Anti-hyperglycemic effect of Torbangun (Coleus amboinicus Lour) leaves extract through liver and muscle glycogen deposits in Streptozotocin-induced hyperglycmic Sprague-Dawley rats model

by Meilla Dwi Andrestian

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Abstract. The association of liver and muscle glycogen deposits with serum insulin levels, β-cells pancreas, and fasting blood glucose (FBG) of streptozotocin (STZ)-induced hyperglycemic rats receiving Torbangun leaves extract (TE) were investigated. Intervention was performed on 25 8-week-old Sprague-Dawley rats that were divided into four groups. Seven rats were separated as a normal group (N) and other rats were injected with streptozotcin (STZ). Confirmation of hyperglycemic was characterized by fasting blood glucose >126 mg/dl. Treatment group which is NG (hyperglycemic rats); N (normal rats); H-IM (62.5 mg/kg BW metformin); and H-IT (620 mg/kg BW TE) for 14 days. This study revealed that TE significantly decreased FBG levels, increased the insulin production, and amount of liver glycogen deposits (α=0.01). However, the intervention did not significantly increase the amount of muscle glycogen deposits. TE administration improves β-cells, increases the liver and muscle glycogen deposits. TE was shown to have antihyperglycemic activity by improving the β-cell, increasing blood serum insulin levels, decreasing blood glucose levels, and increasing the liver glycogen deposits.

Key Words: Diabetes, Glycogen, Hyperglycemia, Torbangun

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INTRODUCTION

Glycogen, which is the main carbohydrate deposit in the body, is mainly localized in the liver and skeletal muscle. It acts as a source of glucose when the body requires glycogen breakdown for energy metabolism, which is referred to as glycogenolysis (Gropper and Smith, 2013).

The liver and muscle glycogen deposits of people with diabetes decrease because the glucagon activity increases with decreased insulin production, which is responsible for stimulating glycogen formation. Individuals with type 1 diabetes mellitus (DM) cannot adequately produce insulin. Meanwhile, individuals with type 2 DM can produce insulin in a fixed amount, but the insulin does not function properly (insulin resistance) because the insulin receptors in the cell membrane are reduced or their structures are altered. Thus, the receptors are unable to respond to the presence of insulin. This condition results in hyperglycemia because glucose cannot be stored in the form of glycogen, either in the liver or muscles.

Oral DM treatment can be performed by taking medications along with natural ingredients. Approximately 1,050 anti-DM plants have been studied (Subramoniam, 2016). One of the plant types used as a functional food and developed to control DM is Torbangun (*Coleus amboinicus* Lour). Torbangun leaves were originally used for increasing breast milk production (galactagogue), which is a local belief of the people of North Sumatra, Indonesia. Lactating mothers in the Batak ethnic group in North Sumatra have a tradition of consuming Torbangun leaves after giving birth for increasing their breast milk production (Damanik *et al.*, 2001; Damanik R, Wahlqvist ML and Wattanapenpaiboon N, 2006; Damanik, 2009). Furthermore, the Torbangun plant has been widely used for its antibacterial and antifungal properties (Bhatt and Negi, 2012; Paul et al., 2014; Sabrina et al., 2014) and for controlling blood pressure, cholesterol levels (Suryowati et al., 2015a; Viswanathaswamy et al., 2011), and DM (Suryowati, 2015).

Torbangun leaves extract reportedly improves the function of pancreatic tissues, and it demonstrates insulinotropic effects (insulin production, secretion, and activity) (Viswanathaswamy et al., 2011). Torbangun (*Coleus amboinicus* Lour) leaves extract administered at a dose of 620 mg/kg body weight for 14 consecutive days has the potential to reduce blood glucose levels and free radicals, increase glucokinase, and protect pancreatic β-cells of streptozotocin (STZ)-induced rats (Suryowati, 2015). STZ is generally used in rat models of diabetes (Kim et al., 2012).

