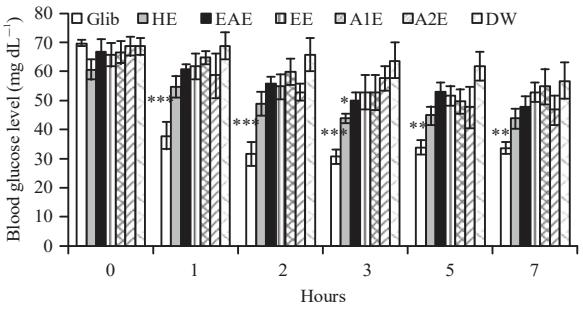

Fig. 1: Effect of *Lawsonia inermis* Linn. leaf extracts on blood glucose level in normal rats

Values were expressed as Mean±standard error of the mean (SEM), Data were analyzed using Kruskall Wallis with Mann Whitney as *post hoc* test, *p<0.05, **p<0.01, ***p<0.001

Table 2: Effect of *Lawsonia inermis* Linn. leaf extracts on blood glucose level of normal rats after glucose loading (1 g kg^{-1}) intraperitoneally

	Blood glucose level (mg dL^{-1})						
Groups	0′	15′	30′	45′	60′	90′	120′
DW	75.5±3.65	129.2±6.15	102.0±8,61	90.8±10.78	93.5±11.06	86.3±8.42	75.5±6.87
M	75.1±2.67	94.8±7.95**	83.6±2.55*	74.8±3.95	76.1±3.08	65.1±6.83	60.5±5.37
HE	63.1±5.87	116.7±4.72	98.7±5.96	85.8±4.05	76.2±5.79	73.2±3.19	65.5±3.57
EAE	66.3±3.65	117.2±5.89	90.8±7.79	77.8±7.75	75.8±7.83	68.8±4.21	68.6±3.94
EE	65.6±3.27	120.5±6.53	98.1±1.73	79.8±0.87	77.3±2.87	73.8±2.89	68.8±1.47
A1E	68.8±3.23	126.2±4.55	103.8±4.22	80.3±2.15	73.2±4.05	70.8±1.62*	67.5±2.07
A2E	64.3±3.11*	108.8±3.18**	88.3±4.39	79.3±2.21	72.6±1.80*	70.1±2.86	62.8±3.62

DW: Distilled water, 10 mL kg^{-1}, M: Metformin, 500 mg kg^{-1}, HE: n-hexane extract, 1 g kg^{-1}, EAE: Ethyl acetate extract, 1 g kg^{-1}, EE: Ethanol extract, 1 g kg^{-1}, A1E: Water extract 1, g kg^{-1}; A2E: Water extract 2, 1 g kg^{-1}, Data were analyzed using Kruskal Wallis with Mann Whitney as the *post hoc* test; values were expressed as Mean±standard error of the mean (SEM) ($n = 6$), *p<0.05, **p<0.01

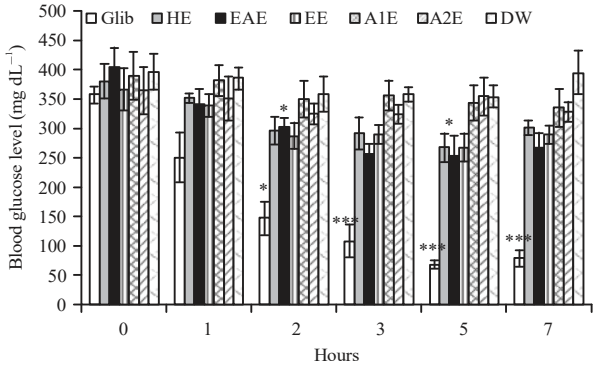

Fig. 2: Effect of single dose administration of *Lawsonia inermis* Linn. Leaf extracts on blood glucose level in streptozotocin-induced diabetic rats

DW: distilled water 10 mL kg^{-1}, HE: n-*hexane* extracts 1 g kg^{-1}, EAE: Ethyl acetate extracts 1 g kg^{-1}, EE: Ethanol extracts 1 g kg^{-1}, A1E: Water extracts 1 g kg^{-1}, A2E: Water extracts 2, 1 g kg^{-1}, Values were expressed as Mean±standard error of the mean (SEM) (n = 6). Data was analyzed using one-way ANOVA and Dunnett's as the *post hoc* test. *p<0.05, **p<0.01, ***p<0.001 versus the control group

glibenclamide-treated group showed significant hypoglycemic activity from the first hour until the end of observation period (p<0.01-0.001) with the highest percentage reduction (55.7±2.7%) was recorded at 3 h.

Intraperitoneal glucose tolerance test (IPGTT) in normal rats: As shown in Table 2, we can see the effect of *L. inermis* leaf exracts on blood glucose level after being loaded with glucose (1 g kg^{-1}) intraperitoneally. Metformin-treated group, acted as the positive control, was able to significantly inhibit the rise of BGL at 15 and 30 min after glucose-loading (p<0.05-0.01). A1E-treated group significantly suppressed the rise in BGL at 90 min, only of 66.3±3.65 to 68.8±4.21 mg dL^{-1}, whereas A2E recorded similar effect at 0 min (p<0.05), 15 and 60 min (p<0.05-0.01) after glucose loading.

