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The effects of dosage variation in black cumin seed oil (*Nigella sativa* L.) use on HbA1c levels and interleukin-17A expression in patients at risks of metabolic syndrome

Vitri Agustiarini[*1,2], Endang Darmawan[`2], Akrom[2]
[1] *Department of Pharmacy, Faculty of Mathematics and Natural Sciences, University of Sriwijaya, South Sumatera, Indonesia*
[2]*Postgraduate Program on Clinical Pharmacy, Universitas Ahmad Dahlan*

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ABSTRACT

Metabolic syndrome causes an imbalance of the immune system and increased levels of HbA1c and IL-17A expression. Black cumin seed oil (BCSO) is known to have antioxidant and immunomodulatory properties. This study reports the effects of dosage variation in BCSO (1.5 and 3 ml per day for 20 days) on HbA1c levels and IL-17A expression in patients at risk of metabolic syndrome at Jetis 1 Public Health Center in Bantul, Yogyakarta. It employed a crossover design in which a total of 66 patients at risk of metabolic syndrome were divided into two groups receiving a sequence of different treatments. Group 1 (N=33) received treatment A first, which was BCSO at a dose of 1.5 ml/day for 20 days. Then, after a washout period of 7 days, it received treatment B, 3 ml of BCSO per day for 20 days. Group 2 followed the same procedure only vice versa, treatment B, then A. The HbA1c levels were measured by the mean plasma glucose (MPG) method, while the IL-17A expression was detected by flow cytometry. The average HbA1c level and IL-17A expression of the treatment groups were statistically analyzed with 95% confidence level. In response to the treatment regime, the HbA1c level of group 1 was 7.34 ± 2.51% (a decrease), and that of group 2 was 7.72 ± 2.44% (an increase). The IL-17 expression in group 1 was 3.74 ± 3.52% (a decrease), and the one in group 2 was 4.07 ± 3.65% (an increase). The effects of administering 1.5 ml and 3 ml BCSO per day for 20 consecutive days on Hb1c level (p=0.17) and IL-17A expression (p=0.67) were not significantly different. Most patients experience a decrease in HbA1c levels and IL-17A expression after the treatment. Insignificant differences between the two groups mean that at the doses of 1.5 ml/day and 3 ml/day for 20 days, BCSO exhibits the same effects in patients at risk of metabolic syndrome registered at Jetis 1 Public Health Center in Bantul, Yogyakarta.

Keywords: metabolic syndrome, BCSO, HbA1c, IL-17A

***Corresponding author**:
Vitri Agustiarini
Department of Pharmacy, Faculty of Mathematics and Natural Sciences
University of Sriwijaya, South Sumatera, Indonesia
Email: vitriagustiarini@yahoo.com

INTRODUCTION

According to the International Diabetes Federation (2006), metabolic syndrome is suspected when a person has at least two of the following factors: abdominal obesity, low HDL cholesterol levels, high blood pressure, and high blood glucose. Metabolic syndrome raises the risk of bowel cancer and cardiovascular diseases because it thickens and stiffens the arterial wall through oxidative stress mechanisms (Stehouwer, 2008). Also, it is associated with 50% coronary heart complications, 30% kidney failure, 30% retinopathy, and 30% prerequisites of amputation (Soegondo, 2008). A systematic review study of some Brazilian populations in the age range of 19-64 years suggests that metabolic syndrome is prevalent, with high-density lipoprotein (HDL) (59.3%) and hypertension (52.5%) as its most frequent component (De Carvalho *et al*., 2013). Novak *et al*. (2013) report that 1,262 Sweden patients aged 50 years old, with the systolic blood pressure ≥140 mmHg and diastolic ≥90 mmHg, are at risk of metabolic syndrome. Moreover, based on the criteria issued by the National Cholesterol Education Programme (NCEP), metabolic syndrome is more prevalent in males (16%) than females (10%).

