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ABSTRACT

Aims: Antidiabetic effect of Inulin from Dioscorea esculenta tubers in streptozotocin-induced diabetic rats was investigated.

Methods: Antidiabetic effect was assessed based on HbA1C level, creatinine level, urea level, and microbiota composition in fecal. Level of HbA1C was determined using spectrophotometry. Level of creatinine and urea in blood serum was determined using enzymatic assay. The colony number of Bifidobacterium sp and Lactobacillus sp was evaluated using the total plate count method.

Result: After 14 days of treatment, there was a significant difference between control and inulin-treated groups in the level of HbA1C, creatinine, and urea. The colony numbers of Bifidobacterium sp and Lactobacillus were higher in inulin-treated groups than those of control group (p<0.05).

Conclusion: Inulin from Dioscorea esculenta tuber can be used as antidiabetic agent.

INTRODUCTION

Diabetes mellitus is the most progressive metabolic disease associated with a constant increase in blood glucose level and impaired kidney function. Constant increased sugar levels may result in increased oxidative stress and production of glico-oxidased products. Reactive oxidative species are
involved in pancreatic β cell dysfunction, apoptotic pathways, impaired insulin production, and insulin resistance. Some studies suggest that type 1 diabetes mellitus is associated with changes in intestinal microbiota composition. These changes occur along with the progression of diabetes leading to unfavorable intestinal microenvironment for certain types of bacteria. Zhang et al., (2013) showed that there was a decrease in the colony number of Bifidobacterium sp and Lactobacillus sp in patients with diabetic mellitus. The dysbiosis condition can be one of the cause in progression of diabetic nephropathy.

Selective modulations of intestinal microbiota by prebiotics have been suggested to decrease inflammation and metabolic dysfunction in rats. Inulin is one of the prebiotics used to modulate intestinal microbiota. Inulin can be isolated from tuber plants such as Dioscorea esculenta. Dioscorea esculenta is one type of tubers belonging to Dioscoreaceae family which is widely grown in Indonesia. Previous studies showed that the Dioscorea esculenta tuber has anti-inflammatory, antioxidant and cytotoxicity effects on breast cancer cells. Another study showed that Dioscorea esculenta has the highest inulin content (14.77% of dry weight) among the other Dioscorea family.

Inulin fermentation has been shown to produce acetic acid of 137±75 mmol, propionic acid of 11±9 mmol and, butyric acid of 20±17 mmol. The products modulates levels of some intestinal hormones involved in glucose and energy homeostasis, including glucagon-like peptide (GLP) -1 and reduces blood glucose levels. Inulin at a dose of 10 g/day has been preferred as a type of prebiotics due its tolerance and increase the number of Bifidobacterium.

The purpose of this study was to investigate the effect of antidiabetic effect of inulin from Dioscorea esculenta based on HbA1C, creatinine and urea level as well as the colony number of Bifidobacterium sp and Lactobacillus sp.

METHODS

All the research procedures have been approved by of Bioethical commission, Faculty of Medicine, Universitas Islam Sultan Agung (registration number: reg58/II/2018).

Authentication of Dioscorea esculenta

Dioscorea esculenta whole plant was obtained from the area of Tugel Mountain, Purwokerto and was authenticated in the plant taxonomy laboratory, Faculty of Mathematics and Natural Sciences, Universitas Negeri Semarang.

Preparation of inulin powder from Dioscorea esculenta

Dioscorea esculenta powder was obtained by using cabinet drying methods. Fresh Dioscorea esculenta was washed and sliced, then added with aquades with ratio of 1: 3, then blend evenly. The result was filtered using a filter cloth and stored in a refrigerator at temperature of -20°C for 24 hours. After 24 hours the frozen blend was thawed at room temperature and centrifuged at 4500 rpm for 10 minutes. The resulting precipitate was transferred into a petri dish and dried at 37°C for 24 hours. After drying, the precipitate was sieved into powder and then filtered.

Concentration of Inulin Test

Concentration of inulin from Dioscorea esculenta was measured using carbazole cysteine method. 1 ml of filtrate of Dioscorea esculenta was added with 0.2 ml of cysteine 1.5% and 6 ml of 70% H2SO4. The mixture was then shaken, then added with 0.2 ml of 0.12% carbazole in an ethanol solution and heated at 60 °C. for 10 minutes. After cold absorbance the sample was measured at a wavelength of 560 nm.

Induction of diabetes in rats

The rats were fasted for 10 h before the intraperitoneal injection of streptozotocin (STZ) at the dose of 45mg/kg BW dissolved in 1 ml of 0.1 M-buffer citrate, pH 4.5. After 72 hours, blood glucose levels of the rats were evaluated using spectrophotometric method. The rats with fasting blood glucose range of above 135 mg/dl were considered as diabetic rats.

Experimental design

The treatment was conducted at Biology Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung. A total of 10 male white Wistar rats were acclimatized for 7 days. During acclimatization, the rats were fed with a normal pellet diet and drinking water ad libitum. Then, 10 rats were divided into 2 groups: P1: 5 STZ induced diabetic control fed with standard and aquades from day 5 to day 28, P2: 5 STZ diabetic rats administrated with inulin from the Dioscorea esculenta tuber at the dose of 180 mg/rat/day from day 5 to day 28.

