INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by the increasing of blood glucose level due to the reduction of secretion and/or activity of insulin. World Health Organisation predicted that DM will become the seventh leading cause of death in the world by 2030 [1]. Treatment of type 2 DM often needs the use of combination therapy, including oral antihyperglycemics and insulin to obtain glycemic goals, because uncontrolled blood glucose levels lead to microvascular complications (neuropathy, retinopathy, and nephropathy) as well as macrovascular complications (cardiovascular risks) [2].

Development and progression of diabetes and its complication related to the increased oxidative stress, with the glucose oxidation as the main source. Reactive ketohydroxyl radicals and superoxide anion radicals are the products of glucose oxidation, which furthermore produce the extremely hydroxyl radicals as well as reactive peroxynitrite radicals [3]. Reactive oxygen species (ROS) are also produced by oxidative phosphorylation, nicotinamide adenine dinucleotide phosphate oxidase (NADPH), H2O2.

Antioxidant defenses, such as Superoxide dismutase (SOD), Glutathione Peroxidase (GSH-Px) and Catalase, were depleted by ROS, lead to the oxidative damage of cells and tissues [4]. Clinical complications of DM due to the hyperglycemic-induced organ damage can be prevented by external antioxidant supplies [5].

Black cumin (Nigella sativa L) seed enhanced the activities of SOD, GSH-Px and reduced the lipid peroxidation. It contains thymoquinone, which plays a role in antioxidant activity by scavenging the ROS and prevention of the tissue damage. Black cumin seed extract was reported to increase the regeneration of pancreas beta cells, increase the serum insulin level as well as reduce serum glucose level on streptozotocin-induced diabetic rats.

It also reported that phenolic compound of methanolic extracts of black cumin shoots and roots have strong free radicals scavenger activity [6-9]. This study aimed to evaluate the antidiabetic, antioxidant and pancreas regeneration activity of N. sativa L seed extract (NSE) in alloxan-induced diabetic rats.

MATERIALS AND METHODS

Materials

N. sativa seeds were obtained from Karanganyar, Central of Java, Indonesia and authenticated by Faculty of Pharmacy, Gajah Mada University, Yogjakarta, Indonesia.

Ethanol, alloxan, normal saline, CMC, Na, EDTA, glibenclamide, a protease inhibitor, reagents for SOD, GSH-Px and Malondialdehyde (MDA) assay, diethylenetriamine-Penta acetic acid, nitroblue tetrazolium (NBT), sodium carbonate, dNan-bathocuproine-di sulphonic acid salt, CuCl2, glutathione, glutathione reductase, NADPH, H2O2.

Preparation of extract

The air dried powered of N. sativa L seeds (500 g) was extracted in ethanol by maceration method for 72h. Ethanol extract was evaporated under pressure to obtain the dry extract.

Animal

The ethical clearance of the experiment have been approved by Medical Health Research Ethics Committee (MHREC) Ref.: KE/FK/64/EC.

Wistar albino rats (150-200 g), 16 w old, were maintained in room temperature, given standard pellet diet and water ad libitum during the experiment period. The extract dose of 125 and 250 mg/kg and a standard drug (glibenclamide 0.1 mg/kg) were given orally.
Antihyperglycemic activity test

The rats were adapted for 5 d and blood glucose levels were measured (T0). Five days after intraperitoneal injection of alloxan (150 mg/kg), the glucose levels were measured to establish the diabetic condition. The rats with glucose levels above 200 mg/dl were used for the experiment. NSE dose of 125 and 250 mg/kg as well as glibenclamide were orally administered for 5 d. Measurement of glucose levels was performed on day 12th, 19th, 26th and 33rd after administration of the extract (T1, T2, T3, and T5, respectively) [10].

Blood glucose levels were determined by standard enzymatic procedures with GOD-PAP reagent and the absorbances were read by UV-Vis spectrophotometer at λ 505 nm.

Antioxidant assays

Standard SOD measurement method with slight modifications was used to measure the superoxide anion radicals scavenging capacity [11]. Liver supernatant (0.06 ml) was reacted with the mixture of 2.70 ml Sodium carbonate buffer (50 mM) containing 0.1 mM EDTA (pH 10), 0.06 ml xanthine (10 mM), 0.03 ml BSA 0.5%, and 0.03 ml [11]. Liver supernatant (0.06 ml) was reacted with the mixture of glutathione reductase enzyme (2.4 units). After the mixture was incubated for 10 min at 37 °C, 200 ml of 1.5 mM NADPH was added, and the absorbance of assay mixture was measured after 30 min at 560 nm. PBS containing 11.5 g/l KCl was used as a control.

Antioxidant activity

Effect of daily administration of NSE for 4 w on antioxidant activity was showed on table 2. Significantly increasing of SOD and GPx activity, as well as decreasing of MDA indicated the potent antioxidant activity of NSE. In both of dose, test groups of ethanol extract of black cumin seed anti hyperglikemi indicate activity, as evidenced by increased levels