Metabolic syndrome is characterized by elevated oxidative stress and high production of, for instance, the hallmark of proinflammatory cytokines, which is interleukin-17A (Nady *et al*., 2009). The antioxidant effect can suppress reactive oxygen species (ROS) (Andrew *et al*., 2009; Dandona *et al*., 2005). Oxidative stress can inhibit glucose u

ptake in muscle and fat cells and reduce insulin secretion by pancreatic β-cells, resulting in cardiovascular disease (Furukawa *et al*., 2004). The metabolic syndrome caused by diabetes is related to glycated hemoglobin type A1c (HbA1c). In a human body, hemoglobin comprises HbA1, HbA2, and HbF (fetus). Hemoglobin A (HbA) accounts for 91-95% of the total hemoglobin count. HbA1c is one of the hemoglobin fractions (HbA) within the red cells to which glucose attaches enzymatically. In it, a binding process between glucose and HbA1, called glycation, occurs and produces glycated hemoglobin or HbA1c. In response to this mechanism, HbA1c concentration is controlled by blood glucose level and the life span of erythrocytes. The glycation of hemoglobin increases proportionally to the blood glucose level throughout 120 days (2-3 months), which is the average lifespan of the red blood cells (Smeltzer and Bare, 2002). It also means that if blood glucose level for the past 120 days is in the normal range, the HbA1c will be within the normal levels as well (Nathan *et al.*, 2008).

High oxidative stress can accelerate disease progression in patients with the risk factors of metabolic syndrome. Known interventions include antioxidant and immunomodulatory therapy. In pre-clinical and clinical trials, BCSO is proven to exhibit pharmacological effects as antidiabetic, antihyperlipidemic, and antihypertensive with the primary mechanism related to the impact of antioxidants and immunomodulators (Heshmati *et al.*, 2015).

Ansari *et al.* (2017) explain that oral administration of 2.5 mL/day of BCSO for 12 weeks can be used as adjunctive therapy in diabetic nephropathy treatment in India. Also, consuming BCSO at a dose of 5 ml/day for eight weeks can reduce systolic and diastolic blood pressures in healthy volunteers with no side effects (Hosseini *et al*., 2013). Randhawa and Al-Ghamdi (2002) state that the tolerated oral dose of black cumin seed powder in mice is 2 g/kg BW per day for five consecutive days, while in humans, the safe dosage is 2 g/kg BW per day for 28 days. Based on a systematic review of 19 articles, *Nigella sativa* L. reportedly has antihyperglycemic effects and causes lipid dysfunction with antioxidant mechanisms, namely insulin secretion, glucose absorption, gluconeogenesis, and gene expression (Heshmati *et al.*, 2015). After investigating 23 articles, Mohtashami *et al.* (2016) conclude that *Nigella sativa* L. can reduce blood pressure, fasting blood sugar, and levels of glycosylated hemoglobin (HbA1c). This paper presents a comparison between the effects of varying doses of BCSO, i.e., 1.5 mL/day and 3 mL/day, administered for 20 days on HbA1c levels in patients at risk of metabolic syndrome who received standard therapy.

RESEARCH METHOD

This research used a crossover design and has been reviewed and approved by the Research Ethics Committee of the Faculty of Medicine and Health of the University of Muhammadiyah Yogyakarta (UMY) in 2016, with a certificate number of research ethics eligibility: 279/EP-FKIK-UMY/VIII/2016. It involved patients at risk of metabolic syndrome at Jetis 1 Public Health Center in Bantul, Yogyakarta, from September to December 2016. After searching through the database, there were 401 patients with diabetes mellitus and 615 patients with hypertension, two risk factors of metabolic syndrome, visiting the center from January to October 2016.

There were 66 at-risk patients (13 male and 49 female) registered at this center who met the inclusion and exclusion criteria and signed an informed consent form. They came from various age groups, 20-70 years old, but most of them were 40-60 years old. The inclusion criteria were patients with (1) obesity (Body Mass Index ≥30 kg/m^2), (2) increased random blood glucose levels up to ≥100 mg/dl and receiving hypoglycemic drug therapy, (3) high blood pressure up to ≥130/85 mmHg or currently receiving hypoglycemic drug therapy, (4) elevated blood triglyceride levels to ≥150mg/dl) or receiving in immunomodulatory treatment, (5) increased LDL levels to ≥160mg/dL or currently participating in antidyslipidemic therapy, and (6) low HDL cholesterol levels (≤40 mg/dL in men and ≤50 mg/dl in women) (International Diabetes Federation, 2006) and (7) male or female patients aged 18-70 years who were willing to be the subject of the research and filled out an informed consent form. Meanwhile, the exclusion criteria were (1) non-cooperative patients during the study (patient dropouts), (2) pregnancy, (3) patients taking corticosteroids, (4) allergy to black cumin seed oil, (5) history of cancer, and (6) patients undergoing tuberculosis treatment.

