

Effect of *lawsonia innermis* (linn) leaves ethanolic extract on blood glucose and malondialdehyde level in alloxan-induced diabetic rats

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Abstract The case of diabetes mellitus (DM) tends to increase worldwide. DM triggers the oxidative stress condition that caused by the increasing of free radical. The present study was conducted to evaluate the effect of giving ethanolic extract of *Lawsonia innermis* (Linn) leaves to the glucose and malondialdehyde (MDA) level in alloxan-induced diabetic Wistar male rats. The powder of dry leaves of *L.inermis* was macerated in ethanol 96% to obtain ethanolic extract (LLEE).Thirty five of rats were divided into five groups, ie. K (normal and given 0.9% NaCl solution), P1-P4 were induced using alloxan (120 mg/kg) intraperitoneally to get diabetic condition. Diabetic rats then were treated as follows: P1 (given 0.9% NaCl solution) P2 (LLEE (200 mg/kg BW), P3 (LLEE (400 mg/kg BW)), P4 (LLEE (600 mg/kg BW)). All groups were treated for 28 days. The fasting blood glucose levels were measured at day 1, 7, 14, 21, 28 whereas MDA levels were measured at the end of treatment. The result showed that LLEE improved blood glucose level (BGLs) of alloxan-induced diabetic rats significantly ($p < 0.001$) at 29th day. However, LLEE could not affect the MDA level ($p > 0.5$). The study concluded that LLEE have antihyperglycemic properties.

Keywords: Diabetes Mellitus, LLEE, BGLs, MDA

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder in the body. The symptom of DM was elevated blood glucose levels because of abnormalities of metabolic pathways of carbohydrates, fats and proteins. It caused by insulin secretion abnormalities, insulin action, or both. Based on the data from the World Health Organization (WHO) the number of people with diabetes mellitus will increase continuously in the future. Around 415 million people were estimated suffering from diabetes mellitus in 2015. This number will keep continuously increase over the last three decades and will continuously be estimated to be 642 million people with diabetes mellitus by 2040 [1]



In 2015, 5 million people aged between 20-79 years died from diabetes mellitus. The aggregate accounts for 14.5% of total deaths in that group age due to various causes [2]. In Indonesia, Diabetes mellitus is increased from 2007 by 1.1% to 2.1% in 2013 [3]. Based on the pattern of population growth, it is estimated that by 2030 there will be 194 million people aged over 20 whose will become DM [4].

The condition of hyperglycemia in people with diabetes mellitus will lead to auto oxidative glucose reactions, protein glycation and activation of polyol metabolism pathways that will accelerate the formation of free radicals (oxidants). Oxidants were reactive compounds that can move electrons from other molecules and produce oxidation in the molecules [5]. Lipids that have double carbon chains can react with oxidants, this process was called lipid peroxidation. Lipid hydroperoxide was the main product of lipid peroxidation process . The lipid hydroperoxide structure was not stable and easy to be malondialdehyde (MDA), 4-hydroxy-2-nonenal (4-HNE), and several other aldehyde forms. The MDA profile in serum serves as a marker (marker) of cellular damage caused by oxidants [6]. Giving antioxidants can bond free radicals.

There were two antioxidants, namely; endogenous and exogenous antioxidants. Endogenous antioxidants come from within the body itself, consisting of Super Oxide Dismutase (SOD), Glutathione Peroxidase (GPx), Catalase, etc. Exogenous antioxidants were obtained from the outside through the foods that we consumed to help the body fight the excess free radicals in the body. Enhanced supply of antioxidants will help prevent the clinical complications of DM [7].

Exogenous antioxidants can be obtained from the plants which rich in antioxidants such as *Lawsonia inermis* (Linn). One part of *Lawsonia inermis* (Linn) plant that commonly used as medicine was its leaves. Phytochemical screening of its leaves demonstrated the present of a phenolic natural antioxidant compound (coumarin, flavonoid, naphthalene, lawsone, gallic acid derivatives) and glycosylated protein [8]. Research from Chikaraddy et al. [9], showed that the ethanolic extract *Lawsonia inermis* (Linn) at three doses i.e. 150, 200 and 400 mg/kg BW for three weeks administration able to decrease the blood glucose levels of diabetic rats. Thus, the decreasing of blood glucose levels will minimize the risk of oxidative stress in cells and tissues [10].

So far, no data has been reported about the correlation effect of *Lawsonia inermis* (Linn) ethanolic extract/ LLEE on blood glucose level and oxidative stress level compound MDA in alloxan-induced diabetic rats model. Therefore, the present study was conducted to investigate its activities on those markers.

2. Methods

2.1 Research design

The study was experimental analytical study using Post Test Control Group Design with the preclinical trial.

2.2 Place of Research

The study was conducted in Integrated Laboratory, Faculty of Medicine, Chemistry Laboratory of Faculty of Mathematics and Natural Sciences (FMIPA) and Biology Laboratory of FMIPA, Universitas Sumatera Utara (USU), in March 2017. The study was conducted after obtaining ethical clearance No 116/KEPH-FMIPA/2017 from the Ethics Committee of the Mathematics and Natural Sciences Faculty (MIPA) Biology USU Medan.

