ORIGINAL ARTICLE

The Expression of *Ins1* Gene in Streptozotocin-Induced Male Sprague Dawley Rats and Treated by Binahong (*Anredera cordifolia*) Leaf Extract and Sambiloto (*Andrographis paniculata*) Leaf Extract

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disease caused by pancreas insufficiency to produce adequate insulin or tissue inability to use existing insulin. Insulin is coded by the Ins gene and produced by the pancreas and extra-pancreatic organs such as the liver. Binahong and sambiloto are traditional medicinal plants they could provide antidiabetic effects, which play a role to help DM condition/disease. The study was conducted to find out how the *Ins1* gene's expression in the liver and pancreas compared in Binahong- and Sambiloto-Treated DM rats. **Method:** This is an experimental study. The samples are pancreas and liver from four-grouped Sprague Dawley rats. The expression of the *Ins1* gene is measured by PCR, then analysed using Livak Method. **Result:** The expression of the pancreatic *Ins1* gene was 0.89 times lower in the Binahong-treated group and 0.85 times lower in the Sambiloto-treated group. Meanwhile, the expression of the liver *Ins1* gene is 9.89 times higher in the Binahong-treated group and 27.77 times higher in the Sambiloto-treated group. **Conclusion:** The expression of the *Ins1* gene is at the lowest in the pancreas of both Binahong and Sambiloto-treated groups. In contrast, the liver *Ins1* gene has the highest expression in the Binahong-treated group and Sambiloto-treated group.

Keywords: Rats, Diabetes Mellitus, Pancreas, Liver, Polymerase Chain Reaction

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INTRODUCTION

Diabetes Mellitus (DM) is a chronic disease caused by insufficiency of the pancreas to produce adequate insulin or tissue inability to use the existing insulin in the body. Globally, there are at least 422 million adults living with diabetes in 2014 according to WHO data. It is a group of metabolic diseases with the same phenotype, hyperglycaemia. High levels of glucose result in classical symptoms of DM, such as polyuria, polydipsia, polyphagia, and weight loss. DM has several forms which can be caused by a complex interaction of genetic and environmental factors. There are Type-1 DM, Type-2 DM, Gestational, and other types of DM (1–3).

In diabetes mellitus, when it reaches a hyperglycaemic state, extra-pancreatic organs such as the thymus, liver, spleen, adipose tissue, and kidney could also produce insulin. It was a reaction to tolerate hyperglycaemia and protect the host from diabetes mellitus and destruction of pancreatic-cells. A study showed that some of the extra-pancreatic insulin-producing organs independently do not need plasma insulin and synthesise their insulin. The presence of this gene outside the pancreas serves as the body's adaptive or compensatory ability to produce insulin if the pancreas was damaged. It shows the body can still make insulin from extrapancreatic organs. Yet, liver is not the main factory of insulin synthesis, the amount of insulin produced is not adequate. However, the origin and what they do remain to be questioned (4–6).

Among traditional medicinal plants, binahong (*Anredera cordifolia*) is believed as one of the plants that could offer an antidiabetic effect. Studies showed that Binahong is a vine that contains flavonoid, saponin and triterpenoid in its leaves. Binahong leaf extract is known for its potential as antidiabetic, anti-hyperlipidemic, analgesic and anti-

inflammatory agents. Binahong produces its antidiabetic effect by lowering blood sugar levels (7,8).

Likewise, sambiloto (*Andrographis paniculata*) which contains andrographolide could raise glucose utilisation by increasing the expression of GLUT-4 mRNA and protein. Andrographolide can stimulate insulin release and inhibit glucose absorption through the inhibition of alpha-glucosidase and alpha-amylase enzymes. The antidiabetic activity of these compounds is related to the antioxidant activity and inhibition of NF-kappa B (9).

Insulin is a protein molecule consisting of 51 amino acids in 2 peptide chains connected with disulphide bonds. Insulin plays a role in the process of using and storing energy abundance. In the human system, it is synthesised by pancreas beta cells and encoded by the Ins gene. Unlike humans, rodents have a two-gene system of insulin which are *Ins1* and *Ins2* genes. The *Ins1* gene has been identified as originating from a reverse-transcribed partially processed mRNA of Ins2 and thus retains only one of the two introns, which is homologues to the Ins2's first intron (10–13).