This research is a clinical trial phase II, and since this phase requires 30-300 subjects (Rahmatini, 2010), it examined 30 patients with an additional 10% to anticipate any patient dropouts. The filtered 66 patients were randomly divided into two groups (n= 33 patients each). Group II was given a dose of 1.5 mL BCSO per day for 20 days, while group II received a dose of 3 mL BCSO/day for 20 days. Blood collection was carried out to examine the clinical conditions, namely blood glucose, cholesterol, and triglyceride, HDL, and HbA1c levels and interleukin-17A expression on Day 21. The trial included a washout period of 7 days to allow the drug in the first treatment to be washed out of the patient's system, preventing its effects on the second treatment. On Day 29, patients in group I was given BCSO capsules with a dose of 3 mL/day for 20 days, and group II received 1.5 mL BCSO per day for another 20 days. Then, on Day 49, the blood glucose, cholesterol and triglycerides, HDL, and HbA1c levels and interleukin-17A expression were measured.

HbA1c level measurement

HbA1c measurement consists of several methods, which were classified into three groups. First was separation by loads, including cation exchange chromatography (disposable microcolumn, high-performance liquid chromatography (HPLC)) and electrophoresis. HPLC can detect abnormal hemoglobin and have good reproducibility with a coefficient of variation (CV) of ≤ 1%. The second method was based on chemical analysis, namely colorimetry and spectrophotometry, while the third one relied on structural difference, consisting of affinity and immunoassay methods (Geistanger *et al*., 2008).

The HbA1c level was measured from mean plasma glucose (MPG). The procedure for examining plasma glucose was as follows: (i) 10 µL of blood plasma was inserted into a test tube, (ii) a reagent was prepared and mixed until homogenous with a 1:2 ratio (4.5 mL: 0.5 mL), (iii) 10 µl of blood plasma was mixed with 1 ml of reagent until homogenous, and (iv) the mixture was allowed to stand for 10 minutes, and then the patient's plasma blood sugar was measured using a Spectrophotometer 5010. The HbA1c level (in percent) was calculated by adding the patient's blood glucose with 77.3 and dividing it by 35.6.

Interleukin-17A examination

The interleukin-17A expression was examined by flow cytometry. A vacutainer tube containing anticoagulant and blood drawn from each patient was processed with the following procedure: (i) 50

µl of the specimen was collected using a pipette, (ii) added with 500 ml of lysis and fixative solution, (iii) mixed using a vortex mixer until homogenous, then incubated for 15 minutes at 20-25°C in a dark room, (iv) centrifuged at 2,000 rpm for 5 minutes. Then, (v) the pellet at the bottom of the centrifuge tube was collected, resuspended in 500 µL of permeabilization/wash buffer, and (vi) incubated for 15 minutes at 20-25°C in a dark room. After centrifuged, (vii) the pellet was collected and added with 5 µl of FITC anti-human IL-17A antibody reagent, (viii) mixed using in a vortex mixer until homogenous and then incubated for 15 minutes at 20-25°C in a dark room, (ix) added with 500 µl of permeabilization/wash buffer and centrifuged. Afterward, (x) the pellet (turbid particles) was collected, added with 400 µl of phosphate-buffered saline, and mixed until homogeneous. Finally, (xi) the interleukin-17A expression was analyzed using a FACS flow cytometer.

Data Analysis

The data obtained through laboratory analysis were processed statistically in SPSS software using the feature paired t-test with a 95% confidence level.

RESULTS AND DISCUSSION

This research was conducted in 2006 at Jetis 1 Community Health Center in Bantul, the Special Region of Yogyakarta.

