The Antihyperglycemic Activity of Ethanol Extract of Trigonella foenum-graecum L. and Its Effect on the GLUT-2 Expression of Streptozotocin-Nicotinamide-Induced Rats

Jena Hayu Widyasti, Gunawan Pamudji Widodo, Rina Herowati

The Faculty of Pharmacy, Setia Budi University, Jl. Letjen Sutoyo, Mojokerto, Surakarta, Indonesia

Submitted: 11-11-2017
Revised: 8-12-2017
Accepted: 13-02-2018

*Corresponding author
Jena Hayu Widyasti
Email: Jenahayu89@gmail.com

ABSTRACT
Insulin is an important factor in glucose transport. Glucose transporter (GLUT-2) in pancreatic β cell membranes is responsible for glucose transport that will stimulate insulin secretion. The purpose of this research was to investigate the antihyperglycemic activities of extract of Trigonella foenum-graecum L. (fenugreek) seed and the effects on GLUT-2 protein expression in pancreatic β cells on Streptozotocin-Nicotinamide-induced (STZ-NA) rats. This research utilized five groups of male Wistar rats. Group I was normal control group, while Groups II-V were induced with STZ-NA. Group II was negative control group (CMC Na 1%); group II I was positive control group (Glibenclamide); group IV and V was examined group (fenugreek seed ethanol extract of 100mg/kg bw and 200 mg/kg bw). The test compounds were orally administered for 14 days. Measurement of blood glucose levels and observation of the expression of GLUT-2 protein in pancreatic β cells in rats were conducted by immunohistochemical method. The research results revealed that the examined group dose of 200 mg/kg bw of fenugreek seed extract appeared to show blood glucose level decline that was proportionate to that of the positive control group. The increased density of GLUT-2 protein in pancreatic β cells significantly occurred in the group injected with 200 mg/kg bw compared to the group injected with 100 mg/kg bw. Hence, 200 mg/kg bw of fenugreek seed ethanol extract was able to decrease blood glucose levels and increase the GLUT-2 protein expression in pancreatic β cells in STZ-NA-induced rats with the effectiveness that was comparable to the positive control group.

Keywords: Trigonella foenum-graecum L, Streptozotocin-Nicotinamide, Glucose transporter-2

INTRODUCTION
Diabetes Mellitus (DM) is a metabolic disease characterized by hyperglycemia resulted from the suppression of insulin production or the increase of insulin resistance. Long-term chronic hyperglycemia and other metabolic abnormalities of DM may cause tissue and organ damages, as well as dysfunctions of eyes, kidneys, nerves, and vascular system (Cavallerano, 2009).

A number of recent researches reveal about the administration of natural components as antidiabetic drugs (Qi, et al. 2010). Fenugreek seed contains 4-hydroxyisoleucine (4HI), trigonelline, gallocatechin as well as flavonoid, carotenoid, coumarine, protein, saponin and lipid (Basch E., et al, 2003). Previously, fenugreek seed has been proven to have hypoglycemic and hypocholesterolemic effects on animals with DM type I and type II (Gupta, et al., 2001) induced with alloxan (Raju, et al., 2001). The content of 4-hydroxyisoleucine amino acid in fenugreek seed improves insulin affinity on receptor which causes insulin sensitivity to increase (Broca, et al., 2004).

On the basis of the previous studies about antidiabetic activities of fenugreek seed,
The Antihyperglycemic Activity of Ethanol Extract

The researchers are interested to examine the effect of the administration of fenugreek seed ethanol 96% extract on the activities of GLUT-2 protein, particularly in pancreatic β cells of rats with DM type I induced by STZ. This study aims to investigate the antihyperglycemic activities of fenugreek seed ethanol extract and the more effective dosage of the extract to be used as antihyperglycemic and its effects on the GLUT-2 expression of Streptozotocin-Nicotinamide-induced rats.

MATERIALS AND METHODS

The materials used in this research included fenugreek seed, ethanol 96%, Streptozotocin (Sigma Aldrich, MO, USA), Nicotinamide, Glibenclamide, xylene solvent, HCl 2N, Dragendorff reagent, lead (II) acetate, chloroform, acetic acid anhydride, diluted sulfuric acid, chloric acid, ethanol 90%, ethanol 70%, NaCl 10%, and FeCl₃, PBS (Phosphate Buffer Saline) formalin, Harris haematoxylin, DAB (Diaminobenzidine), and anti-GLUT-2 antibody.

