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Original Article

ANTIDIABETIC ACTIVITY OF *NIGELLA SATIVA* L. SEED POWDER AND ITS COMBINATION WITH GLICLAZIDE IN ALLOXAN INDUCED DIABETIC MICE

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ABSTRACT

Objective: In Indonesia, *Nigella sativa* (NS) has been widely used for the treatment of diabetes mellitus. The aim of this study was to evaluate the antidiabetic activity of its seed powder and its combination with gliclazide.

Methods: NS was used in seed powder suspension form. At study's begin, Oral Glucose Tolerance Test (OGTT) was performed. And then the mice were induced with 60 mg/kg alloxan intravenously and were treated with 1300 mg/kg NS (NS1), 2000 mg/kg NS (NS2), 0,65 mg/kg gliclazide, and combination of NS1-gliclazide that administered orally for 3 weeks. NS1 was also administered daily to mice induced high-fat emulsion for 3 weeks.

Results: The results showed that in OGTT, NS1 and NS2 inhibit the elevation of plasma glucose level after administering glucose. In mice induced alloxan, plasma glucose level in both NS1 and NS2 was significantly lower than diabetic control and gliclazide group. And NS2 showed more significantly less damage in Langerhans than the other groups. The combination did not showed a better effect than the single use. In mice induced high-fat emulsion, NSI improved the sensitivity of insulin by increasing K_{ITT}.

Conclusion: The results suggest that NS has an antidiabetic activity by increasing insulin production and improving sensitivity of insulin. The combination NS with gliclazide was probably antagonism.

Keywords: Antidiabetic, Beta cell, Insulin sensitivity, *Nigella sativa* L, Seeds powder.

INTRODUCTION

Diabetes mellitus (DM) is one of metabolic disorders characterized by hiperglycemia or high plasma glucose level. It is also followed by abnormalities in metabolism of carbohydrate, fat, and protein. DM can cause micro-and macrovascular complications leading to blindness, renal disease, gangrene, and death[1]. The number of diabetes worldwide cases in 2000among adults ≥20 years of age was projected to be171 million. In 2000, diabetes case in Indonesia was 8.4 million and in 2030 isprojected to be 21.3 million [2]. Side effects due to the used of insulin and oral hypoglycemic agents lead to dissatisfaction [3]. Therefore, a lot of patients nowadays use natural products to treat DM[4,5]. NS commonly known in Indonesia as 'jinten hitam' or '*habatussauda*' is distributed in India, Turkey, Middle East, and Egypt. The seeds have been known by its contents, such as flavonoid, saponin, steroid/triterpenoid, quinon and alkaloid [6-7]. NS can inhibit absorption of glucose in gut[8],protect beta cell[9],and increase AMPK pathway in muscle and hepatic cell. There are lot of studies had been done regarding the antidiabetic activity ofNS extract and/or fixed oil. It is not known whether the active substance was more abundant in fixed oil or in extract form. To get all of the active substances, NS seed powder was used in this study. In Indonesia, the use of NS is usually combinated with medicines[10]. Therefore, this study also evaluate antidiabetic activity of combination NS seed powder with gliclazide.

MATERIALS AND METHODS

Drugs and plant material

Gliclazide (Servier, Indonesia), metformin (Hexpharm Jaya, Indonesia), insulin: Actrapid(Novo Nordisk,Indonesia) and NS seeds (Migasauda, Indonesia).

Preparation of seed powder

The seeds of NS were colected, washed, dried, and mashed. The powder was suspended in sodium-CMC 0.5%.

Animals

Healthy adult male Swiss Webster mice (20-35 g) were obtained from school of pharmacy, Institute of Technology Bandung. Mice were maintained on normal mouse chow and tap water *ad libitum*. Animals were acclimatized to laboratory condition for seven days. Experiments were performed according to laboratory standard in Institute of Technology Bandung.

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test was performed to assess the ability of NS to inhibit the elevated plasma glucose level after the administration of glucose. The mice were fasted overnight and then divided into 6 groups, each group consists of 5 mice. The groups were administered sodium-CMC 0.5% per oral (p. o.), gliclazide 0.65 mg/kg bw p. o., *Nigella sativa*1300 mg/kg bw p. o. (NS1), *Nigella sativa*2000 mg/kg bwp. o. (NS2), or combination of NS1 and gliclazide. Plasma glucose level was determined at 0 min and then the mice were administered by the test suspension. Glucose solution (2 g/kg) was administered 30 min after administered the test suspension. Blood was taken from the tail vein at 60, 90, 120, 150 min of suspension administration. Glucose levels were estimated using blood glucose test strips from Accu Chek and a glucometer from Accu Chek Active (Roche Diagnostics GmnH, Mannheim, Germany).