Baseline characteristics

The baseline data (before treatment) showed that the clinical conditions of patients at risk of metabolic syndrome were mostly hyperglycemic (71.0% of the total research subject), hypertensive (59.7%), and with elevated triglyceride (54.8%), and HDL cholesterol levels (37.1%). The clinical baseline of the research subjects was summarized in Table I.

Table I. The description of the baseline condition of patients at risk of metabolic syndrome at Jetis I Community Health Center in Bantul, Yogyakarta in 2016

Clinical Status	The administered doses of BCSO				Total	
	1.5 mL/hari		3 mL/hari			
	N	%	N	%	N	%
Subject (N)	31	50.0	31	50.0	66	100
Obesity (BMI > 30 kg/m^2)	2	33.3	4	66.7	6	9.1
Hyperglycemia (RBG ≥ 80 mg/dl)	22	50.0	22	50.0	44	71.0
Hypertension						
SBP ≥ 140 mmHg	17	45.9	20	54.1	37	59.7
DBP ≥ 90 mmHg	4	40.0	6	60.0	10	16.1
Dyslipidemia						
Triglyceride ≥ 150 mg/dl	17	50.0	17	50.0	34	54.8
HDL ≤ 40 mg/dl	11	47.8	12	52.2	23	37.1

Notes: BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, RBG= random blood glucose, HDL=high-density lipoprotein.

Table I shows that the baseline conditions of patients (i.e., BMI, blood sugar, blood pressure, triglycerides, and HDL) before receiving 1.5 mL/day or 3 ml/day of BCSO during the first treatment were similar. The outcome of the study is expected to be solely the effect of BCSO administration.

The Clinical Examination Results of Patients at Risk of Metabolic Syndrome Receiving BCSO at the Doses of 1.5 mL/day and 3 mL/day

This study used a paired sample t-test in SPSS program. This test sought to compare the effect of oral administration of 1.5 mL/day and 3 ml/day of BCSO on the clinical condition of patients at risk of metabolic syndrome. No significant differences in the BMI, SBP, DBP, random blood sugar, triglycerides, cholesterol, and HDL levels were found between the two treatment groups ($p > 0.05$). The clinical examination results of patients at risk of metabolic syndrome are presented in Table II.

Table II. The mean levels of BMI, SBP, DBP, random blood glucose, triglyceride, cholesterol, and HDL of patients receiving BCSO at the doses of 1.5 ml/day and 3 mL/day for 20 days at Jetis 1 community health center in Bantul, Yogyakarta in 2016

Parameters	Mean ± SD by BCSO doses		*p*-value
	1.5 mL/day (n=62)	3 mL/day (n=62)	
BMI ± SD (Kg/m^2)	24.67 ± 4.16	24.80 ± 4.22	0.16
SBP ± SD (mmHg)	137.73 ± 17.65	136.92 ± 17.65	0.63
DBP ± SD (mmHg)	79.27 ± 13.96	79.95 ± 10.97	0.69
RGB ± SD (mg/dL)	184.18 ± 89.46	197.77 ± 87.14	0.17
TG ± SD (mg/dL)	173.42 ± 108.50	158.58 ± 97.87	0.05
Chol ± SD (mg/dL)	177.02 ± 46.08	180.63 ± 45.40	0.56
HDL ± SD (mg/dL)	46.00 ± 6.91	45.95 ± 6.61	0.91

Note: BMI=body mass index, SBP=systolic blood pressure, DBP=diastolic blood pressure, RBG= random blood glucose, TG=Triglyceride, Chol=Cholesterol, HDL=high-density lipoprotein, SD=standard deviation

