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Antihyperglycemic Activity of Aqueous Extract of Insulin Leaves (*Tithonia diversifolia*) on Hyperglycemic Rats (*Rattus norvegicus*)

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Abstract

Insulin leave (*Tithonia diversifolia* (Hemsl.) A. Gray) empirically used by people as antihyperglycemic drugs. The study was aimed to evaluate antihyperglycemic activity as well as to determine the most optimum dose of *T. diversifolia* to reduce blood glucose levels. Hyperglycemic condition was induced to male *Rattus norvegicus* rats by intraperitoneal injection of alloxan at a dose of 150 mg/kg bw (body weight). In this study, completely randomized design was performed with three treatment groups and five times repetition. Group P1 received glibenclamide 10 mg/kg bw, P2 received aqueous extract of *T. diversifolia* at a dose of 150 mg/kg bw, P3 received aqueous extract of *T. diversifolia* at a dose of 300 mg/kg bw. Administration of both glibenclamide and aqueous extract of *T. diversifolia* was conducted orally for 28 days. Data were analyzed using Anova and Duncan's test with 95% confidence level ($\alpha = 0.05$). The results showed that the mean percentage of decrease in blood glucose levels, drink intake and body weight of all treatment groups were not significantly different ($p>0.05$). Data of feed intake showed that P1 was significantly different from P2 ($p<0.05$), but P2 was not significantly different from P3 ($p>0.05$). Based on the result of this research, it was found that the low doses of aqueous extract of *T. diversifolia* has the same ability to decrease blood glucose level compared to glibenclamide. Futhermore, this study provide some information that can be used as preclinical analysis to determine effective doses of aqueous extract of *T. diversifolia* to decrease blood glucose levels.

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INTRODUCTION

Diabetes mellitus (DM) is a disorder of the endocrine system in carbohydrate metabolism due to insulin secretion deficiency characterized by the occurrence of hyperglycemia. Hyperglycemia is a condition in which the blood glucose levels remain high because of insufficient amounts of insulin or poor cellular response to insulin (Piero *et al.*, 2014). Various agents used to reduce blood glucose levels with different working mechanisms are available, but there are still obstacles to achieve the glycemic targets. Those obstacles include the increase in the risk of hypoglycemia, mortility and body weight (Hasan *et al.*, 2010). Glibenclamide is a hypoglycemic drug from the sulfonylurea class working by stimulating pancreatic beta cells to secrete insulin (Rai *et al.*, 2012). New strategy is needed for the prevention and treatment of diabetes. Alternative therapies that might be done are the using of herbal medicines, because people have relied on traditional medicines for health care since long time ago.

Medicinal plants with antihyperglycemic activity are used as alternative medicines widely in cases of DM. Insulin leaves (*Tithonia diversifolia* (Hemsl.) A. Gray) have been empirically used by the people as a drug for DM, due to its role in binding the free radicals resulted from pancreatic beta cell dysfunction. People use the plant by boiling the leaves in water and drinking it. Sasmita *et al.* (2017) explained that *T. diversifolia* contains two main components that have been an antidiabetic role, namely flavonoids and sesquiterpenes lactone, found mostly in leaves. Flavonoids play a role in increasing glucose absorption because they are able to inhibit α-glucosidase and α-amylase, while sesquiterpenes lactones acts as a protein inhibitor formed during oxidative stress. Fitmawati *et al.* (2001) stated that flavonoid extracted from plant medicine can prevent disease caused by oxidatif stress such as diabetes and artherosclerosis. Olayinka *et al.* (2015) stated that the chemical compounds contained in every 100 g of *T. diversifolia* dried leaves in aqueous extract were 1.535 mg alkaloids; 851.67 mg flavonoids; 761.67 mg saponins and 64.58 mg phenols. Anwar *et al.* (2016) explained that saponin is an antioxidant that can protect the pancreatic beta cells and inhibit the action of α-glucosidase. Alkaloids and phenols play a role in inhibiting the activity of several enzymes that play a role in carbohydrate metabolism such as α-glucosidase and hexokinase, pyruvate kinase and glucose 6-phosphatase (Qi *et al.*, 2010; Patel *et al.*, 2012). Sumarny and Soetjipto (2011) stated that the percentage the decrease in blood glucose levels of mice after the administration of n-hexane extract of *T. diversifolia* at doses of 5.38 mg/kg BW and 10.75 mg/kg BW for 28 days were 24% and 31%. Olukunle *et al.* (2014) also stated that the administration of aqueous extract of *T. diversifolia* at the dose of 400 mg/kg BW for 21 days could reduce the fasting blood glucose levels up to 82.30%. Anwar *et al.* (2016) explained that the administration of petroleum ether fraction of *T. diversifolia* at a dose of 100 mg/kg BW for 7 days was able to reduce blood glucose levels by 41.19%.