Induction of non-insulin-dependent diabetes mellitus (NIDDM)

NIDDM was induced in overnight fasted mice by a single intravenously injection of 60 mg/kg bw alloxan monohydrate (Sigma-Aldrich A7413). Seven days later, development of diabetes was confirmed by determining plasma glucose level from tail vein. Mice with blood glucose level more than 150 mg/dL were considered as diabetic. Glucose levels were estimated using blood glucose test strips from Accu Chek and a glucometer from Accu Chek Active (Roche Diagnostics GmnH, Mannheim, Germany).

Antidiabetic study

Animals were divided into 6 groups; normal control, diabetic control, diabetic treated with NS 1300 mg/kg (NS1), NS 2000 mg/kg (NS2), gliclazide 0,65 mg/kg, and NS1-gliclazide. Each group consists of 6 mice. Normal control and diabetic control were administered 0,5% sodium-CMC suspension. All of them were administered

dailyper oral for 3 weeks. Blood glucose was determined every 7 days. Glucose levels were determined from tail vein blood and used blood glucose test strips from Accu Chek and a glucometer from Accu Chek Active (Roche Diagnostics GmnH, Mannheim, Germany).

Histology

The whole pancreas from two mice in each group was removed after sacrificed and submerged in 10% formaline solution then immediately processed by the paraffin technique. Sections of 5 µm thickness were cut and stained using Gomori's method[11]with chrome alum Victoria blue-phloxin stain. The number of beta cell and alpha cell was analysed.

Assessing insulin sensitivity

Fifteen mice was induced by high-fat emulsion[12]for 14 days to decrease the insulin sensitivity. This mice divided into 3 groups; control diabetic, diabetic treated with NS 1300 mg/kg bw, and metformin 195 mg/kg bb. Each group consists of 5 mice. The suspension was administered daily per oral for 3 weeks. After the last suspension administering, insulin senstivity was determined by IVITT. Insulin (0.1 U/kg bw) was injected intravenously and plasma glucose level was measured at time points of 0, 2, 5, and 10 min after injection. Insulin sensitivity was estimated by glucose disapperance within 10 min that determined from average slope (K) curve. The K-value was determined by linear regression over time points that multiplied by 100 [13].

Statistical analysis

Data are reported as the mean ± SD and evaluated using t-test and paired sample t-test. The values were considered significant when p-value<0.05.

RESULTS

Glucose tolerance test

The effects of seed powder of NS and its combinations on OGTT was shown in Table 1. At minutes 90 after glucose administration, gliclazide inhibited the increase of plasma glucose level. NS1 and NS2 inhibited the elevated of plasma glucose too, and NS2 was better than gliclazide, although this inhibiting was not significant compared to control diabetic. At minutes 90 and 120, NS1 and NS2 could lower plasma glucose level but its not significantly different. NS1 and NS2 also normalized plasma glucose level within 2 hours. The combination did not show inhibiting effect of the elevated plasma glucose level and it could not normalize plasma glucose level within 2 hours. The plasma glucose level at minutes 120 and 150 in the combination group was significantly higher than control group. The AUC of the glyceamic response was also quantified to make sure the difference of glycaemic response of each group (Table 2). The AUC showed that gliclazide have the best effects and then was followed by NS2. NS2 was significantly different compared to control group.

Table 1: Change of plasma glucose in normal Swiss Webster mice in OGTT.

Group	Plasma glucose level (mg/dL)				
	0	60	90	120	150
Control	125.60	242.20	186.00	156.20	126.20
	±31.28	±56.95	±69.04	±47.98	±38.70
NS1	129.60	239.80	180.80	164.4	138.2
	±27.78	±39.54	±24.77	±24.94	±29.97
NS2	128.20	212.60	171.00	153.00	132.20
	±25.52	±32.18	±34.97	±31.72	±26.58
Gliclazide	126.60	227.00	133.20	118.40	111.4
	±20.31	±45.84	±19.52*	±16.18*	±14.79
NS1 + Gliclazide	124.00	243.80	213.40	192.40	166.40
	±29.73	±52.76	±41.77	±20.54*	±20.54*

Values are mean ± SD, n = 5, (*) significantly different from control group, p<0.05 using t-test. NS1 (*Nigella sativa* 1300 mg/kg) and NS2 (*Nigella sativa*2000 mg/kg).

Table 2: Area under the curve (delta plasma glucose level (mg/dL)*time (min)).

Group				
Control	**NS1**	**NS2**	**Gliclazide**	**NS1 + Gliclazide**
11013.2 ± 3756.39	10785.9 ± 3967.31	8988.97 ± 1420.98*	7791.40 ± 1926.08*	13250.80 ± 1743.14

Values are mean ± SD, n = 5, (*) significantly different from control group, p<0.05 using t-test. NS1 (*Nigella sativa* 1300 mg/kg) and NS2 (*Nigella sativa*2000 mg/kg).