The bioactive component of Torbangun leaves extract is the flavonoids (Suryowati et al., 2015b). One of them is quercetin, an antioxidant that plays a role in controlling blood glucose levels and has the ability to correct oxidative stress in diabetic rats (Ilagan et al. 2016; Lee et al. 2015). Quercetin can stimulate glucose uptake in the muscles through an insulin-independent mechanism involving adenosine monophosphate-activated protein kinase (AMPK). AMPK activation in the skeletal muscle increases glucose uptake through the stimulation of GLUT4 glucose transporter translocation to the plasma membrane. Meanwhile, in the liver, AMPK decreases glucose production primarily through the downregulation of the key enzymes of gluconeogenesis, namely, phosphoenolpyruvate carboxykinase and glucose-6-phosphatase (Eid et al., 2015).

This increased glucose uptake promotes glycogen formation (glycogenesis). Conversely, a decrease in gluconeogenesis allows the retention of glycogen deposits. So far, there is no known effect of Torbangun leaves extract on the mechanism of glycogen formation and its deposits when hyperglicemic occurs. Therefore, we aimed to evaluate the association of liver and muscle glycogen deposits of STZ-induced hyperglycemic rats receiving Torbangun leaves extract with serum insulin levels, β-cells pancreas, and blood glucose levels.

MATERIALS AND METHODS

Study design, time, and location

This study used an experimental method with a completely randomized design (CRD) using Sprague-Dawley rats. The study was conducted from March 2017 to April 2018 at Bogor, West Java, Indonesia. The intervention was divided into the following four groups: negative control (NG, six hyperglycemic rats); normal (N, six rats); control group (H-IM, using 62.5 mg/kg body weight of metformin, six rats); and H-IT (620 mg/kg body weight of Torbangun leaves extract, six rats). The dose of Torbangun leaves extract is based on Suryowati et al (2015a).

Materials

Torbangun leaves were obtained from the gardens in Cibeureum area, Bogor City (6°37'31.47"S, 106°47'60.85"E), West Java, Indonesia, at an altitude of 298 m above sea level. The Torbangun seeds were planted in a total area of ±160 m^2 with a spacing distance of 40 cm (Aziz, 2013). Torbangun leaves were harvested at the age of 8 weeks and picked approximately 15 cm from the plant shoot (Andarwulan et al., 2014). Plant samples have been authenticated by the Botanical Garden Conservation

Center, Indonesian Institute of Sciences, Bogor (Number B-2096/sIPH.3./KS/VII/2017). The experimental animals used were 25 8-week-old male Sprague-Dawley rats with a minimum weight of 140 g from PT. Indoanilab Bogor, West Java, Indonesia. The sample size was determined using Federer's formula. The research protocol was implemented upon approval of Ethical Treatment No. 77-2017 from the Animal Ethics Commission, Institute for Research and Community Service, Bogor Agricultural University.

Torbangun leaves extraction

Torbangun leaves extraction followed the steps modified by (Suryowati et al., 2015b). The extraction was performed by sonication method using Powersonic 505 sonicator at 40 KHz for 40 min at room temperature (25°C), and the extract was then filtered (Annegowda et al., 2012). Solvent evaporation of the extract was performed using rotary evaporator at 60°C (Viswanathaswamy et al., 2011). The rotary evaporator used was the Buchi RE-111. The thick extract obtained was stored at 4°C–8°C (Uma et al., 2011).

Animal intervention

We used Sprague-Dawley rats with a CRD. The intervention groups were NG (seven rats), N (six rats), H-IM (six rats), and H-IT (six rats). Except for the N group, the rats were given STZ induction with a dose of 40 mg/kg body weight (Jung et al., 2011) and hyperglycemic validated on day 3 when the fasting blood glucose (FBG) levels reached >126 mg/dl (Akbarzadeh et al., 2007). Food and water were given *ad libitum* with 12/12 hours light/dark conditions (Kim et al., 2012). Torbangun leaves extract and metformin were both orally administered for 14 days for comparison. Data regarding FBG levels were collected on days 0, 4th, 7th, 11th, and 14th using a glucometer. Blood was obtained through the tip of the tail (Kwon et al., 2013). Insulin levels were measured from the blood serum and glycogen content was measured from the rat liver and muscles that had been necropsied on day 15th.

