



## The activity of torch ginger's (*Etilingera elatior*) flower methanol extract to reduce the levels of free fatty acid, blood glucose, and triglyceride in obese wistar rats

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### Abstract

Torch ginger (*Etilingera elatior*) is a plant rich in antioxidants such as flavonoids, tannins, polyphenols, saponins, and steroids. Torch ginger has activity as antidiabetic. This study aims to determine the potential of torch ginger flower methanol extract in reducing levels of free fatty acids, blood glucose, and triglycerides in obese rats. The study was conducted *in vivo* using 18 female Wistar rats, which were divided into three groups, namely a normal control group, a high-fat diet control group, and a treatment group (high-fat diet + 100 mg/kg BW torch ginger flower extract) and the phytochemical test was carried out *in vitro*. The treatment lasted for 30 days, and after that, the rats were fasted for 10-12 hours to take their blood which would later be used to examine free fatty acids, blood glucose, and triglycerides. The results showed that the methanolic extract of torch ginger flower contained polyphenols, flavonoids, alkaloids, saponins, and steroids. *In vivo* results showed that a dose of 100 mg/kg BW of torch ginger flower extract was able to reduce levels of free fatty acids, blood glucose, and triglycerides with a percentage reduced in free heavy acid levels by 26.60%, glucose levels by 43.53%, and triglyceride levels by 57.39% compared to the high-fat diet control group.

**Keywords:** blood glucose, free fatty acids, torch ginger, phytochemical test, triglycerides

### Introduction

A high-fat diet and lack of activity are the causes of obesity. Obesity is a condition in which the body has excess fat levels. Obesity results from excess fat accumulation that causes degenerative diseases such as type II diabetes, hypertension, dyslipidemia, insulin resistance, atherosclerosis, and arthritis. Diabetes mellitus is a disease caused by metabolic disorders that cause abnormalities in insulin secretion and impaired insulin work. Usually, diabetes mellitus is characterized by high levels of glucose in the blood. Type II diabetes is obesity which can increase oxidative stress and accelerate atherosclerosis (buildup of fat in artery walls).

Efforts to prevent and treat diabetes mellitus are mostly made using synthetic drugs but have many side effects. The bad side effects caused by synthetic drugs trigger most diabetics to be interested in other alternative treatments that use natural ingredients. Medications with herbal ingredients have fewer side effects than synthetic drugs. Alternative medicine to treat diabetes comes from plants that contain secondary metabolites such as flavonoids, polyphenols, steroids, tannins, triterpenoids, alkaloids, and saponins. All these compounds were reported to have antioxidant activity. Flavonoids decrease blood glucose levels by inhibiting damage to pancreatic beta cells. Besides that, flavonoids also act as antioxidants that can bind free radicals <sup>[1]</sup>.

Torch ginger is one of the plants originating from Indonesia. Researchers have recently become the center of attention because of their antioxidant, anti-inflammatory, anticancer, and antibacterial activities. Handayani *et al.* (2014) <sup>[2]</sup> reported that torch ginger has antifungal pharmacological effects; Also, torch ginger is registered as anti-aging. Another study on torch ginger showed that torch ginger flower extract could reduce blood glucose levels in male Swiss Webster mice with hyperglycemia <sup>[3]</sup>.

Based on this background, the researchers intend to reveal the effect of torch ginger flower methanol extract in reducing levels of free fatty acids, blood glucose, and triglycerides in obese Wistar rats, which has not been done much.

### Materials and Methods

#### Material used

Torch ginger flower, methanol, distilled water, Wagner reagent, NaOH, FeCl<sub>3</sub>, concentrated HCl, dilute HCl, PP indicator, Mg powder, H<sub>2</sub>SO<sub>4</sub>, anhydrous acetic acid, chloroform, oxalic acid.

#### Method

##### Torch ginger flower processing stage

Fresh samples of torch ginger flowers (*Etilingera elatior*) were washed in running water and drained. Torch ginger flowers are cut into small pieces and then dried by aerating. The dried torch ginger flowers are then mashed using a blender.

### Torch ginger flower extraction

The method used to extract torch ginger flowers is the maceration method with 1 kg of torch ginger flower powder soaked using 96% technical methanol solvent for 24 hours. Then the pulp and the filtrate are separated. The filtrate was accommodated, and the dregs were macerated similarly until extracted the compounds. The filtrate obtained was collected and evaporated using a vacuum rotary evaporator to get a thick extract. The thick methanol extract was weighed and put into a sterile container, followed by activity tests using Wistar rats and phytochemical tests.

