Analysis of the *Moringa oleifera* Seed Oil Extract on Insulin Level in Alloxan-Induced Diabetic Rat (*Rattus norvegicus*)

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Abstract

Moringa oleifera is a plant that can be used and grown intensively in several Southeast Asian countries. This study aims to determine the effect of *Moringa oleifera* seed oil extract on the levels of the insulin and blood glucose levels of *Rattus norvegicus* induced by alloxan. This study used 30 female Wistar white rats induced by alloxan. Rats were divided into 6 groups. Groups 3 to 6 were injected 100, 200, 300, 400 mg/kg BW extract of *Moringa oleifera* seed oil, respectively. Group 1 neither had alloxan induction nor treatment while group 2 had been induced alloxan but no treatment. Data were analyzed using the Kruskal-Wallis test with a significance level of 0.05. The results showed that there was no effect of *Moringa oleifera* seed oil extract on insulin level (p value = 0.161). Conversely, there was an effect on blood glucose levels in alloxan-induced *Rattus norvegicus* (p value = 0.036).

Keywords: *Diabetes mellitus, Moringa oleifera, Rattus norvegicus.*

Introduction

Diabetes mellitus (DM) is a metabolic disorder of various etiologies characterized by chronic hyperglycemia due to absolute or relative deficiency in insulin secretion/insulin action or both. The number of DM patients was 171 million in 2000, which might increase to 360 million by 2030. As the number of people with DM has doubled worldwide, national and international health care budgets have increased[1,2].

Despite the many advances in therapy using expensive synthetic drugs, herbal medicines are growing. It is called not only effective with low side effects in clinical experience but also relatively spend the low cost. Herbal medicines or extracts are widely prescribed, even when the biologically active compounds are unknown[3,4]. Traditional medicine is generally obtained from natural ingredients[5]. One of the plants that has potential for traditional treatment is the *Moringa oleifera* plant.

Moringa oleifera is a plant that grows intensively in several Southeast Asian countries. This plant has been used for a long time since its compositions are good for traditional medicine. Almost all parts of the *Moringa* plant can be utilized especially the *Moringa oleifera* seeds. The use of *Moringa oleifera* seed oil extract can reduce blood glucose levels in hyperglycemic mice[6]. Szkudelski found that the main phytochemical element in *Moringa oleifera* seed extract is flavonoids. Bioflavonoids are known for their multi-directional biological activities including hypoglycemic effects[7].

Flavonoids are one of the most common groups of secondary metabolites found in plant tissues. Flavonoids act as an antioxidants by donating hydrogen atoms or chew metal in the form of glucosides which contain glucose side chains) or aglycones[1,2,3,4]. Extract of *Moringa oleifera* seed significantly can reduce alloxan-induced hyperglycemia because it contains not only elements like phytochemicals and micronutrient but also quercetin and kaemferol. Besides, kaemferol also have hypoglycemic activity[8].

Considering that *Moringa oleifera* plants are very potential but it is a lack of data showing the effect of *Moringa oleifera* seed oil can increase the blood insulin levels, this research aims to have further study. An extract of *Moringa oleifera* seed oil will be investigated at a dose of 100 mg/kg BW, 200 mg/kg BW, 300 mg/kg BW and 400 mg/kg BW to observe its effect on insulin levels in alloxan-induced diabetic rats. The effect of *Moringa oleifera* seed oil extract on the insulin and blood glucose levels of alloxan-induced *Rattus norvegicus* will be investigated.

Materials and Method

This study had a laboratory experimental research using a completely randomized design (CRD) in six treatment groups with five replications (6x5). This study used 30 female Wistar white rats with 30 treatment that consist of: Group 1 (K1): control animals that are only given food and drink without alloxan induction and treatment; Group 2 (K2): animals induced by alloxan without treatment; Group 3 (K3): alloxan-induced animals, and injected 100 mg/kg BW of *Moringa oleifera* seed oil extract orally; Group 4 (K4): alloxan-induced animals, and injected 200 mg/kg BW of *Moringa oleifera* seed oil extract orally; and Group 5 (K5): alloxan-induced animals, and injected 300 mg/kg BW of *Moringa oleifera* seed oil extract orally.

Alloxan monohydrate induction was given using intraperitoneal injection methods. Alloxan monohydrate powder was diluted using 0.95% NaCl saline water at a dose of 150 mg/Kg BW by measuring the body weight of experimental animals first. Injection was applied into the abdominal cavity through muscular tissue using a 1 mL syringe. *Moringa* seed oil injection was applied using the per oral method. The density of *Moringa* seed oil was created by having 1 ml of oil and measuring its mass through analytical balance. Injection volume is adjusted to the dose and weight of an experimental animal. Administration of seedling extracts was carried out for 14 days and then on the 15th day all the rats were euthanized, their blood was drawn for analyzing insulin levels using the ELISA method.

Results

Animal insulin levels after 14 days treatment were measured using INS ELISA Kit 96 and the wells from MyBiosource. The following table show the results:

Table 1. Levels of Rat Insulin (*Rattus norvegicus*).