Necropsy

All rats were administered general anesthesia with a combination of 90 mg of ketamine and 10 mg of xylazine. The necropsy was performed by cutting through the skin and abdominal muscles until the abdominal cavity was open. Rat blood was removed until the heartbeat stopped, placed in a tube, and then centrifuged at 3000 rpm for 10 min (Rahmawati et al., 2014). A separate serum was obtained to measure

insulin levels. The liver and soleus muscle samples were collected for glycogen content measurement.

Measurement of insulin levels

Insulin levels were measured using ELISA method (Salarinasab et al., 2017). Working procedure followed the manual of ELISA kit (Cat. No E0707Ra) from the Bioassay Technology Laboratory, China.

Histopathological observation of the pancreas

Pancreatic tissues were collected and fixed in 10% formalin neutral buffer solution, and the tissues were then cut. Tissues were dehydrated using alcohol solution and were then infiltrated using paraffin xylene and vacuum dried. Thereafter, tissues were cut using a microtome, and tissue slices were attached to the glass preparation. Objects were stained using hematoxylin and eosin dye before observation under a microscope (Slaoui and Fiette, 2011). The microscope used was Leica DM 750 Hiplan with Optilab camera.

Glycogen content measurement

Glycogen content was measured using spectrophotometric method (Sruthi et al., 2014). The spectrophotometer used was Genesys 20 Vis with λ: 200-1100 nm.

. Liver and muscle organ preparation were initiated by isolating the organs in 15 ml of 5% trichloroacetic acid solution in centrifuge tubes containing 5 ml of ethanol, and the organs were stored overnight at room temperature. The tubes were then centrifuged at 3000 rpm for 15 min. The clear liquid formed was the glycogen contained in the samples. The samples were dissolved in 2 ml of distilled water. A blank solution was made by preparing 2 ml of distilled water into the centrifuge tubes. A standard solution was prepared by adding 2 ml of glucose solution containing 0.1 mg of glucose. Anthrone reagent was added to all tubes, with 10 ml for each tube. After the solution became homogenous, all tubes were soaked in a cold water bath until the temperature dropped. The tubes were then soaked in boiling water, soaked again in cold water, and cooled at room temperature. The solution was transferred to a colorimetric tube, and its absorbance was measured at a wavelength of 620 nm. The glycogen content was calculated by the following formula:

$$\text{Glycogen content} = \frac{\text{DU/DS} \times \text{volume of the extract} \times 0.9 \times 0.1}{\text{Tissue weight (g)}}$$

where DU is the optical density of the sample; DS indicates the standard optical density; 0.9 denotes the factor for converting the glucose value to glycogen value; and

0.1 is the amount of glucose in 2 ml of the standard solution. The glycogen contained in the tissue was calculated and expressed in mg per 100 mg of sample.

Data analysis

Data obtained from the results of parameter the measurements were analyzed using paired sample T-test for FBG and analysis of variance for glycogen deposits at a 99% confidence interval. If there was a variance, the analysis was continued with Tukey's honest significant difference test.

RESULTS AND DISCUSSION

Effects on fasting blood glucose levels

Table 1 presents the effects of Torbangun leaves extract administration, which reportedly improves pancreatic tissue function and demonstrates insulinotropic effects (insulin production, secretion, and activity) in hyperglycemic rats. The high FBG levels in the STZ-induced groups at the beginning of intervention indicated that the study objects had hyperglycemia as one of the DM symptoms (>126 mg/dl). The FBG levels at the end of the study showed a decreased glucose level toward normal conditions. Meanwhile, the NG group was still hyperglycemic. Statistical analysis results revealed a significant difference between blood glucose levels at the beginning and end of intervention in the H-IM group (p=0.002, α=0.01) and H-IT group (p=0.005, α=0.01)