Body weight is used as one of the health indicators. The condition of high extracellular glucose levels (hyperglycemia) causes the cell to become deficient in glucose so that the cell has decreased energy. The decrease of energy in cells will cause an increase in lipid catabolism so that body weigh decreases (Yuniwarti *et al.*, 2018). Based on previous studies, the dose and length of treatment time were known to affect the ability of medinical plant extract in decreasing blood glucose levels. This study aimed to analyze the response of blood glucose levels, food and drink intake as well as the body weight of hyperglycemic rats after administration of aqueous extract of *T. diversifolia* leaves at doses of 150 mg/kg BW and 300 mg/kg BW for 28 days. This research is expected to be useful to obtain the dose of insulin leaves exctract as an herbal medicine to reduce blood glucose levels.

METHODS

Extraction of the Plant

T. diversifolia leaves were obtained from Semarang. The leaves were washed, followed by oven dried at 60^0 C for 1-2 days and mashed using a blender. Powdered *T. diversifolia* (100 g) was first extracted via maceration in 1.000 mL of distilled water for 24 hours at room temperature. The filtrate was evaporated using a rotary evaporator at $80\text{-}90^0$C resulted in viscous paste. Aqueous extract of *T. diversifolia* with a dose of 150 mg was made by dissolving 150 mg of the plant extract into 3 mL distilled water, while the dose of 300 mg was made by dissolving 300 mg of the the plant extract into 3 mL distilled water.

Animal Model Samples

The samples used in this study were fifteen male white rats type *R. norvegicus*, that were about 3 months old and had 200 g weight. Rats were obtained from the Biology laboratory of Semarang State University (UNNES). Rats were acclimatized for 7 days in room temperature (25-

28^{0}C) and controlled the humidity (60-80%) in the dark-light cycle fo 12 hours. The food and drink were given *ad libitum*, the composition of diet are 3.05% crude lipid, 19.44% crude fiber and 19,99% crude protein in dry sample. All experimental animal procedures were approved by the Faculty of Medicine's Ethics Committee, Diponegoro University with certificate number 82/EC/H/FK-RSDK/VI/2018.

Experimentals Design

This study used a completely randomized design (RAL). Rats were grouped into three groups randomly. Each group consisted of 5 rats.
Group 1 : rats were induced by alloxan + glibenclamide at a dose of 10 mg/kg BW rat/days
Group 2 : rats were induced by alloxan + aqueous extract *T.diversifolia* at a dose of 150 mg/kg BW rat/days
Group 3 : rats were induced by alloxan + aqueous extract *T.diversifolia* at a dose of 300 mg/kg BW rat/days.
The treatment was given for 28 days by orally.

Hyperglycemic rats were obtained by giving injection of alloxan monohydrate at a dose of 150 mg/kg BW intraperitoneal (Yuniwarti *et al.*, 2018). In three consecutive days, blood glucose levels were examined to ensure that alloxan was working well and the glucose level was stable. Blood glucose levels before treatment were calculated at the fourth day after alloxan injection using Easy Touch GCU glucometer via intravenous tail. The percentage decrease in blood glucose levels is calculated using the formula below.

$$\text{The percentage of blood glucose level} = \frac{\Delta \text{ blood gucose}}{\text{Blood glucose before treatment}} \times 100\%$$

(Yuniwarti *et al*, 2018).

Variable Test

Measurement of rat's weight was carried out before treatment and it was continued once a week until the end of the treatment using a scale (Ohause), so that the data obtained a gram per a week.