2.3 *Lawsonia inermis* Ethanolic Extract preparation [11].

The fresh leaves were obtained from Medan Denai area and were dried in temperatue room. The dried leaves then were blended in machine into powder. The extraction was done by maceration method. The powder was macerated with ethanol 96% (1:10 (w/v)). After 5 days the extract was filtered, the pulp was squeezed and washed with enough liquid to obtain 100 parts. Maserate was transferred into a closed vessel, left in a cool place that was protected from sunlight for 2 days. The concentrated extract was obtained using evaporator and then was kept in the freeze until used.

2.4 Experimental animals

The 35 males of healthy Wistar rats (*Rattus norvegicus*), ranging 150-200 g, and have never been used for any experiments previously.

The rats were individually stacked in the FMIPA Biology Laboratory and maintained at room temperature 25 ± 5 ° C. Giving sufficient food and beverage according to the treatment group with a 12 hour light cycle and 12 hours of dark cycle. After one week of acclimatization, the rats were divided into 5 groups randomly. Based on the sampling formula, the number of Wistar rat in each group was 5 rats. To avoid the risk of drop out of the Wistar rat, then 2 rats were added in each group [12], namely:

- a Group I (K), negative control (normal) was not given any treatment, just like normal rats in general given feed and drink (*ad libitum*) in the cage.
- b Group II (P1), Positive control (diabetic) were feed and drink (*ad libitum*) in the cage
- c Group III (P2): LLEE 200 mg/kg BW, orally, for 28 days.
- d Group IV (P3): LLEE 400 mg/kg BW, orally, for 28 days.
- e Group V (P4): LLEE 600 mg/kg BW, orally for 28 days

The diabetic condition was obtained by injecting the rats using alloxan (120 mg/kg BW) in 0.1M citric buffer intraperitoneally (P1-P4) [13]. The blood glucose levels (BGL) were measured after 7 days using glucose meter (GlucodrTM). Rats with BGL of 250 mg/dl above were included in the study[14]

After the rats have BGL >250mg/dl, group P2-P4 were given extract ethanol of Lawsonia inermis Linn leaves in each 200 mg/kg BW, 400 mg/kg BW, 600 mg/kg BW [9][10]. The fasting blood was taken from rats tail were conducted by the using glucose test strips at day1, 7, 14, 21 and 28. After 28 days (4 weeks), the rats were sacrificed by surgical dislocation of the neck after light chloroform anesthesia. The 12 hours fasting blood of the rats was taken 3 ml from the heart and blood serum was stored at -80°C before examined.

2.5 Determination of MDA Level

The examination of blood MDA levels used Spectronic 21 with a commercial kit and read with at wavelength 532 nm.

2.6 Process and Data Analysis

All data were expressed as mean \pm standard deviation (SD). Mean differences between the groups were statistically analyzed by one-way analysis of variance (ANOVA) at the alpha value of 0.05 followed by Post Hoc Test. Mean differences between pre-treatment and post-treatment were analyzed by paired *t*-test. Differences were considered significant at $p < 0.05$

3. Results and Discussion

The present study is evaluate the BGL of rats in several times i.e. before and after alloxan induction, at P1-P4 groups.

Table 1. The BGL fasting of rats before and after induced alloxan 120mg/kg BW

Group	Before (mg/dl)	After (mg/dl)	p*
P1	152 \pm 17.08	350 \pm 100.92	0.000
P2	119 \pm 14.57	440 \pm 21.22	
P3	112 \pm 14.50	487 \pm 101.79	
P4	105 \pm 13.21	426 \pm 149.46	

* paired t test

From the table 1 can be seen the BGL before and after induced alloxan 120mg/kg BW. The paired t-test of P1 –P4 showed the correlation of the BGL before and after alloxan induction ($p < 0.001$). Alloxan compounds were one of the most toxic diabetogenic substances, especially to pancreatic beta cells, and when given to experimental animals such as rats can cause diabetic to the rats. The alloxan toxicity mechanism begun with the entry of alloxan into the pancreatic β cells and the retrieval rate will determine the alloxan diabetogenic properties. The damaging of β cells occurs through several processes simultaneously, through the oxidation of sulfhydryl groups and the formation of free radicals. The mechanism of action of the alloxan produces damage to pancreatic β cells primarily invading cellular compounds containing sulfhydryl groups, amino acids cysteine and proteins binding to SH groups (including enzymes containing SH groups). The alloxan reacts with two SH groups binding to the sides of proteins or amino acids to form disulfide bonds to inactivate proteins that result in impaired protein function [13].

Giving extract ethanol of *Lawsonia inermis* Linn leaves was done starting on the first day. The mean value of fasting blood glucose levels of animals experiment after given extract ethanol of *Lawsonia inermis* Linn leaves on day: 7, 14, 21, and 28 this time can be seen in table 2.