Table 1. Rats' FBG levels before and after the intervention

Groups	Initial glucose levels (mg/dl)	Final glucose levels (mg/dl)
NG	153.00±3.56	167.33±14.58
N	100.86±6.20	91.29±8.24
H-IM	147.83±6.84	103.17±2.21**
H-IT	155.83±9.56	105.17±5.04**

**) significant at α=0.01
Cut off point FBG>126 mg/dl
NG=negative, N=normal, H-IM=metformin drug control, H-IT=Torbangun leaf extract intervention

The results of this study are in accordance with the results reported by Suryowati (2015) who intervened in extracts of Torbangun leaves extracted by maceration method. Suryawati (2015) reported that Torbangun leaves extract at a dose of 620 mg/kgBW can significantly reduce blood glucose levels through intervention for 14 days.

The maintenance of blood glucose concentration is an important homeostatic function, and it is the primary function of the liver, skeletal muscles, and adipose tissues, representing majority of the body tissues. This process is hormonally

controlled, especially by hormones produced by the pancreas (i.e., insulin and glucagon) and glucocorticoid hormones produced by the adrenal cortex in the kidneys (Gropper and Smith, 2013). Insulin is secreted by pancreatic β-cells and has a broad spectrum of anabolic effects in various tissues. In response to food intake, insulin stimulates the uptake and storage of carbohydrates, fat, and amino acids (Langlais et al., 2015). DM is a condition wherein the regulation of glucose homeostasis throughout the body is impaired, leading to various complications. Additionally, the ability of insulin to regulate glucose metabolism decreases (Henriksen, 2013).

Statistical analysis findings revealed a significant difference between blood glucose levels at the beginning and the end of the intervention in the H-IT group and showed the effectiveness of Torbangun leaves extract in reducing blood glucose levels in hyperglycemic rats.

Effects on blood serum insulin levels

An increase in blood glucose levels after carbohydrate consumption triggers insulin release and decrease glucagon secretion. Insulin is the only hormone that can stimulate a decrease in blood glucose levels, and it is a primary anabolic hormone. It stimulates the uptake of glucose, amino acids, and lipids. Next, it directs the conversion of these substances for storage in the muscles and adipose tissues (Gropper and Smith, 2013).

Table 2 presents the comparison of the blood serum insulin levels in rats receiving Torbangun leaves extract intervention and other interventions. Statistical analysis results revealed a significant difference between groups (p=0.000, α=0.01). The NG group showed the lowest insulin production and secretion compared with those in the other groups.

There was a significant increase in serum insulin production in the H-IM and H-IT groups. This shows an improvement in pancreatic cell-β function by giving Torbangun leaves extract almost as effective as metformin. The study conducted by Ismail et al (2015) reported that metformin plays a role in improving pancreatic cell-β, resulting in an increase in insulin expression. In addition to increasing serum insulin levels, Cheng et al (2006) also reported that metformin was able to improve insulin receptors, which led to improvements in GLUT4 gene expression and decreased expression of PEPCK genes. Bösenberg and Zyl (2008), metformin has the ability to increase insulin sensitivity, thereby reducing gluconeogenesis and glycogenolysis. In

addition, metformin also increases GLUT-4 and GLUT-1 regulation to increase glucose storage.

The pathogenesis of DM is characterized by a metabolic disorder, that is, a decrease in peripheral tissue response to insulin (Kangralkar et al., 2010). The damage in the peripheral tissue occurs due to an increase in free radicals in the body. According to Viswanathaswamy *et al.*, a decrease in blood glucose levels in rats that were administered Torbangun leaves extract occurred through the restoration of pancreatic tissue function and insulinotropic effects (Viswanathaswamy et al., 2011). This occurs because the antioxidants present in the Torbangun leaves extract can capture the free radicals that are formed as a result of hyperglycemia. The decreased levels of free radicals caused the insulin receptors to function properly; thus, glucose could be transported into cells, resulting in glycogenesis and decreased gluconeogenesis.