Measurement food intake was done once a week, while drink intake measurements were carried out every day. Measuring the food intake was that each rat provided 1000 feed stock. Rats every day were fed *ad libitum* taken from the feed stock. Food intake data were calculated by weighing stock feed minus the remaining feed (g/week). Drink intake data were calculated by measuring the initial volume of distilled water found in the drinking area (mL/day) (Mardiati and Sitasiwi, 2016).

Data Analysis

Body weight, food intake and drink intake were presented in graphical form. Data percentage decrease blood glucose levels, body weigh, food intake and drink intake are analyzed statistically using Anova analysis and if there were significant differences followed by Duncan test with a 95% confidence level (α = 0.05).

RESULTS AND DISSCUSIONS

Insulin leaves (*T. diversifolia*) can be used to reduce the blood glucose level because they have antihyperglycemic activity. The percentage decrease in blood glucose levels of hyperglycemic rats are showed by Figure 1.

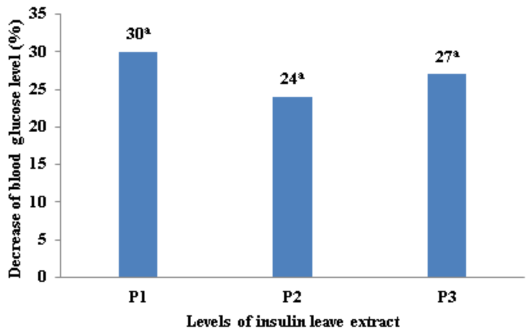

Figure 1. Percentage decrease of blood glucose levels in hyperglycemic rats.

The percentage in decreasing of blood glucose levels in the treatment aqueous extract of *T.diversifolia* both doses of 150 mg/kg BW/days and 300 mg/kg BW/days is lower than glibenclamide treatment, but they have no big differences. Decrease of blood glucose levels in glibenclamide treatments is thought to be due to an increasing in insulin secretion. Glibenclamide is DM drug from the sulphonylureas group which plays a role in stimulating insulin. The role is to release insulin secretion through potassium ATP (kATP) channel inhibition in pancreatic β cells. Sen *et al.* (2016), explained that glibenclamide causes the inhibition of kATP through the ability to depolarize the pancreatic β cells membrane. The pancreatic β cells depolarization of the pancreas will stimulate the opening of intracellular Ca^{2+} channels and an increasing in Ca^{2+} stimulates insulin release. The percentage of decreasing in blood glucose levels obtained was not more effective than the studied of Olukunle *et al.* 2014 which stated a decrease in blood glucose levels is 82.30%. This is presumably because the dose used in the study is lower. Lower doses can avoid the risk of toxicity from a plant, especially that is used for a long term. Passoni *et al.* (2013) said that sesquiterpenes lactones and chlorogenic acid derivatives are toxic because they have anti-inflammatory ability. This anti-inflammatory ability causes a decrease in the number of leukocytes and neutrophils so that it is harmful to health.

The decrease in blood glucose levels in hyperglycemic rats was given by aqueous extract of *T.diversifolia* caused by chemical compounds such as flavonoids, phenols, and lactone sesquiterpenes. Flavonoids are polyphenol compounds that can inhibit the action of α-glucosidase and amylase and act as antioxidants. Hasan *et al.* (2010), the hypoglycemic effect is caused by a decrease in the absorption of glucose in the intestine by inhibiting the activity of the α-glucosidase enzyme which acts to degrade polysaccharides into monosaccharides. If the rate of glucose absorption from intestines decrease, this can lead the glucose homeostatis to diabetes. Antioxidant protect pancreatic β cells from oxidation through inhibition of chain reactions of lipid peroxidation, superoxide radical binding (Thongsom *et al.*, 2013). The antioxidant found in *T. diversifolia* leaves act as a superoxide radical binder and increase glutathione peroxidase activity. Inhibition of oxidation reactions is done through the mechanisms of catching free radicals by donating one unpaired electron in free radicals so that free radicals become reduced (Pasaribu *et al.*, 2015). Glutathione peroxidase is an antioxidant enzyme that serves to bind peroxidation (H_20_2 or lipid peroxidation). Gradually decreasing free radicals can repair damage to the pancreatic β cells so that the pancreas can secrete insulin. The normal insulin secretion decrease the level of glucose levels in blood.