***Nigella sativa* can reduce plasma glucose level and protect pancreatic cell in diabetic mice induced-alloxan**

Daily treatment with NS1 and NS2 for 3 weeks reduced plasma glucose level in diabetic mice induced-alloxan. Plasma glucose level in group NS1 and NS2 was significantly different compared to diabetic control and not significantly different compared to normal control. This result showed that NS1 and NS2 normalized the plasma glucose level reached the similar level to normal control (Figure 1). This reduction of plasma glucose level was better than gliclazide group. The combination group did not show a better reduction in plasma glucose level than the single use of NS1.

Pancreatic cells were stainned with Gomori to understand the mechanism behind its antidiabetic activity (Figure 2). Staining with Gomori can show Langerhans clearly and show the difference of beta and alpha cell. At the end of treatment, control group had pancreatic cell damage with reduction of beta cell. Among all groups, NS2 showed less damage of pancreatic cells and an increased number of beta cell. NS1 and NS2 inhibited the elevation of alpha cell. Gliclazide and NS1-gliclazide group increased number of alpha cell that significantly different from diabetic control.

***Nigella sativa* improve insulin sensitivity**

Intravenous insulin tolerance was tested to know the insulin sensitivity in mice. Insulin sensitivity was showed from K_{ITT} value. NS1 increased K_{ITT} that significantly different from diabetic control. The elevation of K_{ITT} showed improvement in insulin sensitivity.

DISCUSSION

In this study, antidiabetic activity of NS seed powder and its combination was evaluated in beta cell damage diabetic mice. This study was begun with oral glucose tolerance test as the preliminary test. This test was a model for pre-diabetic mice. In this test, NS1 and NS2 reduced plasma glucose level but not significantly different to control group. NS2 inhibited the elevation of plasma glucose level in first 30 minutes that showed NS2000 mg/kg bw could inhibit the absorption of glucose. Combination NS 1300 mg/kg bw and gliclazide could not reduce plasma glucose level to normal and the

plasma glucose level was significantly different from control and gliclazide (p<0,05). This showed that NS had antidiabetic activity by inhibiting the elevation of plasma glucose level whereas the combination of NSand gliclazide could not normalize plasma glucose level.

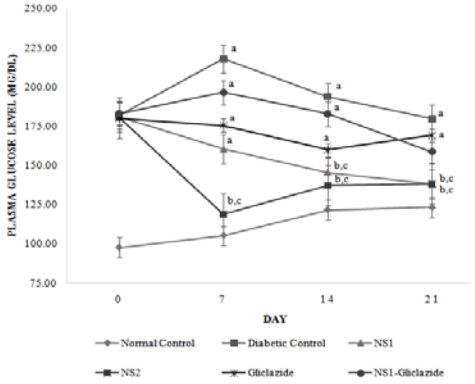

Fig. 1: Plasma Glucose Level in Mice Induced Alloxan Before and After Treatment. NS1 (*Nigella sativa* 1300 mg/kg) and NS2 (*Nigella sativa* 2000 mg/kg), n = 6, with (a) significantly different from normal control group using t-test, (b) significantly different from diabetic control using t-test, and (c) significantly different from initial plasma glucose level using paired sutdent's t-test, p< 0.05.

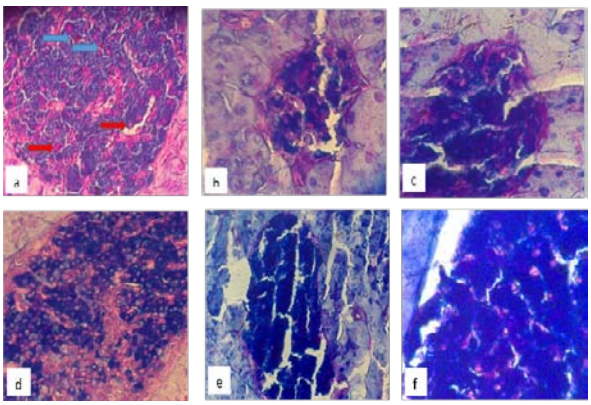

Fig. 2: Histopathological Studies of Pancreas at Day 21 After Treatment: a = normal group, b = control group, c = NS 1300 mg/kg bw, d = NS2000 mg/kg bw, e = Gliclazide gruop, and f = NS 1300 mg/kg bw + Gliclazide. Beta cell appeared pink (red arrow) and alpha cell appeared dark red (blue arrow) at the periphery of the islet. (X1000).

Table 3: Total number of B and A cell per islet pancreas after treatment.

