



## THE EFFECT OF A COMBINATION OF BLACK SEED (*NIGELLA SATIVA LINN.*) AND GLIBENCLAMIDE ON BLOOD GLUCOSE LEVELS

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### ABSTRACT

**Background:** Diabetes Mellitus was considered one of the most alarming pandemics of the twenty-first century before the advent of the COVID-19 pandemic. Glibenclamide (glyburide) is an anti-DM drug of the sulfonylurea class. Several studies have examined one of the medicinal plants, black seed (*nigella sativa linn.*) as a complementary for DM control. **Objective:** This study determines the effect of the combination of black seed (*nigella sativa linn.*) and Glibenclamide on reducing blood glucose levels. **Methods:** This research design was an experiment using a pretest-posttest design with control group design. This study used 24 mice (*mus musculus*) which were divided into four groups, namely a negative control group that was only given aquadest, a positive control group that was given alloxan as an induction of hyperglycemia and anti-DM drug glibenclamide, treatment group 1 was given alloxan and Black Seed, and treatment group 2 was given alloxan and a combination of black seed and glibenclamide. Data were analyzed using the t-test with  $\alpha = 0.05$ . **Results:** Blood glucose levels in the black seed and glibenclamide combination treatment group decreased significantly ( $p=0,00$ ). **Conclusion:** the combination of black seed (*nigella sativa linn.*) and Glibenclamide is effectively lowers in lowering glucose levels.

**Keywords:** effect, *Nigella sativa*, Glibenclamide, blood glucose levels

### INTRODUCTION

There are 175 million people out of 382 million people with Diabetes Mellitus (DM) in the world have not been diagnosed, so they have not carried out proper management and are at risk of progressively developing into complications. DM sufferers in the world will increase to 592 million people by 2035. The International Diabetes Mellitus Federation Atlas states that 415 million adults in the world suffer from DM and it is predicted that by 2040 DM sufferers will increase to 642 million people.<sup>1</sup> DM was considered one of the most alarming pandemics of the twenty-first century before the advent of the COVID-19 pandemic.<sup>2</sup>

Some anti-DM drugs respond to reduced blood glucose levels, but their safety and effectiveness require further study.<sup>3</sup> The use of anti-DM drugs usually requires long-term use with considerable side effects such as liver disorders, abdominal pain and kidney injury.<sup>4</sup> Some anti-DM drugs do not always produce good results and cause serious side effects such as weight gain, hypoglycemia, gastrointestinal problems, bone microarchitecture disorders, vitamin B12 biochemical deficiencies, and urinary tract infections.<sup>5</sup> To reduce the side effects of anti-DM drugs tablets are used with a mixture of anti-DM drugs glibenclamide and metformin.<sup>6,7</sup>

Glibenclamide (glyburide) is an anti-DM drug of the sulfonylurea class, which causes insulin secretion by binding to the SUR1 regulatory subunit of the ATP-sensitive potassium channel of pancreatic beta cells.<sup>8</sup> Glibenclamide is significantly metabolized by the liver and excreted by the kidneys. Glibenclamide has a long duration of action that leads to an increased risk of prolonged hypoglycemia. Glibenclamide has serious cardiovascular effects, including effects on ischemic preconditions and blunting of ST elevation in acute coronary syndrome.<sup>9,10</sup>

Several studies have examined one of the medicinal plants, black seed (*nigella sativa linn.*) as a complementary treatment in the for DM control. Black seed has been shown to significantly lower glucose levels oral glucose tolerance test, fasting plasma glucose, insulin resistance, increased serum fasting insulin, and hemoglobin A1c (HbA1c) levels.<sup>11</sup> The action of insulin on muscle cells and fat cells can be increased by black seed, resulting in an increase in basal glucose uptake. In particular, the hypoglycemic effect of black seed is also due to the content of Thymoquinone which acts as a powerful antioxidant for the pancreas as an insulin producer.<sup>12</sup> Black seed plays a role in increased sensitivity to insulin, inhibition of intestinal amylase, decreased electrogenic adsorption of glucose in the intestine,



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triggering the AMP-activated adenosine monophosphate (AMPK) protein kinase pathway and amplification of IV muscle glucose transporters as a result of increased phosphorylation of acetyl-CoA carboxylase.<sup>13</sup>

People with diabetes are at high risk for taking many drugs (polypharmacy). This is done to overcome other DM comorbidities such as dyslipidemia, hypertension, depression, and others plus anti-DM drugs regularly.<sup>14,15</sup> Black seed is recommended as an adjunct therapy to manage DM10. Blood glucose levels are expected to decrease further in the administration of a combination of anti-DM drugs and black seed.<sup>16</sup> This study aims to study the effect of the combination of black seed (*nigella sativa linn.*) and Glibenclamide on reducing blood glucose levels.