The resultant clinical conditions were measured with a paired t-test. Table II shows that the levels of BMI, SBP, DBP, random blood sugar, triglyceride, cholesterol, and HDL between the two treatments (BCSO at the doses of 1.5 mL/day and 3 mL/day) were not significantly different ($p > 0.05$). Patients receiving BCSO at the dose of 1.5 mL/day had a blood pressure level of 137.73/79.27 mmHg, while the ones given 3 mL/day of BCSO had 136.92/79.95 mmHg. According to the International Diabetes Federation (2006), the target blood pressure of patients with metabolic syndrome is 135/85 mmHg. Najmi *et al.* (2012) assert that consuming 500 mg of black cumin seed powder per day for eight weeks can reduce blood pressure in patients with metabolic syndrome in India. Another study confirms this ability of black cumin seed extract at the dosage of 100 and 200 mg twice per day for eight weeks to lower blood pressure in patients with moderate hypertension in Iranian hospitals (Dehkordi *et al.*, 2008). After taking BCSO at a dose of 2x2.5 mL for eight weeks, healthy volunteers with blood pressures ranging from 110/60 to 140/90 mmHg report lowered blood pressure levels and no side effects (Fallah *et al.*, 2013). In another study, 6 out of 10 trials did not show a significant reduction in blood pressures after receiving black cumin seed products (Mohtashami *et al.*, 2016). When consumed for eight weeks, *Nigella sativa* L. extract in a capsule dosage form is known to significantly reduce the systolic blood pressure of patients with moderate hypertension but insignificantly lower the diastolic pressure. According to Jaarin *et al.* (2015), black cumin seed lowers blood pressure by lessening oxidative stress on cardiac and angiotensin-converting enzyme activity, intensifying cardiac heme oxygenase-1 activity, and preventing the release of plasma nitric oxide. Black cumin seeds contain thymoquinone, thymol, fatty acids, lipases, and tannins, all of which can reduce blood pressure through calcium channel inhibition and diuretic mechanism (Najmi *et al.*, 2012).

Aside from blood pressure, this study measured the random blood glucose level of patients belonging to two treatment groups. Patients receiving 1.5 ml and 3 ml of BCSO per day had random blood glucose levels of 184.18 mg/dL and 197.77 mg/dL, which were insignificantly different. The expected therapeutic target is ≤ 180 mg/dL (ADA, 2017). BCSO contains active compounds, such as thymoquinone, nigellone, and thymol, which play a role in lowering blood glucose levels. Also, the

presence of these compounds in adipose cells can stimulate the secretion of insulin by pancreatic β cells and is shown to leave no effect on the sensitivity of tissue to glucose (Andaloussi *et al*., 2008).

The HbA1c levels and interleukin-17A expression after receiving BCSO at the doses of 1.5 mL/day and 3 mL/day

The HbA1c levels and interleukin-17A expression obtained from the examination were analyzed using the paired t-test. This test compared the effects of oral administration of 1.5 mL/day and 3 ml/day of BCSO on HbA1c levels and interleukin-17A expression of patients at risk of metabolic syndrome. The results showed insignificant differences between the two treatments (p> 0.05). The examination results of HbA1c levels and interleukin-17A expression are presented in Table III.

Table III. The average HbA1c levels and interleukin-17A expression of patients at risk of metabolic syndrome who received BCSO at the doses of 1.5 mL/day and 3 mL/day for 20 days at Jetis 1 community health center in Bantul, Yogyakarta in 2016

Parameters	Mean ± SD by BCSO doses		p-values
	1.5 mL/day (n=62)	3 mL/day (n=62)	
HbA1c	7.34 ± 2.51	7.72 ± 2.44	0.17
Interleukin-17A	3.74 ± 3.52	4.07 ± 3.65	0.67

Based on Table III, the HbA1c levels and interleukin-17A expression of patients at risk of metabolic syndrome who received 1.5 ml BCSO per day did not significantly differ from the ones given 3 ml BCSO per day (p> 0.05). Patients consuming BCSO at the doses of 1.5 ml/day and 3 ml/day had HbA1c levels of 7.34±2.51 % and 7.72 ± 2.44%, respectively. As for the Interleukin-17A expression, the former had 3.74 ± 3.52 while the latter 4.07 ± 3.65. According to the American Diabetes Association (ADA, 2017), the target level of HbA1c is ≤7%. Bamosa *et al.* (2010) claim that when administered at a dose of 2-3 mg/day for eight weeks, BCSO, as adjunctive therapy for patients receiving oral anti-hypoglycemic drugs, can reduce the levels of fasting blood sugar, two-hour postprandial glucose, and glycosylated hemoglobin (HbA1c) by 1.52%. Hosseini *et al*. (2013) add that 2x2.5 mL of BCSO per day for three months, in addition to 500 mg metformin and 5 mg glyburide, can lower HbA1c levels in patients with type 2 diabetes mellitus by 3.4%.