Experimental induction of diabetes

Diabetes induction was carried out by intraperitoneal injection of STZ and NA. STZ was dissolved in 0.1M citrate buffer pH 4.5 (Kulkarni, et al., 2012). NA was dissolved in saline solution. The experimental animals were induced with the combination of STZ at the dosage of 50mg/kg rat’s bw and NA was applied at the dosage of 10mg/kg once, so that it caused DM within 5 days after induction. NA was administered 15 minutes before the of STZ.

Experimental design

In this research, group I was the normal control group (only given pellet feed and water), group II was the negative control group (administered with CMC Na 1% + STZ-NA solution), group III was the positive control group (administered with 0.45mg/kg bw of Glibenclamide + STZ-NA), group IV and V were extract groups, was administered with 100mg/kg rat’s bw of fenugreek seed extract dose of 100 mg/kg bw + STZ-NA (group IV), and 200mg/kg bw + STZ-NA (group V). Administration of the test compounds were conducted for 14 days with the duration of per oral administration, once a day. The measurement of fasting blood glucose levels was carried out in day 12 (T₂) and day 19 (T₃) (Figure 1).

Immunohistochemistry

The procedure of Immunohistochemistry (IHC) was conducted in three stages, namely (1) preparation of pancreatic β tissue sample slide; (2) optimization of the dilution and operating time of anti-GLUT-2 antibody; and (3) Immunohistochemistry (IHC) on sample, photomicroscopy and density semi-quantitative of GLUT-2 protein expression.

RESULTS AND DISCUSSIONS

The effect of fenugreek seed extract on serum glucose level in STZ-NA-induced rats

The measurement results of rats’ blood glucose levels after the administration of test preparation within 14 days indicated that positive control group (Glibenclamide) appeared to have the highest average percentage, which means that positive control group performed highest activities in reducing blood glucose level. The significant decrease of blood glucose levels then occurred to rats after the induction of fenugreek seed ethanol extract at the dosage of 200 mg/kg bw, followed by the induction at the dosage of 100 mg/kg bw. The average percentage of the decrease of rats’ blood glucose levels after they were induced with test preparation (hypoglicemic activities) (Table I).

The percentage of blood glucose level decrease in day 12 and day 19 was analyzed using one-way ANOVA statistical analysis. The results indicated significance = 0.000 (p<0.05), which means that there was a significant difference among treatment groups. The results of the percentage of blood glucose level decrease in day 12 and day 19 indicated that there was not any significant difference between normal negative control group, both did not experience significant decrease of blood glucose levels. There was a significant difference among positive control group and normal and negative control groups, but there was not any significant difference between positive control group and extract group induced with fenugreek seed extract at the dosage of 200mg/kg bw.
The result of immunohistochemical observation on GLUT-2 in rats’ pancreatic β cells

The examination of GLUT-2 protein was conducted by calculating semi-quantitatively brown the color density in rats’ pancreatic β cells which had been colored immunohistochemically using anti-GLUT-2 antibody. The pictures of immunohistochemical observation (Figure 2). The result of GLUT-2 coloration which was done using immunohistochemistry on pancreatic β cell was indicated with brown color on the area of cytoplasm cell. The brown color was resulted from GLUT-2 protein in pancreatic β cells which secreted insulin, β cells in pancreatic Langerhans insula. This was in line with a study conducted by Xiong, et al., (2011) that was coloring GLUT-2 protein in pancreatic islets immunohistochemically.

Pancreatic β cells secreted insulin as a response to high blood glucose levels. GLUT-2 performed as transporter which brought glucose in, and therefore an increase in the glucose concentration in Langerhans insula occurred, causing ATP production to increase and then Potassium ion increased and caused Ca²⁺ depolarization which then led pancreatic β cells to secrete insulin (Henquin, et al., 2003).

The result of observation on GLUT-2 protein was carried out by calculating semi-quantitatively the brown color density of rats’ pancreatic β cells which had been colored using immunohistochemical method with anti-GLUT-2 antibody. The measurement result of GLUT-2 protein semi-quantitatively among treatment groups (Figure 3).