## METHOD

The design of this study was an experiment using a pretest-posttest design with control group design. This study used 24 mice (*Mus musculus*) which were divided into four groups, namely a negative control group that was only given aquadest, a positive control group that was given alloxan as an inducer of hyperglycemia and the anti-DM drug glibenclamide (0.65 mg/ KgBB)/ day), treatment group 1 was given alloxan and black seed (0.011 ml/ 20 grBB), and treatment group 2 was given aloxan combined with glibenclamide (0.65 mg / kg bb / day) and Black Seed (0.011 ml / 20 grBB). The inclusion criteria of mice used in this study were 2-3 months old with a body weight of 20-25 g, healthy, actively moving, male, not disabled and not used for other studies. The criteria for exclusion of mice are sick or dead mice, the blood glucose level of mice after alloxan-induced is less than 200 mg/ dl.

All test animals were adapted for seven days to lower the stress levels of test animals under laboratory conditions. The animal cage is sufficiently ventilated and lighted, subsequently the cage is fed with sawdust. Test animals are satisfied for 10-12 hours. After that, test animals in the positive control groups and 2 treatment groups were injected intraperitoneally alloxan 120 mg / kg body weight to increase glucose levels. On the 3rd day after alloxan induction, blood glucose levels were measured in all mice as glucose level data before treatment.

Measurement of blood glucose levels in mice is done by holding the tail of the mouse using the right hand. Let its front legs grip the wire of the cage cover, and then clamp the nape of the mouse with the thumb and forefinger of the left hand. After that, the mouse's tail clasp from the right hand is transferred to the left hand, and then pulled slightly so that the stomach stiffens. The tail of the mouse is cut (0.2 cm) from the tip of the tail using sterile scissors. The blood that comes out is dripped on the measuring strip, then the strip is inserted into the Autocheck® glucose level measuring device.

The black seed (*nigella sativa linn.*) used in this study has been identified by the Medical Laboratory of Universitas Nahdlatul Ulama Surabaya. Habatussauda extract is made by pounding 1000 grams of Habatussauda in a dry and clean state with a grinder. After that Habatussauda was soaked using 96% ethanol solvent as much as 5000 mL, then shaken using a mixer for 2-3 hours, then let stand for 24 hours. After that, filtration is carried out producing filtrate for further processing in the Rotary Evaporator. When in the Rotary Evaporator, 96% ethanol solvent is vacuumed, then distilled to become liquid. Distilled liquid is collected. If all 96% ethanol solvent has evaporated, black seed extract will be obtained. The dose of black seed given to mice in all treatment groups was 0.0117 ml / 20 grams of body weight per day. After being given Habbatussaida, the combination treatment group 2 was also given Glibenclamide (0.65mg/kg body weight) daily. Some were administered orally using a syringe to each rat for 14 days. After 14 days, blood glucose levels were measured in all mice as glucose level data after treatment.

Statistical analysis was conducted to examine differences in blood glucose level data before and after treatment in 2 treatment groups. The data is normally distributed, so statistical analysis uses the t-test. The difference test is considered meaningful if  $p < 0.05$ .

## RESULTS

This study used a study sample of 24 male white mice (*Mus musculus*), each group consisted of 6 mice and each group had one mouse as a reserve. Adaptation was carried out for 7 days, then 20 mice were made hyperglycemic by inducing alloxan intraperitoneally into the body of male mice on the first day 1 time and waiting 3 days later to get a



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hyperglycemic condition. After that, the blood glucose levels of mice are measured using a glucometer. Blood samples of mice to measure their glucose levels are taken from the tail of the mice. If the blood glucose level of mice after alloxane reaches  $> 200$  mg / dl, it can be ascertained that mice have hyperglycemia and can be given treatment. Treatment is given for 14 days.

Blood glucose in the black seed treatment group decreased significantly after being given black seed ( $p = 0.000$ ). Blood glucose in the Habatussauda and Glibenclamide treatment group experienced a significant decrease after being given Glibenclamide and black seed ( $p = 0.000$ ). The decrease in glucose levels in the combination treatment group of Glibenclamide and Habatussauda ( $130 \pm 1.14$ ) was significantly greater ( $p = 0.02$ ) than the positive control group given only Glibenclamide ( $72 \pm 0.14$ ) and the group given only Habatussauda ( $63 \pm 0.22$ ).