Acute anti-hyperglycaemic test in STZ-induced diabetic rats (SDR): The effect of single dose administration of *L. inermis* leaf extracts on STZ-induced diabetic rats can be observed in Fig. 2. Metformin-treated group showed significant antihyperglycemic effect from 1-7 h study period (p<0.05-0.001) with the highest BGL reduction of 81.0±6% was recorded at 5 h. EAE-treated group showed significant decrease (p<0.05) in BGL at 3 h observation, as compared to diabetic control with the percentage reduction of 36.5±16%. Current findings showed that only EAE managed to lower BGL of the diabetic rats to a significant level (p<0.05). Hence, it is considered as the most active extract of *L. inermis* leaf.

DISCUSSION

Medicinal plant, as a source of alternative medicine has potency for new drug discovery because of it's active compound diversity[16,17]. *L. inermis* Linn has been used cosmetically and medicinally[5] for over 9,000 years[16]. It is traditionally used to treat a variety of diseases such as arthritis, leprosy, ulcers, cardiac disease and diabetes[5]. In this study, the trace of those compounds presented in *L. inermis* leaf extracts may contribute to its antidiabetic activities, as seen in normal and diabetic rats (Fig. 1-2 and Table 2). The antidiabetic activity of the phytochemicals has been reported in many studies. Phytochemical screening of the methanolic extracts of *L. inermis* leaf studied by Raja *et al.*[18] showed the presence of glycosides, phytosterol, steroids, saponins, tannins and flavonoids. The extracts showed antibacterial activity. In another study, Tiong *et al.*[19] showed that alkaloids isolated from *Catharanthus roseous* L. are able to enhance the uptake of glucose in β-TC6 and C2C12 cells and inhibit protein tyrosine phosphatase-1B (PTP-1B), thus suggesting their antidiabetic potential activity. A 28N or-22® Witha 2,6,23-trienolide, a steroid, that fractionated from the acetone extract of *Elephantopus scaber*, significantly decreased high blood glucose level in diabetic rats[20]. Flavonoids, polyphenolic compounds found in many plants, have been reported to act against free-radical compounds and normalize hyperlipidemia and hyperglycemia conditions[21-23]. It was also reported to

inhibit the activity of alpha-glucosidase enzyme[24]. This action lead to suppression of the post prandial hyperglycemia. Uma *et al*. [25] succesfully quantified the total phenolic compound in *L. inermis* leaf extracts. Triterpenoids were reported to exert antidiabetic activity through PPARg activation and alpha-glucosidase enzyme inhibition[26,27]. Whereas, tannin was reported to inhibit not only alpha-glucosidase activity but also alpha-amylase[28]. In addition, *Syzygium cumini* extracts that contain triterpenes, steroids, glycosides, alkaloids, flavonoids, saponins and tannins demonstrated the ability to reduce fasting blood glucose levels of streptozotocin-induced diabetic rats after 3 weeks oral administration[29].

Hypoglycemic test showed that BGL of all treated groups tended to decrease as no food was given during the 7 h of study period. A significant reduction in BGL (less than 40 mg dL^{-1}), were recorded in glibenclamide-treated group, thus verified the hypoglycemic effect of this conventional oral antidiabetic drug. Similarly, HE showed significant hypoglycemic effect as glibenclamide. This result suggested the possible antidiabetic activity of HE. Hypoglycemic is the condition wherein blood glucose level goes below the normal levels (less than 60 mg dL^{-1})[29]. This agent acts on pancreatic β-cell by inhibiting the ATP-K-ase channel. The inhibiton of this channel will result in depolarization of plasma membrane of β-cell pancreas, followed by an increasing of the intracellular calcium concentration which finally contributed to the exocytosis of insulin[30]. An antihyperglycemic agent, on the other hand, could decrease high BGL but not lower than the normal BGL, so this agent is unlikely to induce hypoglycemia. Example of antihyperglycemic agent is metformin. Metformin is used as the positive control in the present study. Metformin acts by enhancing the sensitivity of insulin in the muscle and adipose tissues and decreasing gluconeogenesis in the liver by activating adenosine monophosphate-activated protein kinase (AMPK)[31,32]. The present study showed that A1E and A2E were able to inhibit the rise of BGL after being loaded with glucose intraperitoneally which proven their ability as antihyperglycemic agents. These findings suggested that A1E and A2E might act in the same way as metformin, the positive control.

As mentioned of the results above, it can be assumed that all the extracts of *Lawsonia inermis* Linn. leaf, except for EE, have shown certain degree of antidiabetic activity with different mechanisms of antidiabetic action. This implicated that each type of extract may contain different active compounds that contributed to their specific bioactivity. The present study, however, only identifies the compounds qualitatively. Indeed, further study is needed not only to identify and to quantify the active compounds involved but also to clarify the possible mechanism of antidiabetic action of these extracts.

CONCLUSION

HE of *L. innermis* leaf showed functional effect as a hypoglycemic agent, A1E and A2E alleviated post prandial hyperglycemia and lastly, EAE showed its pharmacological activity as an antihyperglycemic.

SIGNIFICANCE STATEMENT

This study discovered antidiabetic activity of different extracts of *L. inermis* Linn. leaf that can be beneficial as an alternative treatment of diabetes mellitus. This study will help the researcher to uncover the critical areas of active principle ingredients of *L. inermis* leaf that many researchers were not able to explore. Thus a new theory on the possible mechanisms of antidiabetic action may be arrived at.

ACKNOWLEDGMENTS

The authors are especially grateful to RISTEKDIKTI for providing a grant for this study. The research work was supported by HIBAH BERSAING Grant Scheme 2016 (175/UN5.2.3.1/PPM/SP/2016).

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