In this study, expression of GLUT-2 protein was stated in the percentage of brown color density. Expression of GLUT-2 protein in pancreatic β cells decreased as a result of STZ-NA induction which caused toxicity in pancreatic β cells, and therefore led to the damage and decrease of insulin secretion and the increase of blood glucose levels. This result (Figure 3) in which the lowest percentage of brown color density of GLUT-2 protein in negative control group was 32.31%.

Table I. The average percentage of the decrease of rats’ blood glucose levels

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>The average percentage of blood glucose level decrease in day12</th>
<th>The average percentage of blood glucose level day 19</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>5.33±2.43</td>
<td>5.52±1.53</td>
</tr>
<tr>
<td>Negative control group</td>
<td>2.36±1.72</td>
<td>4.90±1.36</td>
</tr>
<tr>
<td>Positive control group</td>
<td>20.72±3.25</td>
<td>56.41±2.69</td>
</tr>
<tr>
<td>Extract group (100mg/kg BW)</td>
<td>11.80±1.91</td>
<td>34.58±2.42</td>
</tr>
<tr>
<td>Extract group (200mg/kg BW)</td>
<td>20.63±0.38</td>
<td>54.03±2.39</td>
</tr>
</tbody>
</table>

![Figure 1. The results of blood glucose level measurement after test preparation induction](image-url)

![Table I. The average percentage of the decrease of rats’ blood glucose levels](image-url)
On the other hand, the percentage of brown color density of GLUT-2 protein in normal control group was the highest of all and even considered 100%, since it was not induced STZ-NA, and therefore it did not experience the damage of pancreatic β cells.

The percentage of GLUT-2 protein density of pancreatic β cells in positive control group and both of extract groups (dosage of 100 and 200 mg/kg bw) were lower than that in normal control group. This was related to the duration of therapy. Therapy with fenugreek...
seed extract on GLUT-2 protein density was not comparable to GLUT-2 protein density in normal control group since the duration of therapy was only 14 days and STZ-NA induction was not given, and therefore, pancreatic β cells condition stayed normal. Positive control group and extract group dosage of 200 mg/kg bw indicated proportional percentage of brown color density of GLUT-2 protein, 67.67% and 65.85%. This revealed that both control groups had proportional GLUT-2 protein expressions which were able to produce insulin in rats’ pancreatic β cells. The administration of fenugreek seed extract could increase 4-HI (Hydroxyisoleucin) amino acid which had been verified to play important role in insulinotropic action to improve insulin secretion and reduce blood glucose levels in rats with DM type II (Henquin, et al, 2003). This is presented in Figure 17 that there was an increase of GLUT-2 protein density in pancreatic β cells of rats induced with STZ-NA (positive control group, extract at the dosage of 100mg/kg bw and extract at the dosage of 200 mg/kg bw) compared to negative control group.

A research conducted by Ferrer et al. (2011) compared the expression of GLUT-2 in human with DM and without DM. The expression of GLUT-2 in pancreatic islets in human with DM type II was lower than that in human without DM type II. A research carried out by Arya et al. (1995) revealed that Pseudovaria macrophylla extract in rats with DM induced with STZ and NA could reduce blood glucose levels indicated in the increase of expression of GLUT-2 in pancreatic β cells. The secretion of insulin from pancreatic β cells was a complex process involving integration and interaction of various external and internal stimuli as a response to the change of blood glucose levels (Henquin, et al, 2003). Molecularly, the mechanism of glucose inducing insulin secretion occurred in some stages: glucose level among pancreatic β cells increased; and then the glucose entered pancreatic β cells through diffusion which was facilitated by GLUT-2 protein. Intracellular glucose in metabolism produced ATP, causing the increase of ATP/ADP ratio, the high level of intracellular blood glucose levels caused depolarization of cell membranes and induced the closing of K⁺ATP channel on cell surface, followed by the opening of cell–surface voltage dependent Calcium channels (VDCC), Ca²⁺ influx into β cells, addition of free cytosolic Calcium which triggered insulin secretion, and insulin molecules entering blood circulation were bond to receptor. The bond of insulin and its receptor required glucose transporter to enter muscular cell and adipose tissue, and efficient glucose intake, so that blood glucose levels could be reduced (Henquin, et al, 2003).

CONCLUSIONS

On the basis of the research results, it can be concluded that the fenugreek seed ethanol extract is able to reduce blood glucose levels and increase the excretion of GLUT-2 protein in pancreatic β cells.

REFERENCES