Table 2. Profile of rats' blood serum insulin levels

Groups	Insulin levels (IU/l)
NG	7.33±0.40
N	9.31±0.99
H-IM	12.87±1.25**
H-IT	13.67±0.51**

**) significant at $\alpha=0.01$
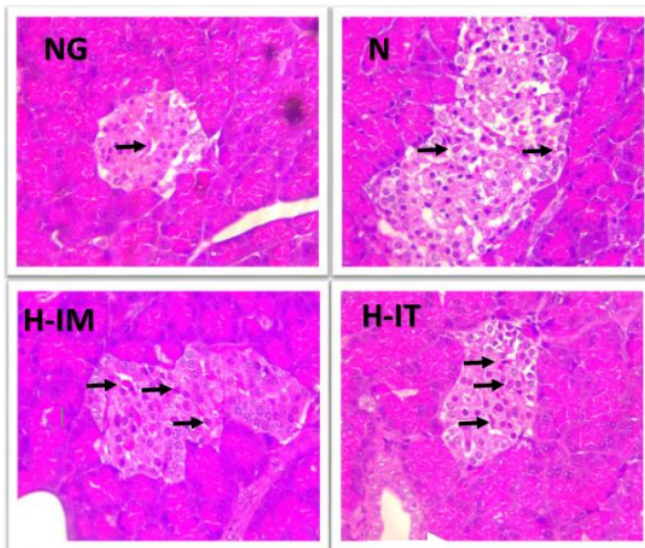NG=negative, N=normal, H-IM=metformin drug control, H-IT=Torbangun leaves extract intervention

Torbangun leaves extract contains flavonoid compounds, with potential antioxidant effects (Patel et al., 2010). Flavonoids are frequently found in plants and are involved in α-glucosidase inhibition (Lee et al., 2015). The flavonoids contained in the Torbangun leaves extract can inhibit enzymes, resulting in a decrease in carbohydrate hydrolysis and glucose absorption (Suryowati, 2015). Furthermore, it is assumed that the flavonoid that play a role is quercetin. Quercetin has the ability to decrease oxidative stress in diabetic rats (Lee et al., 2015; Suryowati et al., 2015b) and quersetin also a powerful antioxidant that directly catches reactive oxygen species and activates antioxidant enzyme functions (Jeong et al., 2012). However, based on studies conducted on the role of antioxidants in DM recovery, the antihyperglycemic potential in plant extracts is related to one type of flavonoids or the synergistic effects of various flavonoids contained (Jung et al 2011).

Histopathological observation of the pancreas

Figure 1 presents the results of histopathological observations in all treatments with 400 times enlargement. The results revealed that the NG group had decreased number and size of β-cells and decreased size of islets of Langerhans. Islets of Langerhans in group N appeared normal, β-cells were in the direction of the medulla with a clear hexagonal shape, whereas α-cells were in the cortical part of the islets of Langerhans. The H-IM group showed normal pancreatic conditions with Langerhans Island, and although β cells and α-cells were difficult to distinguish, there was a residual bent toward β-cells. The H-IT group still showed α-cell dominance, but β-cells repair was detected.

NG=negative, N=normal, H-IM=metformin drug control,
H-IT=Torbangun leaves extract intervention

Figure 1. Rats' pancreatic histology in all groups that showed Langerhans Island and β-cells indicated by arrows

This is in line with the study conducted by Suryowati (2015) who reported changes in β-pancreatic cells by treating Torbangun leaves extract resulting from maceration extraction. Changes occur because endocrine cells begin to regenerate towards normal forms, although there are still some endocrine cells that are degenerated.

The increase in serum insulin levels of H-IM and H-IT groups indicated an improvement in pancreatic β-cells that had previously been damaged by STZ induction.

The liver of diabetic rats receiving Torbangun leaves extracts had regeneration changes to normal conditions (Suryowati, 2015). Torbangun leaves extract administration protected endocrine cells so that insulin secretion can be increased.