The involvement of rodents/rats as an experimental subject has been widely used in experimental studies about diabetes mellitus. Streptozotocin (STZ) is one of the most common compounds that is used to induce diabetes mellitus in experimental animals. It is a neoplastic agent which contains active substances, including 2-Deoxy-2-[[(methylnitrosamino)-carbonyl] amino]-D-glucopyranose and citric acid. STZ has the ability to selectively destruct pancreatic β cells, also develop hypoinsulinemic- and hyperglycaemic-state. Low doses in multiple administrations of STZ stimulate immune and inflammatory reactions related to lymphocyte infiltration into the pancreatic islets. Meanwhile, a high dose of STZ in one-time administration is cytotoxic to the cell and alkylates DNA fragments of pancreatic β cell. STZ also creates Reactive Oxygen Species (ROS) which contribute to more DNA damage (14–16).

Thus far, there is no further information about the expression of the *Ins1* gene in Streptozotocin-induced diabetic rats treated with Binahong and Sambiloto extract. Therefore, this experiment was conducted to explore how antidiabetic effects that binahong and sambiloto provide on DM.

MATERIALS AND METHODS

Study Design

An experimental design was held from March 2018 to March 2020. This study is a continuation of the previous research conducted by Astarina, et al.

Animals

A total of 20 male Sprague Dawley rats aged 12 weeks with 120 – 160 grams body weight were obtained from IRAT.co Animal Facility and Modelling Provide, Bogor Agricultural University. The rats were kept in the Animal House Laboratory, Faculty of Medicine, State Islamic University. The protocol was ethically approved by the Institutional Ethics Committee Faculty of Medicine UIN Syarif Hidayatullah Jakarta University No. B-036/F12/KEPK/TL00/11/2020.

Experimental Groups

A total of 20 animals were randomised into four groups. The groups were named Normal group as the negative control, DM group as the positive control, Binahong-treated group and Sambiloto-treated group as the experimental targets. Each group consisted of five rats. The rats were adapted to the environment and nursed with free access to water and food for the first 14 days. The three groups (DM group, Binahong-treated group, and Sambiloto-treated group) were fasted over a day and were injected with 50 mg/kg BW of Streptozotocin (STZ) intraperitoneally at day-15 to induce Diabetes Mellitus.

Blood glucose levels were measured before and 4 days after the induction (day-19) to confirm the blood glucose values of the STZ-injected groups (blood glucose levels > 200 mg/dL). Glucose levels among the Normal group remained within the normal range. The Binahong-treated group was given 100 mg/kg BW per day of 70% ethanol extract from Binahong leaves (*Anredera cordifolia*) orally and the Sambiloto-treated group was given 400 mg/kg BW per day of 96% ethanol extract from Sambiloto leaves orally, once daily for 14 days (day-20 to day-33). The animals were euthanised by inhalation of ether and the organs (pancreas and liver) were isolated, then kept at the temperature -80^{o}C

RNA Isolation & *Ins1* gene expression quantification

Insulin mRNA was isolated from pancreatic and liver tissue using Direct-zol TM RNA Miniprep Plus. Isolated mRNA then was converted into cDNA using SensiFAST™ cDNA Synthesis Kit. Real-Time PCR LightCycler® 480 machine with SensiFAST™ SYBR® No-ROX One-Step kit was used to determine *Ins1* gene Cycle Threshold (CT), with β-actin gene acting as a housekeeping gene. CT Values were quantified using $2^{-\Delta\Delta CT}$ to relatively determine the expression of the *Ins1* gene.

RESULTS

The diabetic was confirmed by measuring the blood glucose values of the STZ-injected groups, as shown in Table I (blood glucose levels > 200 mg/dL). The data collection was conducted by using Real-time PCR which generates CT (Cycle Threshold) Values. Then, the CT Values were processed using a semiquantitative quantification Livak Method and the result was presented in the form of $2^{-\Delta\Delta CT}$ as a relative comparison of gene expression.