Black cumin seeds contain thymoquinone that can increase insulin sensitivity and reduce HbA1c levels. Metabolic syndrome patients experience pancreatic β-cell dysfunction and insulin hypersecretion, resulting in insulin resistance. Damage to pancreatic β-cells can decrease insulin production and cause hyperglycemia (Anggraeni, 2007). Black cumin seeds lower HbA1c through the following mechanisms: reducing superoxide dismutase (SOD), malondialdehyde (MDA) and DNA damage, mitochondrial vacuolization, and fragmentation and maintaining the function of β-cells in, for instance, streptozotocin-induced diabetic mice. If β-cells can preserve their function, insulin can be produced. Black cumin seeds intensify insulin sensitivity, which is produced by attenuating the phosphorylation of acetyl-CoA carboxylase through the signaling pathway of mitogen-activated protein kinase and normalizing glucose transporter-4 in the muscle so that the HbA1c level can decrease (Islam, 2016).

BCSO contains antioxidants, chiefly thymoquinone, that has anti-inflammatory effects (Ahmad, 2013). Zareian *et al.* (2014) state that, based on a case study in Iran, the concentration of interleukin-17A is significantly higher in patients with type 2 diabetes mellitus than in healthy people and more elevated in women and men. The high expression of a pro-inflammatory cytokine, that is, interleukin-17A, is attributable to high glucose that is induced via the signaling pathways leading to lymphocyte activation and relevant to the pathogenesis of diabetic complications (Kumar *et al*., 2014). Interleukin-

17A induces pro-inflammatory cytokine, such as interleukin-6, which is shown to cause insulin resistance (Kern *et al*., 2001). According to Cohen *et al.* (2014), the normal interleukin-17A expression is 0.89 pg/mL, and black cumin through its anti-inflammatory properties inhibit the NF-KB and iNOS pathways and, consequently, prevent the damage to pancreatic β-cells.
In Keyhanmanesh *et al*. (2015), α-Hederin pretreated 48 guinea pigs that receive fluticasone and thymoquinone have significantly reduced levels of IL-4, IFN-γ, and IL-17.

This study has several limitations. It only compares two doses of BCSO, namely 1.5 ml/day and 3 ml/day. Also, several uncontrolled variables may affect the results of the study. For instance, there is a considerable distance between the location of the blood sample collection and the laboratory. The subjects carry out their daily activities without any rules or restrictions related to the research procedures. The uncontrolled factors that potentially affect the results of this study include the patient's diet, medication compliance, physical activity, and exercise, as well as sample distribution and storage. Besides, concomitant diseases related to the immune system (such as inflammation, autoimmune, tumors, and cancer), genetic factors, and psychological conditions receive less attention in this study.

The crossover design in this study can address problems arising from uncontrolled variables that are related to the subject, namely by using the research subject also as control. Subjects may develop adaptive responses to the research procedure, causing their conditions at the first and second stages (treatment) to differ. During the trial, the researcher realized that there were still deficiencies, mainly due to uncontrolled variables. For this reason, a further clinical study of BCSO requires a more significant number of subjects and more specific inclusion and exclusion criteria.

CONCLUSION

The administration of BCSO at the doses of 1.5 and 3 ml/day for 20 days has the same effect on patients at risk of metabolic syndrome at Jetis 1 Community Health Center in Bantul, Yogyakarta, that is, decreased HbA1c and interleukin-17A expression. No significant differences have been detected between the two doses ($p > 0.05$).

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REFERENCES

Ahmad, A., Husain A., Mujeeb M., Khan., Najmi A.K., Siddique N.A., Damanhouri Z.A., Anwar F., 2013, A Review On Therapeutic Potential Of Nigella Sativa: A Miracle Herb, *Asian Pacific Journal of Tropical Biomedicine*, 3(5): 337-352.

American Diabetes Association., 2017, Standards of Medical Care in Diabetes: Promoting Health and Reducing Disparities in Populations, *Diabetes Care*, 40 (1): S6-S10.