**Table 1.** Glucose Levels in the Research Group

| Group                          | Glucose Level Before Treatment (mg/dl) | Glucose Levels After Treatment (mg/dl) | Decrease in glucose levels | P value |
|--------------------------------|--|--|----------------------------|---------|
| Negative Control               | $214 \pm 0.65$                         | $198 \pm 0.87$                         | $146 \pm 0.26$             |         |
| Positive Control               | $214 \pm 1.06$                         | $142 \pm 1.20$                         | $74 \pm 0.18$              | 0.000   |
| Habatussauda                   | $229 \pm 2.78$                         | $159 \pm 1.56$                         | $65 \pm 0.24$              |         |
| Habatussauda and glibenclamide | $239 \pm 3.78$                         | $99 \pm 1.64$                          | $131 \pm 1.14$             |         |

## DISCUSSION

This study showed that the combination of Glibenclamide with Black Seed (*Nigella sativa*) effectively caused a decrease in blood glucose levels until normal glucose levels. This study is in line with the research of Ali, et al (2021) and Moustafa (2019) who have examined the combination of other anti-DM drugs, namely metformin with black seed in reducing glucose levels. The combination can lower blood glucose levels to normal blood glucose levels in mice. This is different from the black seed treatment group in this study which can also lower blood glucose levels, but not up to normal levels of mouse blood glucose. The study also found that the use of Glibenclamide in combination with black habatussauda lowered glucose levels significantly, compared to the decrease in glucose levels that

occurred with the administration of only glibenclamide.<sup>17,18</sup>

This study proves that black seed has a hypoglycemic effect by increasing the action of insulin on muscle cells and fat cells, so that basal glucose uptake can be higher. In particular, the hypoglycemic effect of black seed is produced by Thymoquinone (TQ). TQ is a major component in Black Seed (almost 50%). It is a powerful antioxidant that can reduce the oxidative effects of the pancreas.<sup>19</sup> TQ has an antidiabetic role through reducing free radicals (ROS) thereby defending pancreatic  $\beta$  cells from injury.<sup>20</sup> The results of this study are also in line with Hoda, et al (2023) research which found anti-hyperglycemic postprandial effects on Black Seed by inhibiting carbohydrate absorption and increasing insulin secretion.<sup>21</sup>

The hypoglycemic effect of black seed from this study is in line with Solemaini et al (2022) research which found increased levels of antioxidant factors (NRF2 and FGF21) in diabetic conditions, then significantly reduced after treatment with black seed. This effect occurs because black seed causes a decrease in oxidative stress and expression levels of transcription factors that stimulate insulin genes (MafA and PDX1 genes), so that they are close to normal conditions.<sup>22</sup>

The role of black seed (*nigella sativa linn.*) in the complementary medicine of DM includes reduction of glucose levels, increased insulin levels, increased cell metabolism and increased expression of glucose metabolism-related genes, as well as decreased early and late complications of DM.<sup>23</sup> The antidiabetic mechanisms of black seed can be stimulated through changes in oxidative status, through increased endogenous antioxidants and free radical reduction, as well as reduction of inflammation, and improvement of lipid profiles.<sup>24</sup>

This study's result align the research of Basile, et al (2022) who explained that Glibenclamide can increase the ability of beta cells from pancreatic islets of Langerhans, thereby increasing insulin secretion. In addition, Glibenclamide can affect glucose transport proteins, resulting in an increase in the sensitivity of pancreatic beta cells to blood glucose levels.<sup>25</sup> The results of this study are also in line with Leisegang's (2021) research which found other synergistic antidiabetic activities from the anti-DM drugs Metformin and black seed. The combination of



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Metformin with Thymoquinone from Black Seed given to people with DM, resulted in reduced levels of HbA1c and blood glucose.<sup>26</sup> Thymoquinone enhances the antidiabetic effect of Metformin by preventing complications of DM due to hyperglycemia and oxidative damage. Such antidiabetic effects also increase the activity of Glut-2 and insulin receptors in hepatocytes.<sup>27</sup>

### CONCLUSION

The combination of black seed (*nigella sativa* linn.) and glibenclamide is effective in lowering glucose levels. The effectiveness is recommended to be clarified in future studies to ascertain the mechanism that occurs in the use of a combination of black seed (*nigella sativa* linn.) and glibenclamide, effective doses and the use of research subjects in patients with DM.

### ETHICAL APPROVAL

This research has passed the ethical test at Nahdlatul Ulama University Surabaya with no 328/EC/KEPK/UNUSA/2023.

### CONFLICTS OF INTEREST

There is no conflict of interest.

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### AUTHOR CONTRIBUTIONS

All authors have contributed to this research process, including conception and design, analysis and interpretation of the data, drafting of the article, critical revision of the article for important intellectual content, final approval of the article, collection and assembly of data.

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