Number	K1	K2	K3	K4	K5	K6
1	4,192	8,804	4,404	0,142	1,364	7,322
2	4,192	11,884	1,246	3,450	3,009	3,708
3	4,192	8,823	1,259	3,450	3,262	1,789
4	1,509	3,230	0,644	3,450	11,936	0,314
5	19,453	17,064	1,259	17,149	3,262	9,117
Mean	6,708	9,961	1,762	5,528	4,567	4,450

Table 2. Data Normality Test.

	Kolmogorov-Smirnov[a]			Shapiro-Wilk		
Insulin	Statistic	df	Sig.	Statistic	df	Sig.
	.282	30	.000	.814	30	.000

a. Lilliefors Significance Correction

Tabel 3. Kruskal Wallis Test

	Insulin
Chi-Square	7.923
df	5
Asymp. Sig.	.161

This study revealed that the DM condition arises caused by alloxan, alloxan injection can affect pancreatic cells so that type 2 DM occurs with blood glucose levels increased. Measurement of blood glucose levels was carried out after alloxan was induced as much as 150 mg/kg BW intraperitoneally in 5 treatment groups (K2-K6). Besides, glucose levels were measured routinely until reaching hyperglycemic levels ≥126 mg/dL. On the 15th day after the treatment of *Moringa oleifera* seed oil extract, blood glucose levels were measured again with the following results:

Table 4. Blood Glucose of Rats (*Rattus norvegicus*).

Number	K1	K2	K3	K4	K5	K6
1	86	116	147	435	130	111
2	79	126	132	121	118	141
3	132	189	425	112	443	153
4	108	600	118	336	124	122
5	88	346	152	118	168	128
Mean	98.60	275.40	194.80	224.40	196.60	131.00

Table 5. Data Normality Test.

	Kolmogorov-Smirnova			Shapiro-Wilk		
Blood Glucose	Statistic	df	Sig.	Statistic	df	Sig.
	.336	30	.000	.687	30	.000

a. Lilliefors Significance Correction

Table 6. Kruskal-Wallis Test.

	Blood Glucose
Chi-Square	11.938
df	5
Asymp. Sig.	.036
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

Discussion

Table 1 shows that the highest average insulin level is in group K2 or alloxan-induced animals without *Moringa oleifera* seed extract. Then, the lowest result was in the group K3 (alloxan-induced animals, and injected 100 mg/kg BW seed oil extract orally). The increase of hormone levels due to antioxidant levels in *Moringa oleifera*. *Moringa* seed oil regenerates β-pancreatic cells in pancreatic organs. Based on Chumark *et al*., phytochemical screening results from *Moringa oleifera* plant extracts showed the presence of secondary metabolites such as flavonoids, terpenoids, saponins, and tannins. Study has shown that terpenoids and flavonoids have hypoglycemic activity[9]. Anti-inflammatory and antioxidant effects reported from flavonoids can also play an important role in reducing insulin resistance[10,11,12,13,14].

Table 2 shows that Kolmogorov-Smirnov and Shapiro-Wilk test reveals data were not normally distributed on insulin levels (p value 0.000 < α 0.05). Then the data were statistically proceed using Kruskal-Wallis test as in Table 3. The Kruskal-Wallis test obtained a significance value of 0.161 > 0.05. Since the significance is greater than specified value then H_0 is accepted and H_a is rejected. It means that there is no difference in blood insulin levels on alloxan-induced mice.

Hypothesis called that administration of *Moringa oleifera* seed is possible to increase blood insulin levels. Ethanol extract of *Moringa oleifera* leaves has strong anti-diabetic activity because it not only decreases the lowers blood glucose levels but also increases insulin sensitivity and functional beta cells in diabetic mice[14]. There are some possible mechanisms for hypoglycemic action such as stimulation of pancreatic insulin secretion and improving tissue insulin resistance. Further research is needed to ascertain the right mechanism for the anti-diabetes effect of plant extracts and secondary metabolites involved.

The therapeutic use of *Moringa oleifera* leaves has been evaluated in diabetes since their capacity to reduce blood glucose concentration. It contains polyphenols such as quercetin-3-glycosides, routine, kaempferol and glycosides. Blood sugar decreased due to *Moringa oleifera* therapy can be observed on fasting blood glucose, oral glucose tolerance test and post prandial glucose in diabetic rats, in an average decrease of 25% or more. The antidiabetic activity of *Moringa oleifera* seed powder has been observed.

In mice, glucose and amelioration of lipid peroxide levels decreased. It reduced IL-6 level and immunoglobulin A compared with positive control of diabetes in both insulin-resistant and insulin-deficient bioassays. Meanwhile Sandanamudi *et al*. showed that *Moringa oleifera* contained soluble fiber which increased amelioration of glucose levels, lymphocyte proliferation and nitric oxide induced from macrophages[15]. Another study observed *Moringa oleifera* fortification in diabetes can reduce fasting blood glucose. It not only reduces the entry of glucose into the mitochondria and but also releases reactive oxygen species. Besides, it also promotes glycation end products (AGEs) which increase cell adhesion and inflammation in diabetic patients. Treatment with *Moringa oleifera* has shown that after histological examination of the pancreas of diabetic rats, significant damage is reversed in the histoarchitecture of the islets of Langerhans[16].