Biochemical assays

HbA1C, Urea and creatinine level were evaluated on day 29. Blood sample collected for estimation of HbA1C, creatinine, and urea was taken from the retro-orbital plexus, as much as 3 ml. Serum creatinine and urea serum levels were examined at Semarang Laboratory of Health Laboratory by enzymatic method was used to determine the serum creatinine and urea levels. Serum creatinine was measured using the Jaffe method without deproteinization, whereas serum urea levels were measured using the enzymatic Endpoint/Berthelot method. HbA1C levels was measured using the immunoassay and expressed in percentage.

Bacterial species identification and quantitative colony count
Identification of Bifidobacterium sp. and Lactobacillus sp. was based on gram staining on culture of fecal bacteria grown on MRS medium, whereas the of bacterial colony counting was determined by total plate count method (TPC). The identification and quantity calculations were performed at Microbiology Laboratory, Faculty of Medicine, Universitas Islam Sultan Agung.

Lactobacillus sp. on MRS media was observed as flat edge (smooth), convex elevation, small colony shape, creamy color, and smooth shiny surface. Type Bifidobacterium sp. was identified by observing non-spore forming bacteria, positive gram characteristics, catalase negative, non-motile, pairs of V-shaped or Y-shaped pairs, in gram-positive staining. Colonies of Bifidobacterium sp. Was observed as a gray in color.

**Table 1.** Level of HbA1c, creatinine and urea level in treated groups (mean SD).

<table>
<thead>
<tr>
<th>Groups</th>
<th>HbA1C level in blood serum (%)</th>
<th>Creatinin level in blood serum (mg/dl)</th>
<th>Urea level in blood serum (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>7.62±0.50&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.81±0.06</td>
<td>83.17±14.8</td>
</tr>
<tr>
<td>P2</td>
<td>6.14±0.35&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.62±0.04</td>
<td>51.20±0.58</td>
</tr>
</tbody>
</table>

Note: the letters<sup>a,b</sup> which differ between treatment groups (P1 and P2) showed significant differences for each variable (p <0.05)

Data analysis

Results were presented as mean (SD). Mean difference of HbA1C level of urea, creatinine, Bifidobacterium sp and Lactobacillus sp colony number between treatment group and control was analyzed using unpaired T test with 95% confidence interval.

**RESULTS**

This study showed that inulin from Dioscorea esculenta lowered the level of of HbA1C, the creatinine, and urea in diabetic rats. Table 1 showed mean percentages of HbA1C, the creatinine, and urea level in treated group and diabetic control rats.

![Figure 2. Number of Bifidobacterium sp. and Lactobacillus sp. colony (mean SD)](data:image/png;base64,iVBORw0KGgoAAAANSUhEUgAAAJwAAAD4CAYAAABnnaOwAAAAGXRFWHRTb2Z0d2FyZQBBZG9iZSBJbWFnZVJlYWR5ccllPAAAAyJpVFh0WE1MOmNvbS5hZG9iZS54bXAAAAAAADw/eHBhY2tldCBiZWdpbj0i77u/IiBpZD0iVzVNME1wQ2VoaUh6cmVTek5UY3prYzlkIj8&h=525&w=525&functionalId=110755&format=png&width=525&height=525&fit=1&crop=1&rs=1)

**DISCUSSION**

There was a significant reduction in levels of HbA1C, creatinine and urea in inulin treated animals as compared with diabetic control. Heinemann and Freckmann (2015) have reported that the decrease in HbA1C levels is closely associated with the decrease in blood glucose levels, which can be estimated at 6.5% (47.5 mmol/mol) HbA1C levels equivalent to blood glucose levels of 140 mg/dl, while HbA1C 8.6% (70.5 mmol/mol) is equivalent to a blood glucose level of 200 mg/dl<sup>13</sup>.

This present study showed that the inulin treatment significantly increase the number of Bifidobacterium sp. and Lactobacillus sp in the experimental group (p< 0.05) as compared to animal
control. This effect was likely due the capability of the inulin to stimulate the growth of bacteria *Bifidobacterium sp.* and *Lactobacillus sp.* as previously demonstrated by Winarti et al., (2013) that the inulin from gembili (*Dioscorea esculenta*) can stimulate the growth of *Bifidobacterium breve* BRL-131, *Bifidobacterium bifidum* BRL-130, *Bifidobacterium longum* ATCC-15707 and *Lactobacillus acidophilus* in vitro.\(^4\) This finding is also supported by the study of Pompei et al., (2008) showing that inulin can increase the growth of *Bifidobacterium adolescentis*, *Bifidobacterium infantis*, *Bifidobacterium breve*, *Bifidobacterium longum*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus*, *Lactobacillus reuteri*, *Lactobacillus delbruechi* and can inhibit the growth. *E. coli* and *Clostridium sp.*\(^5\). The increase in the number of the two intestinal microbiota bacteria in our study might have been associated with the increased production of short chain fatty acids (SCFA) such as lactate, acetate, propionate and butyrate affecting glucose metabolism leading to increase production of insulin. This possible mechanism has been proposed by zhang et al (2013) in there study on the effect of the intestinal bacteria on the level of SCFA levels and the increase of SCFA have been shown to increase insulin level via GLP-1.\(^1\)\(^6\)\(^5\).

**CONCLUSION**

Inulin from *Dioscorea esculenta* tuber has an antidiabetic effect in streptozotocin-induced rats by decreasing HbA1C, this may be due to a certain modulation effect on bacteria *Bifidobacterium sp.* and *Lactobacillus sp.* which is associated with decreased levels of creatinine and urea.

**ACKNOWLEDGMENTS**

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**REFERENCES**


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