Liver and muscle glycogen deposits

The profile of insulin production was also manifested in the rat glycogen deposits in all intervention groups. The H-IT group with the highest insulin content also showed the most glycogen deposits than other groups, either in the liver or muscles. Glycogen deposits, especially in the liver, showed a significant difference ($p = 0.000$, $\alpha = 0.01$) (Table 3).

Table 3. Rats' glycogen deposits at the end of the intervention

Groups	Liver glycogen deposit (mg/g)	Muscle glycogen deposit (mg/g)
NG	5.65±1.02	2.91±0.70
N	7.02±1.78	3.36±0.89
H-IM	7.21±1.74	3.67±0.83
H-IT	10.65±0.54**	3.71±0.73

**) significant at $\alpha = 0.01$ (p=0.000)
NG=negative, N=normal, H-IM=metformin drug control, H-IT=Torbangun leaves extract intervention

The glycogen deposits in the liver comprise 7% of the liver weight, while the glycogen deposits in the muscles comprise only 1% of all skeletal muscles. Although the relative concentration of glycogen in the liver is higher than in the muscles, the highest total glycogen deposit in the body is in the skeletal muscles (Gropper and Smith, 2013).

The liver plays an important role as a guardian of postprandial hyperglycemia through glycogen synthesis. DM is a result of the failure of the liver in performing glycogenesis. In STZ-induced rats, DM occurs because of the deactivation of enzyme glycogen synthase phosphatase (Suarsana et al., 2010). The amount of glycogen deposits in individuals with DM decrease because of reduced insulin production or sensitivity; thus, glycogenesis is inhibited and glycogenolysis occurs in the liver, which contributes to an increase in blood glucose levels. Therefore, DM therapy is expected to increase insulin production so that glycogenesis occurs properly and glycogenolysis can be inhibited to attain glucose homeostasis in the body.

The profile of insulin production was also manifested in rat glycogen deposits in all intervention groups. The H-IT groups that had the highest insulin content also showed the most glycogen deposits compared with other groups, either in the liver or muscles. The liver glycogen deposits in the H-IT group even showed a significant

difference compared with the liver glycogen deposits of other groups. This is because the glycogen deposits in the liver directly contributed to blood glucose levels.

Increased insulin production in the H-IM and H-IT groups tends to also increase muscle glycogen storage. This occurs because insulin can stimulate an increase in GLUT4 transporter activity which plays a role in glucose translocation from the blood to muscle cells (Jung et al., 2017). Unlike the glycogen deposits in the liver, glycogen deposits in the muscles are a source of energy used by the muscles themselves and do not directly contribute to blood glucose levels (Gropper and Smith, 2013). Thus, DM therapy, through either medications or Torbangun leaves extract, did not provide a significant difference in muscle glycogen deposits.

Diabetic rats that were provided tempeh extract intervention showed an increase in glycogen deposits in the liver or muscles (Jeong et al., 2012). An increase in glycogen deposits was also detected in diabetic rats receiving NIDDWIN (formulation of 11 types of anti-DM herbs) therapy. This occurred because of an increase in the conversion of blood glucose to glycogen. The decreased blood glucose levels could be associated with the anti-DM activity of those herbal mixtures (Sruthi et al., 2014). This is likewise the case in hyperglycemic rats administered Torbangun leaves extract. There was a decrease in glucose levels in line with the increase in insulin levels and glycogen deposits, especially in the liver. This indicates that the Torbangun leaves extract at dose 620 mg/BW can hasten glycogenesis, and its effectiveness is nearly similar to metformin and several other types of anti-DM herbal therapy.

In conclucion, the administration of Torbangun extract to rats increases the liver and muscle glycogen deposits. These increased deposits in the liver and muscles are in line with increased blood serum insulin levels and decreased blood glucose levels. Therefore, it can be concluded that Torbangun leaves extract have antihyperglycemic activity by improving the β-cell, increasing blood serum insulin levels, decreasing blood glucose levels, and increasing the liver glycogen deposits.

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