T. diversifolia has an ability to increase insulin sensitivity which is responsible for reducing the hyperglycemia, decreasing food intake and increasing body weight in hyperglycemic rats. Figure 2 (A) shows the treatment given affects feed consumption. In Table 1. shows food consumption between glibenclamide treatment and aqueous extract of *T. diversifolia* at doses of 150 mg/kg BB was significantly different, but the treatment of aqueous extract of *T. diversifolia* was not significantly different. Food intake on seven days of treatment is relatively higher, it is suspected that during this time polyphagia occurred in the hyperglycemic rats. Polyphagia results from cells experiencing a lack of energy because glucoe cannot be absorbed into the cell. Fauziah *et al.* (2018) explained when the cells deficient glucose will activate the hunger center located in the hypothalamus section so increase food intake. When the cells in the condition lack of energy, adipose

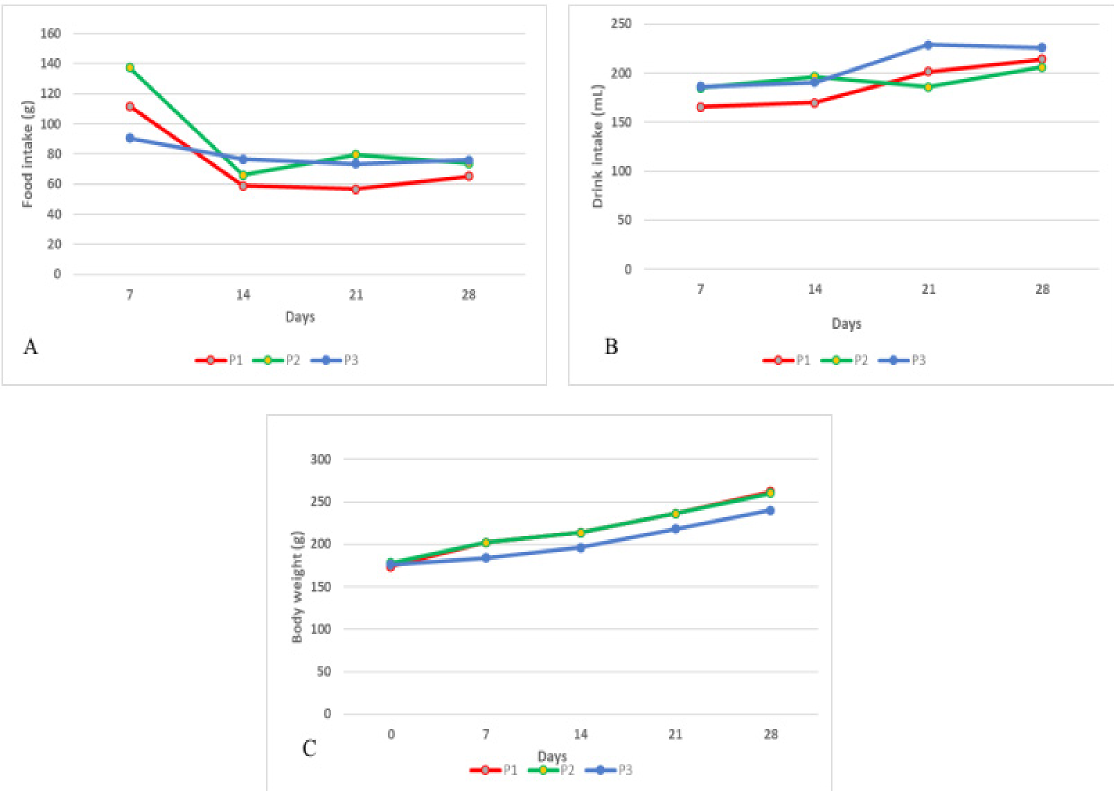

Figure 2. Graphic of food intake (A), drink intake (B) and body weight (C) of various treatments.