Table 2. The blood glucose level of alloxan-induced diabetic rats on day 7,14, 21, 28 after *Lawsonia inermis* (Linn) ethanolic extract administration

Group	The mean value of fasting blood glucose levels of rats (mg/dl)			
	Day 7	Day 14	Day 21	Day28
K	155± 9.16	81± 2.30	88 ± 19.00	112 ± 25.05
P1	379± 18.58	250±100.92	228 ± 64.08	309 ± 71.14
P2	421 ± 30.51	399±46.18	380.66 ± 55.89	450 ± 93.66
P3	402 ± 66.77	341± 41.52	253.66 ± 21.22	236 ± 66.57
P4	351 ± 2.30	359.66 ± 95.82	270.33 ± 31.64	256 ± 41.25

Table 2 showed that P1-treated group on day 7 still hyperglycemia. On day 14 and day 21 the BGLs of this group were decreased then tended to increase at day 28. Meanwhile, the decreasing of BGLs were demonstrated on P2-, P3-, and P4-treated group at day 7, 14, 21 and 28 measurements. Effective decreasing can be seen in giving the ethanolic extract of *Lawsonia inermis* (Linn) leaves 400mg/dl dose.

To determine the significance of the difference between BGLs on day 28 in all groups were done by ANOVA test with average blood glucose levels in each group that can be seen in table 3

Table 3. The mean blood glucose on all groups on day 28

Group	The Mean Blood Glucose (mg/dl)	p*
K	107.33	0.000
P1	196.00	
P2	119.67	
P3	112.33	
P4	105.67	

*ANOVA test

In this study there was an association between the extract ethanol of *Lawsonia inermis* (Linn) leaves with the decreasing of blood glucose levels in the group of experimental rat ($p < 0.05$).

Lawsonia inermis (Linn) is native to tropical and subtropical plants such as Asia, South Africa, East Africa, North Africa, Northern Australia, which naturally grow also in tropical regions. Leaves are

small, greenish brown to the dull green, opposite in arrangement along the branches with short petioles, about 1.5-5cm long and 0.5cm wide to 2 cm. The main component of the leaf is a dye substance known as lawsone, 2-hydroxy-1,4-naphthoquinone-C₁₀H₆O₃ [16].

Based on phytochemical screening, the leaves of *Lawsonia inermis* (Linn) contained phenolic compounds (coumarins, flavonoids, naphthalene and gallic acid derivatives) glycosylation proteins. Flavonoids can reduce the activity of hydroxy radicals, superoxide anions and peroxide radicals by protecting lipid membranes against damaging oxidation reactions. Giving the antioxidants can increase the mass of pancreatic β cells and maintain insulin content in it [8].

Based on Choubey's et al. research [17], concluded that giving extract ethanol of *Lawsonia inermis* Linn leaves 500 mg/kg BW for 28 days effectively decreased blood glucose levels of rats. Research from Chikaraddy et al. [9], stated that the extract ethanol of *Lawsonia inermis* (Linn) leaves doses 150 mg/kgbw, 200 mg/ kg BW and 400 mg/kg BW for 3 weeks can decrease BGLs of albino rats. The decreasing blood glucose levels will decrease the risk of oxidative stress in cells and tissues [10].

Oxidative stress of rat's tissue in this study can be seen by measuring of rat's blood levels. In this study the ratio of blood MDA levels of rats in each group before and after giving extract ethanol of *Lawsonia inermis* (Linn) leaves can be seen in Table 4.

Table 4. The blood MDA level of rats on day 28

Group	MDA ($\mu\text{mol/gram}$)	P*
K	2.58	0.398
P1	2.74	
P2	2.43	
P3	2.31	
P4	2.40	

*Anova test

The increasing of MDA levels was found in alloxan-induced rats group at P1. The MDA level P2- was lower than K- and P1-treated groups. The P3-treated group showed the lowest level of MDA compared to the entire group. Normality test of MDA data of the present study showed $p = 0.513$, then one-way Anova analyzed result $p > 0.05$ that concluded there was no association of giving LLEE to MDA level in the present study. This result was different from the previous study that the composition of flavonoid from the herbs able to decrease the level of MDA rats which was induced oxidative stress [18,19].

MDA is one of the product compounds of lipid peroxidation product used as a marker of oxidative stress. In high oxidative stress, there was a significant increase in serum MDA levels. The damaging to cells or tissues due to increased MDA in oxidative stress conditions can be slowed by the administration of antioxidants. Anti-oxidants are vital substances that can protect the body from free radical attack by neutralizing or reducing its negative effects. There are several antioxidant groups of endogenous antioxidants and exogenous antioxidants.

The flavonoid compounds in the leaves can not act as natural exogenous antioxidants with the ability to eliminate the free radical compounds in the body to inhibit the reaction of free radical in the lipid oxidation [8]. Thus, the mosaic blood level of alloxan-induced rats can be suppressed.

In the present study, although there was decreasing of MDA levels in the treatment group that received LLEE (P2-P4), statistically no significance. This result could be affected by the duration of LLEE administration that should be more than 28 days. The assume was supported by the decreasing of the BGLs that have not reached to normal level on the 28th days of the treatment.

4. Conclusions

Ethanol extract of *Lawsonia inermis* (Linn) leaves at dose 400mg/kg BW able to decrease blood glucose level of alloxan-induced diabetic rats. MDA levels were not improved after LLEE administration.

Acknowledgments

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