Andaloussi, AB., Martinean, LC., Spor D., Vuong T., Leduc, C., Joly E., 2008, Antidiabetic Activity Of Nigella Sativa Seed Extract In Cultured Pancreatic-B Cells, Skeletal Muscle Cells And Adipocytes. *Pharmaceutical Biology Journals*, 46(1-2): 96-104.

Andrew, S.P., Van Belle, T., Wurster, A.L., Suto A., Michaud, M., Zhang, D., Grusby, M.J., Herrath, M.V, 2009, Interleukin-21 Is Required For The Development Of Type I Diabetes In NOD Mice, *Diabetes*, 58(5):1144-1155.

Anggraeni, D., 2007, Mewaspadai Adanya Sindrom Metabolic. (Online). (Retrieved from http://labcito.co.id. on December 24, 2016).

Ansari, Z.M., Mohammad, N., Rahat, A.K., Shahzad, F.H, 2017, Protective Role Of Nigella Sativa In Diabetic Nephropathy: A Randomized Clinical Trial, *Saudi Journal of Kidney Diseases and Transplantation*, 28(1):9-14.

Bamosa, A.O., Kaatabi, H., Lebdaa, F.M., Elq A.M., Al-Sultanb A., 2010, Effect Of Nigella Sativa Seeds On The Glycemic Control Of Patients With Type 2 Diabetes Mellitus, *Indian journal of physiology and pharmacology*, 54(4):344-54.

Cohen, Joseph M., 2014, Top 27 Scientific Health Benefits of the Panacea Black Cumin Seed (*Nigella sativa*), Retrieved on September 24, 14:27 WIB

De Carvalho, V.F., Bressan, J., Babio, N., Salas-Salvado, J., 2013, Prevalence Of Metabolic Syndrom In Brazilian Adults: A Systematic Review, *BMC Public Health*, 13:1198.

Dandona, P., Aljada, A., Chaudhuri, A., Mohanty, P., Garg, R., 2005, Metabolic Syndrome: A Comprehensive Perspective Based On Interaction Between Obesity And Diabetes, *Circulation,* 111(11):1448-1454.

Dehkordi, F.R, and A.F. Kamkhah., 2008, Antihypertensive Effect Of Nigella Sativa L. Seed Extract In Patients With Mild Hypertension, *Fundam Clin Pharmacol*, 22(4):447-452.

Fallah H.H., Amini M., Mohtashami R., Gramarchehre M. E., 2013, Blood Pressure Lowering Effect Of Niglle Sativa L. Seed Oil In Healthy Volunteers: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial, *Phytother Res*, 27(12): 1849-1853.

Furukawa, S., Fujita, T., Shimabukuro, M., Iwaki, M., Yamada, Y., Nakajima, Y., Nakayama, O., Makishima, M., Matsuda, M., Shimomura, I., 2004, Increased Oxidative Stress In Obesity And Its Impact On Metabolic Syndrome, *The Journal of Clinical Investigation*, 114(12):1752-1761.

Geistanger, A, Arends S, Berding C., 2008, Statistical Methods For Monitoring The Relationship Between The IFCC Reference Measurement Procedure For Hemoglobin A1c And The Designated Comparison Method In The United States, Japan, Sweden. *Clinical Chemistry*, 54(8):1379-85.

Heshmati, J., Namazi, N., 2015, Effects of black seed (Nigella sativa) on metabolic parameters in diabetes mellitus: a systematic review, *Complementary Therapies Medicine*, 23(2):275-282.

Hosseini, M.S., Mirkarimi, S.A., Amini, M., Mohtashami, R., Kianbakht, S., Fallah, Hosseini H., 2013, Effects Of Nigella Sativa L. Seed In Type II Diabetic Patients: A Randomized, Double-Blind, Placebo-Controlled Clinical Trial, *Journal of Medicinal Plants*, 12(47): 93-99.

International Diabetes Federation., 2006,The IDF Consensus Worldwide Defenition of the Metabolic Syndrome, (online) (www.idf.org) 25 September 2017: 14:27, Indonesia.