Table 1. The Effect of treatments on food intake, drink intake and body weight of hyperglycemic rats

Parameter	P1	P2	P3
Food intake	73.12^{a}±10.18	89.12^{b}±9.02	79.07^{ab}±8.38
Drink intake	187.90^{a}±22.81	193.46^{a}±19.17	208.11^{a}±38.94
Body Weight	217.60^{a}±12.52	218.00^{a}±12.00	202.80^{a}±13.90

Note: The same superscript letters indicates no significant different result (P<0.05).

cells will be overhauled to get energy. Overhauled of adipose cell causes the signals to stimulate satiety (leptin) decrease. The decrease of leptin signals make easily hunger. The administration of glibenclamide or aqueous extract of *T. diversifolia* at different doses in hyperglycemic rats was able to suppress polyphagia, which was shown to decrease on day 14 to day 28 of treatment. Decrease in food intake is because the glibenclamide or aqueous extract of *T. diversifolia* capable of repairing insulin secretion so that the glucose can enter the cell. Adequate glucose availability in the cell activates the satiety in the hypothalamus so that hunger is reduced. The reduced hunger will directly reduce the food intake. In addition, another factor that affect the decrease in food intake is an increase of drink intake. Increasing of drink intake can reduce the appetite.

The antihyperglycemic ability of *T. diversifolia* in the treatments had no effect on drink intake of hyperglycemic rats (shown in Figure 2B). Statistically, consumption of drinks in glibenclamide treatment or aqueous extract of *T. diversifolia* were not significantly different. Glibenclamide acts to increase insulin secretion, increased insulin secretion will increase glucose absorption in cells. Chemical compounds found in *T. diversifolia* work together to improve glucose availability in cells. Flavonoids play a role in increasing insulin release by regenerating pancreatic Langerhans cells and inhibiting aldose reductase inhibitors, polyphenols play a role in reducing free radicals in the body, while tannins are thought to play a role in inducing phosphorylation of insulin receptors with glucose transporters 4 (GLUT 4) (Sandhar *et al.*, 2011; Pasaribu *et al.*, 2015; Liu *et al.*, 2005).

Figure 2 describes glibenclamide or aqueous extract of *T. diversifolia* treatment can increase the body weight of hyperglycemic rats. Statistically the increase on body weight of both treatments was not significantly different. The increase in body weight in the treatment of aqueous extract of *T. diversifolia* was thought to be related to the ability of glucose to be absorbed into the cell. Sesquiterpen lactone that can found in *T. diversifolia* can increase the sensitivity of insulin. Sesquiterpen lactone compounds and tagitinin A can increase peroxisome proliferator-activated receptors (PPARs) ϒ activity so as to increase insulin sensitivity (Lin, 2012). PPARs ϒ activation can increased the expression of a number of genes associated with glucose metabolism and regulated insulin transcription in pancreatic β cells (Fauziah *et al.*, 2018). Increasing body weight in glibenclamide treatments was thought to be a side effect of the ability to reduce blood glucose levels. The ability of glibenclamide to stimulate insulin secretion causes more glucose to be absorbed by the cell, so that the body weight increases. Glucose that can enter the cell can prevent the occurrence of lipolysis in adipose tissue so that there is no breakdown of fat and body weighy will gradually increase. The center of feeling full in the hypothalamus will also be activated because glucose in the cells is sufficient so that polyphagia can be reduced. Increased absorption of glucose into cells, reduction of lypolisis will be able to produce a decrease in blood glucose levels.

Based on the data obtained, the result can be used as a preclinical analysis to determine the most effective dose of aqueous extract *T. diversifolia* for decrease blood glucose levels. The potential dose ranged from 150-400 mg/BW rats. The aqueous extract of *T. diversifolia* on 150-400 mg/BW of rats can reduce blood glucose levels, reduce feed consumption and increase body weight as good as glibenclamide effect. So, this research can be recommended for herbal medicines to reduce blood glucose levels.

CONCLUSION

Aqueous extract of *T. diversifolia* leaves have potential as antihyperglycemic herbal medicine. Doses 150 mg aqueous extract of *T. diversifolia* leaves/kg of rat's BB have the same ability with glibenclamide to decrease blood glucose levels in hyperglycemic rats.

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