Having several studies carried out, it is revealed that the administration of *Moringa oleifera* seed oil extract can increase blood insulin levels in rats through the mechanism of pancreatic insulin secretion stimulation. It also improves insulin resistance in tissues by active compounds contained therein such as flavonoids, sterols, triterpenoids, alkaloids, saponins, and phenolics. Statistically, the rise of insulin hormones in treated mice still occurred although it was not significant. The increase of hormone levels almost reaches a significant value since the levels of antioxidants in *Moringa oleifera*. *Moringa oleifera* seed oil regenerates β-pancreatic cells in pancreatic organs. These cells produce insulin which is secreted when the blood sugar levels rise. In addition, glucose change to fat requires insulin which is produced by langerhans, a group of cells in the pancreas. High glucose levels with insulin increase cholesterol levels in the blood. Vitamin D is important for secreting insulin in the pancreas. This study shows that individuals with low vitamin D levels are very bad at handling blood sugar and a higher risk of having diabetes[17].

Insulin resistance is a condition showing a low potential for endogenous and exogenous insulin. An assessment of homeostatic model (HOMA) β cell function and insulin resistance (IR) was first described in 1985[18]. This technique is a method for assessing the function of β and IR cells from basal glucose and insulin or C-peptide concentrations. This model has been widely used since it was first published. Therefore, we present

here an overview of the model and its proper use and limitations in clinical science.

Insulin resistance is a disruption of insulin in metabolic response where blood glucose levels increase. It requires more levels of insulin to make blood sugar levels back to normal (normoglycemic). Insulin resistance occurs in the target cell receptors on skeletal muscle tissue and liver cells. Damaged receptors increase insulin secretion which is called hyperinsulinemia[19]. This study reveals that the highest blood glucose levels were in the K2 group which is called having positive control of DM rats (275.40 mg/dL). Meanwhile, the lowest glucose levels in the DM rats group were given *Moringa oleifera* seed oil extract (*Moringa oleifera*) dose 400 mg/kg BW (131 mg/dL). The Kolmogorov-Smirnov and Shapiro-Wilk tests determine whether the data distribution was normal or not. The results of Kruskal-Wallis test shows significance value of 0.036<0.05. since the the significance is smaller than specified then H_0 is rejected and H_a is accepted. It means that there is a differences in blood glucose levels on alloxan-induced mice.

Aloxan monohydrate will reach pancreatic tissue and attack β-pancreatic cells. The mechanism of aloxan monohydrate action cause oxidative stress on pancreatic tissue. It permanently damage the ß-pancreas cells. Damage to β-pancreas cells decrease insulin levels and increase blood sugar (hyperglycemia). The aloxan monohydrate is used to make animals experienced with blood sugar increased (hyperglycemia)[20]. Statistical analysis showed that there was an increase in blood sugar levels due to the addition of *Moringa oleifera*. *Moringa oleifera* seed oil. It make fluctuation in the control until the treatment on group 5 (K1-K5). The highest increase occurred in group 6 (K6). Blood sugar increased because of *Moringa oleifera* L. level. *Moringa oleifera* seed oil is no longer effective even though it contains antioxidants.

The therapeutic use of *Moringa oleifera* leaves has been evaluated in reducing blood glucose because they contain polyphenols such as quercetin-3-glycosides, routine, kaempferol and glycosides. The blood sugar decreased can be noticed in different tests: fasting blood glucose, oral glucose tolerance test, and postprandial glucose in diabetic rats with an average decrease of 25% or more[4,20]. The antidiabetic activity of *Moringa oleifera* seed powder has been observed in mouse models. It shows decreased glucose, amelioration of lipid peroxide levels, reduced levels of IL-6, and immunoglobulin which was compared between positive control of diabetes in both insulin-resistant and insulin-deficient bioassays.

Meanwhile Sandanamudi *et al*. showed that moringa oleifera contained soluble fiber which increased amelioration of glucose levels, lymphocyte proliferation and nitric oxide-induced from macrophages. In another study, fortification of *Moringa oleifera* in diabetes can cause fasting blood glucose decrease[15]. It reduces the entry of glucose into the mitochondria, reactive oxygen relief, and advance glycated end products (AGEs)[20]. Therefore, cell adhesion and inflammation increase in diabetic patients. Treatment with *Moringa oleifera* has shown that the pancreas of diabetic rats which is significantly damaged is reversed in histoarchitecture of islet cells after histological examination. Based on the explanation and some previous studies, it is called that there is no effect of *Moringa oleifera* seed oil extract in increasing rat blood insulin levels. It all carried out through stimulation of pancreatic insulin secretion and/or improvement of insulin resistance with flavonoids, sterols, triterpenoids, alkaloids, saponins, and phenolics.

Conclusion

In sum, *Moringa oleifera* seed oil extract can reduce blood glucose levels and can be used as a potential plant recommendation for traditional treatment of DM.

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Ethical Approval: This study was approved by the Animal Care and Use Committee, Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia.

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