Table I: Diabetes Induction Blood Glucose Levels

Groups	Initial Blood Glucose Levels (mg/dl)	STZ-Injected Blood Glucose Levels (mg/dl)
Normal	91.40±14.43	100.8±16.9*
DM Group	128.0±10.2	386.0±120.3
Binahong-Treated and Sambiloto- Treated	131.2±22.0	596.6±4.9

*No STZ was injected to Normal group; DM = Diabetes Mellitus; STZ = Streptozotocin

Binahong (*Anredera cordifolia*)

The pancreatic *Ins1* gene expression level in the Binahong-treated group was lower (Mean $2^{-\Delta\Delta CT}$ = 0.19) than Normal and DM group gene expression (p=0.013) (Figure 1 A). Expression of liver *Ins1* gene in the Binahong-treated group was the highest (Mean $2^{-\Delta\Delta CT}$ = 9.89) compared to the *Ins1* gene expression in the Normal and DM Group (p=0.082) (Figure 1 B).

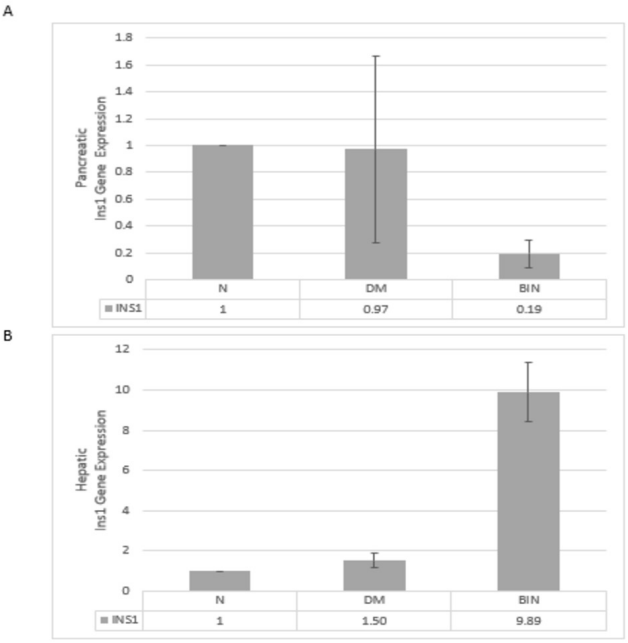

Figure 1: Expression of *Ins1* gene (A) Pancreatic Tissue and (B) Liver tissue. The ratio of pancreatic *Ins1* gene expression in the Binahong-treated group was significantly lower than in other groups (p=0.013). The ratio of hepatic *Ins1* gene expression in the Binahong-treated group was significantly higher than in other groups (p=0.082).

Sambiloto (*Andrographis paniculata*)

In the Sambiloto-treated group, the level of pancreatic *Ins1* gene expression was also lower (Mean $2^{-\Delta\Delta CT}$ = 0.15) compared to the Normal and DM group gene expression (p=0.009) (Figure 2 A). However, on the liver sample, the level of *Ins1* gene expression was the highest in the Sambiloto-treated group (Mean $2^{-\Delta\Delta CT}$ = 27.77) compared to Normal and DM groups (p=0.006) (Figure 2 B).

DISCUSSION

The Normal group was used as a calibrator where the -ΔΔCT values were considered as zero. Therefore, the