Group	The Number of Cell per Islet	
	Beta cell	Alpha cell
Normal control	95 ± 7	25 ± 7
Diabetic control	8 ± 3[a]	4 ± 1[a]
NS1	8 ± 2[a]	2 ± 2[a]
NS2	28 ± 7[a,b]	4 ± 1[a]
Gliclazide	11 ± 3[a]	5 ± 1[a,b]
NS1 + Gliclazide	10 ± 4[a]	5 ± 3[a,b]

NS1 (*Nigella sativa* 1300 mg/kg) and NS2 (*Nigella sativa* 2000 mg/kg), n = 2, with (a) significantly different from normal control group using t-test and (b) significantly different from diabetic control using t-test, p< 0.05.

The study was continued by inducing alloxan. Alloxan is derivate urea which can produce superoxide radical and causes rapid destruction to beta cell [14,15]. Destruction of beta cell can decrease insulin secretion and lead to hyperglycaemia. This method was model for diabetes type 1. NS 1300 mg/kg bw and 2000 mg/kg bw reduced plasma glucose level significantly compared to diabetic control. This plasma glucose level was not significantly different from normal control that showed *Nigella sativa* could reduce plasma glucose level close to normal.

Table 4: Result of insulin sensitivity assay intravenous insulin tolerance test before and after treatment

Group	K_{ITT} ± SD	
	Before	After
Normal control	7.57 ± 0.50	6.63 ± 0.59
Diabetic control	4.20 ± 1.31[a]	3.03 ± 0.87[a]
NS1	4.12 ± 1.18[a]	4.71 ± 0.58[a,b]
Metformin	3.79 ± 1.04[a]	5.15 ± 1.68[a,b,c]

Value are mean ± SD, n = 5, NS1: *Nigella sativa* 1300 mg/kg. (a) Significantly different from normal control using t-test, (b) significantly different from diabetic control using t-test, and (c) significantly different from K_{ITT} before treatment using paired student's t-test, p< 0.05.

NS 1300 mg/kg bw reduced plasma glucose level by increasing the secretion of insulin from beta cell and inhibiting the alpha cell, while NS 2000 mg/kg bw incresed the density of beta cell. Gliclazide and combination group could not reduce plasma glucose level significantly compared to diabetic control. In gliclazide and combination group, beta cell was increased but not significantly different from diabetic control, otherwise alpha cell was increased too and significantly different from diabetic control. The elevation of alpha cell increased secretion of glucagon. It made the reduction of plasma glucose level in both of groups were not significant. From this method, the combination of NS and gliclazide still could not reduce plasma glucose level significantly compared to diabetic control. This showed that NS and gliclazide interacted and decreased the antidiabetic effect. Interaction between NS and gliclazide occurred consistently in both of an experimental model. This showed that there will be an interaction if NSand gliclazide were used at the same time.

In this study, antidiabetic activity was evaluated too in type 2 diabetic mice to know another mechanism of NS. Diabetes type 2 is caused by insulin resistance and/or relative insulin deficiency. Diabetes type 2 is the most common case of diabetes. Insulin resistance mice were induced by a high-fat-glucose emulsion. High-fat-glucose emulsion increase GLUT2 and α-glucosidase in small intestinal epithelium and decrease GLUT4 in skeletal muscle. NS significantly increased K_{ITT} value compared to diabetic control that means NScould improve insulin resistance. The possible mechanism maybe by decreasing GLUT2 and α-glucosidase in small intestinal epithelium or/and by increasing GLUT4 in skeletal muscle. Similar results showed that NSextract can increase GLUT4 present in muscle of diabetic mice [16].

This study showed that NShas antidiabetic activity by inhibiting the elevation of plasma glucose level, increasing insulin release, improving beta cell pancreas, inhibiting the increasing of alpha cell pancreas, and improving insulin sensitivity. Some of these mechanismsis similar to agonist glucagon-like-peptide-1 (GLP-1) or dipeptidyl-peptidase-IV (DPP-IV) inhibitor that can increase insulin secretion and inhibit glucagon [17,18]. Furthermore, NSis also similar to metformin that can improve insulin sensitivity.

CONCLUSION

NS 1300 mg/kg bw and 2000 mg/kg bw can reduce plasma glucose level. NS 1300 mg/kg bw can increase secretion of insulin from existing beta cell and inhibit the alpha cell. NS 2000 mg/kg bw can inhibit the absorption of glucose in small intestine, increase secretion of insulin and increase density of beta cell. Combination of gliclazide and NS can not reduce plasma glucose level significantly compared to diabetic control and showed interaction between them that can decrease the antidiabetic effect.

NS greatly inhibit absorption of glucose in gut, increase secretion of insulin, inhibit alpha cell, increase insulin sensitivity, and increase density of beta cell.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGEMENT

ABBREVIATIONS

DM – Diabetes Mellitus

NS – *Nigella sativa*

NS1 – *Nigella sativa* 1300 mg/kg bw

NS2 – *Nigella sativa* 2000 mg/kg bw

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