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood glucose level (mg/dl)</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>T4</th>
<th>T5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>77.78±1.7*</td>
<td>77.67±1.6*</td>
<td>77.84±2.1*</td>
<td>78.07±1.3*</td>
<td>78.28±1.3*</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>21.66±7.4*</td>
<td>21.53±7.8*</td>
<td>21.79±7.8*</td>
<td>21.32±7.3*</td>
<td>21.36±7.4*</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>21.54±7.3*</td>
<td>19.57±6.6*</td>
<td>16.75±5.5*</td>
<td>124.43±6.5*</td>
<td>104.26±1.3*</td>
<td></td>
</tr>
<tr>
<td>NSE 125 mg/kg</td>
<td>207.55±3.6*</td>
<td>156.85±2.0*</td>
<td>157.37±1.7*</td>
<td>142.16±1.5*</td>
<td>130.99±2.0*</td>
<td></td>
</tr>
<tr>
<td>NSE 250 mg/kg</td>
<td>209.4±2.0*</td>
<td>191.5±2.0*</td>
<td>136.86±1.9*</td>
<td>121.47±1.5*</td>
<td>107.91±1.3*</td>
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</tr>
</tbody>
</table>

N = 5; Values were expressed as mean±SD; a: significantly different to diabetic control (P<0.05); b: significantly different to Glibenclamide as positive control (P<0.05)

<table>
<thead>
<tr>
<th>Group</th>
<th>Antioxidant activity</th>
<th>SOD (%)</th>
<th>GPx (U/mg)</th>
<th>MDA (nmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>81.09±4.38a</td>
<td>72.65±0.73a</td>
<td>2.69±0.15a</td>
<td></td>
</tr>
<tr>
<td>Diabetic control</td>
<td>17.82±3.50</td>
<td>9.14±0.36</td>
<td>9.15±0.52</td>
<td></td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>65.82±5.0a</td>
<td>58.56±0.41a</td>
<td>3.36±0.14a</td>
<td></td>
</tr>
<tr>
<td>NSE 125 mg/kg</td>
<td>38.43±6.29a</td>
<td>46.27±0.54ab</td>
<td>6.85±0.14ab</td>
<td></td>
</tr>
<tr>
<td>NSE 250 mg/kg</td>
<td>59.22±4.68a</td>
<td>51.09±0.74ab</td>
<td>4.39±0.24ab</td>
<td></td>
</tr>
</tbody>
</table>

N = 5; Values were expressed as mean±SD; a: significantly different to diabetic control (P<0.05); b: significantly different to Glibenclamide as positive control (P<0.05)
of SOD and GPx serata, decreased levels of MDA. Anti hyperglycemic activity of the extract dose, indicating the potential of the metabolites contained in the ethanol extract of black cumin seeds.

The phenolic compound of methanolic extract of *N. sativa* shoot and root was reported to show strong free radicals scavenging activity [9]. Some secondary metabolites in plants that can decrease blood glucose levels are flavonoids, quercetin, quinolizidine, anthocyanins, catechins and flavones and coumarin [16]. It was reported that tannins, flavonoids, and phenolic glycosides are natural antioxidants that play a role in pancreatic β cells protection from free radicals [17].

**Pancreas regeneration activity**

Profiles of H&E histopathology of normal rat showed the photograph average diameter β islets of Langerhans of the pancreas, the normal group showed normal or healthy picture of the condition of the islets of Langerhans, which is supported by IHC picture islet cells of the Langerhans in which the population of the islet cells that dominate the islets of Langerhans (fig. 1 and 2).

**DISCUSSION**

Significantly increasing of SOD and GPx activity, as well as decreasing of MDA indicated the potent antioxidant activity of NSE. In both of dose, test groups of ethanol extract of black cumin seed antihyperglycemic indicate activity, as evidenced by increased levels of SOD and GPx serata, decreased levels of MDA. Antihyperglycemic activity of the extract dose, indicating the potential of the metabolites contained in the ethanol extract of black cumin seeds.

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It was reported that tannins, flavonoids, and phenolic glycosides are natural antioxidants that play a role in pancreatic β cells protection from free radicals [17]. Increased levels of SOD and GPx and the decreased levels of MDA after administration of glibenclamide as well as both doses of NSE, indicated the influence of the enhanced adaptive defense mechanisms against free radicals and protection of the pancreas from oxidative stress [15].

Histopathology profiles of normal rat showed that the β islets cells were distributed in all the part of Langerhans of the pancreas, with the normal size. The diabetic group profiles indicated the reduced amount and size of the β islets cells. Administration of glibenclamide and both doses of NSE increased the amount and size of β islets cells. This results indicated the increasing of insulin production in the islet cells in pancreatic β, so they lowered blood glucose levels.

The IHC images of the positive control group and the NSE doses of 250 mg/kg bw (fig. 2) showed the positive reaction appearance Ag and Ab in the pancreatic β islet cell populations that dominate the islets of Langerhans. Immunohistochemistry profile of NSE dose of 250 mg/kg clearly illustrated the populations of islet β cells of pancreatic Langerhans in comparison to that’s of NSE dose of 125 mg/kg. This indicated the dose-effect correlation.

Results of the analysis of multiple comparisons of ethanol extract of black cumin seed compared with glibenclamide result that there was no significant difference in the level of 95%. These data indicated that NSE had the potential effect to regenerate or repair the damaged of islet cells where insulin production in the islets of Langerhans. It was also supported by data from blood glucose levels, the data increased levels of antioxidant enzymes SOD, GPx and data decreased levels of MDA in rat liver.
CONCLUSION

*N. sativa* seeds extract dose of 125 and 250 mg/kg showed antihyperglycemic effect, enhanced antioxidant activity, as well as pancreas regeneration from organ damage on an alloxan-induced diabetic rat. Further studies are needed to investigate and elucidate the mechanism of action of active compounds of NSE.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

Declared None

REFERENCES