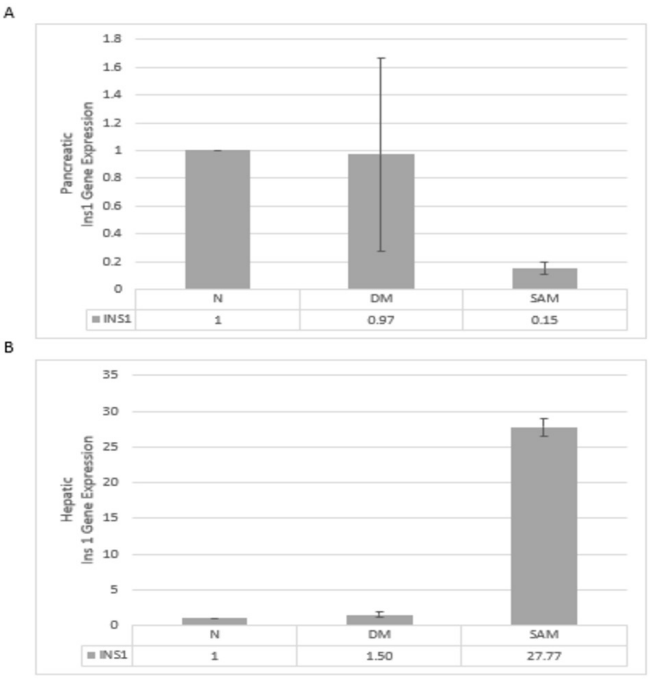

Figure 2: Expression of *Ins1* gene (A) Pancreatic Tissue and (B) Liver tissue. The ratio of pancreatic *Ins1* gene expression in the Sambiloto-treated group was significantly lower than in other groups (p=0.009). The ratio of hepatic *Ins1* gene expression in the Sambiloto-treated group was significantly higher than in other groups (p=0.006).

$2^{-\Delta\Delta CT}$ returned a value of one. The DM group had lower pancreatic *Ins1* gene expression (Mean $2^{-\Delta\Delta CT}$ = 0.97) than the Normal Group. In the DM rats, Streptozotocin induction generates the formation of Reactive Oxygen Species (ROS) which alter DNA fragmentation and damage pancreatic β cells (14). STZ causes the expression of pancreatic *Ins1* has decrease and degraded pancreatic β cells structure. As a result, the production of insulin will drop and lead to a high level of glucose (17).

Binahong leaves contain flavonoids, phenol compounds and ascorbic acid as antioxidants which prevent oxidative damage, defend the cells and accelerate cell repair (7,8,18). An antioxidant is thought to play a role in scavenging free radicals from the diabetes inducer agent and affects lowering the blood sugar levels (7). Andrographolide contained in sambiloto leaf extract has antioxidant and inhibitory properties of NF-kappa B. In addition, these compounds can stimulate insulin release and inhibit glucose absorption and alpha-amylase enzymes (9).

In this study, the pancreatic *Ins1* gene expression in the Binahong-treated group was the lowest amongst the normal and DM group. On the other hand, the hepatic *Ins1* gene expression in the Binahong-treated group had the highest level of expression between the Normal and DM group. The expression of the pancreatic *Ins1* gene of the Sambiloto-treated group was lower compared to the normal and diabetic group. However, in the hepatic tissue, the *Ins1* gene expression of the Sambiloto-treated

group was higher compared to the other group.

From the previous study, the Binahong-treated and Sambiloto-treated groups showed higher levels of blood glucose compared to the Normal and DM group. There was also a two-week absence of treatment before the organ isolation. The absence of treatment might leave the cells unprotected from the oxidative stress in hyperglycemia. It is possible that during 2 weeks without administration of sambiloto and binahong leaf extracts, the cells become unprotected by the antioxidant properties of both extracts and are thought to be deteriorating which relates to the expression of the pancreatic *Ins1* gene. On the other hand, the expression *Ins1* on hepatic tissue (binahong-treated and sambiloto-treated groups) was higher compared to the other groups. This is thought to be due to the continuous availability of high levels of glucose and nitric oxide which will be detected by hepatocytes. (4) This will induce insulin synthesis by increasing proinsulin gene expression. In addition, there is an effect of Andrographolide as a hepatoprotective so that hepatocytes can be protected from damage caused by ROS (Reactive Oxygen Species) (19). The presence of liver gene expression data shows the insulin synthesis can actually occur in extra-pancreatic organs, because insulin genes are found in several cells, but gene expression in each cell is different due to the mechanism of gene regulation in each tissue or organ. All cells have genes related to the formation of certain tissues or proteins, such as hormones or others. However, the ability of these genes to express their products will be influenced by the regulatory mechanisms that exist in the cells or tissues in the organ. Yet, there is still no further studies on the effect of binahong leaves extract on *Ins1* gene expression and little information regarding extra-pancreatic insulin. Studies related to the antidiabetic effect of binahong and sambiloto to *Ins1* gene expression need to be conducted to enrich the information about their antidiabetic effect.

CONCLUSION

Pancreatic *Ins1* gene expression in both the Binahong-treated group and Sambiloto-treated group is the lowest compared to the Normal group and DM group. Whereas, the liver *Ins1* gene expression in the Binahong-treated group and Sambiloto-treated group is higher than the Normal group and DM group.

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