Islam, Muhammad, T., 2016, Biological activities and therapeutic promises of Nigella sativa L., International journal of pharma sciences and scientific research, Departement of pharmacy Southern University Bangladesh, Bangladesh, 2(6): 242-243.

Jaarin, K., Foong, W.D., Yeoh, M.H., Kamarul, Z.Y.N., Qodriyah, H.M.S, Azman, A., Zuhair, J.S.F, Juliana, A.H., Kamisah, Y., 2015, Mechanisms Of The Antihypertensive Effects Of Nigella Sativa Oil In L-Name- Induce Hypertensive Rats, *Clinics*: 70 (11):751-757.

Kern, PA, Ranganathan S, Li C, Wood L, Ranganathan G., 2001, Adipose Tissue Tumor Necrosis Factor And Interleukin-6 Expression In Human Obesity And Insulin Resistance. *American Journal of Physiology-Endocrinology and Metabolism*, 280 (5): 745-751.

Keyhanmanesh, R., Saadat, S., Mohammadi, M., Shahbazfar, A.A., Fallahi, M., 2015, The Protective Effect of α-Hederin, the Active Constituent of Nigella sativa, on Lung Inflammation and Blood Cytokines in Ovalbumin Sensitized Guinea Pigs, 29(11):1761-1767.

Kumar, N.P., George, P.J., Kumaran, P., Dolla, C.K., Nutman, T.B., Babu, S., 2014, Diminished Systemic And Antigen-Specific Type 1, Type 17, And Other Proinflammatory Cytokines In Diabetic And Prediabetic Individuals With Latent Mycobacterium Tuberculosis Infection, The *Journal of Infectious Diseases*, 210(10):1670-1678.

Mohtashami, A., Mohammad, H.E., 2016, Effects Of *Nigella Sativa* Supplementation On Blood Parameters And Anthropometric Indices In Adults: A Systematic Review On Clinical Trials, *Journal of Research in Medical Sciences*, 7(04): 213-219.

Nady, S., Hoover, J.I., Shata., Mohamed, T., 2009, Interleukin-12 Is the Optimum Cytokine To Expand Human Th17 Cells In Vitro, *Clinical and Vaccine Immunology*: 16(6): 798-805.

Najmi, A, Nasiruddin, M., Khan, R.A., Haque, S.F., 2012, Therapeutic Effect Of NS (*Nigella Sativa* L.) In Patients Of Poor Glycemic Control, *Asian Journal of Pharmaceutical and Clinical Research*, 5:224-228.

Nathan, D.M., Kuenen, J., Borg, R., 2008, A1C-Derived Average Glucose Study Group. Translating the A1C Assay into Estimated Average Glucose Values. *Diabetes Care*, 31: 1473-1478.

Novak, M., Bjorck, L., Welin, C., Manhem, K.., Rosengren, A., 2013, Gender Differences In The Prevalence Of Metabolic Syndrome In 50-Year-Old Swedish Men And Women With Hypertension Born In 1953, *Journal of Human Hypertension*, 27(1): 56-61.

Rahmatini., 2010, Evaluasi Khasiat Dan Keamanan Obat (Uji Klinik), *Majalah Kedokteran Andalas*, 1(34) : 31-38.

Randhawa, M.A., Al-Ghamdi, M.S., 2002, A Review Of The Pharmaco-Therapeutic Effects Of Nigella Sativa, *Pakistan Journal of Medical Research*, 41 (2): 77-83.

Smeltzer, suzanne C., Barebrenda G., 2002, *Buku Ajar Keperawatan Medikal Bedah Brunner dan Suddarth* (Ed.8, Vol.1,2) EGC: Jakarta.

Soegondo, 2008. *Hidup Secara Mandiri dengan Diabetes Melitus, Kencing Manis, Sakit Gula*, FK UI, Jakarta.

Stehouwer, C. D., Henry, R. M., Ferreira, I., 2008, Arterial Stiffness In Diabetes And The Metabolic Syndrome: A Pathway To Cardiovascular Disease, *Diabetologia*, 51(4):527-539.

Zareian, P., Dizgah, I.M., 2014, Serum Interleukin 17 in Type 2 Diabetes Mellitus, *Journal of Archives in Military Medicine*, 2(4):e24689.