### Phytochemicals Screening

One milligram of methanol extract from the flowers of torch ginger (*Etilingera elatior*) was subjected to phytochemicals screening following the methodology of Harborne, 1987 [4].

### Animal test

A total of 18 female Wistar rats were separated into three groups: normal control, high fat diet control, and treatment group. Normal control rats were fed a standard CP550 diet and drinking water ad libitum, while control rats on a high-fat diet were fed a high-fat diet (lard and egg yolk), and the treatment group was given a high-fat diet and 100 mg/kg BW of torch ginger flower methanol extract. The treatment lasted for four weeks, and on the last day, the rats were fasted for 10-12 hours to collect their blood which would later be used to examine free fatty acids, blood glucose, and triglycerides.

### Measurement of blood glucose

Blood glucose levels were measured using a glucometer in mg/dL. A blood sample is taken and then touched to a test strip on a glucometer and left until the blood glucose level is read and recorded. Normal fasting blood glucose levels in rats are 50-145 mg/dL; if more than 145mg/dL, the rat is declared to have diabetes.

### Measurement of FFA

Determination of free fatty acid levels in rats was carried out using acid-base titration using the Folch method. 10 mL neutral solution of methanol and chloroform (1:2) v/v plus 200 L (0.2ml) of blood serum and 2-3 drops of phenolphthalein indicator, then titrated with NaOH until the color changes to pink.

### Measurement of triglyceride

Triglyceride levels were tested using the ERBA-XL 100 instrument using the working principle of spectrophotometry with Sekisui Medical triglyceride reagent No. Lots. R1: 105R10 and R2: 105R10.

## Result and Discussion

### Phytochemical test result

In this study, 1 kilogram of torch ginger flower powder was extracted by the maceration method using methanol as a solvent to produce 116.24 grams of thick brown torch ginger flower extract with a yield value of 11.62% and a water content value of 9.90%. The results of the phytochemical tests are presented in Table 1.

**Table 1:** Phytochemical test result of torch ginger flower methanol extract

No	Phytochemical test	Reagent	Result	Description
1	Polyphenol	FeCl <sub>3</sub>	+	Changes in color from light green to blackish green
2	Flavonoids	Mg and HCl. Powder	+	Changes from light green to orange
3	Saponins	HCl 1%	+	The foam does not disappear after the addition of HCl
4	Alkaloids	Wagner	+	A brown precipitate is formed
5	Steroids	Liebermann-Burchard	+	Change from light green to dark green

+ (detected)

- (not detected)

Torch ginger flower methanol extract showed positive reactions in all secondary metabolite tests. These secondary metabolites have been reported to have antioxidant, antiobesity, and antihyperglycemic activity [5].

### The activity of Torch ginger Flower Extract

Rats treated for 30 days had their blood taken to analyze blood glucose levels, free fatty acids, and triglycerides. The average value of the post-test results for each parameter is presented in Table 2.

**Table 2:** Average of blood glucose, FFA, and triglycerides

Factor	Glucose (mg/dL)	FFA (mmol/dL)	Triglycerides (mg/dL)
Control	80,67 ± 2,944 <sup>b, c</sup>	11,306 ± 1,531 <sup>b</sup>	134,17 ± 3,312 <sup>b, c</sup>
HF	231,67 ± 9,136 <sup>a, c</sup>	17,005 ± 0,746 <sup>a, c</sup>	209,67 ± 2,160 <sup>a, c</sup>
P100	130,83 ± 3,869 <sup>a, b</sup>	12,483 ± 1,696 <sup>b</sup>	89,33 ± 4,456 <sup>a, b</sup>

Results are expressed in mean  $\pm$  SD. SD = standard deviation. <sup>a</sup> indicates a significant difference from control; <sup>b</sup> shows a significant difference from HF (high fat); <sup>c</sup> shows a significant difference from Interest P100.

The post-test results showed a significant difference between the high-fat and the torch ginger flower treatment in each parameter (blood glucose, FFA, and triglycerides). It indicates that administering torch ginger flower methanol extract at a dose of 100 mg/kg BW every day for four weeks can reduce blood glucose levels, free fatty acids, and triglycerides.

Rat's blood glucose levels

### Rat's blood glucose levels

Glucose is a carbohydrate that plays an important role in the body. Glucose is a source of energy and carries out the normal functions of various tissues. Glucose levels are influenced by several factors, one of which is obesity. Obesity can increase oxidative stress, which causes metabolic disorders in the body resulting in pancreatic beta cell failure and, in the long term, will result in hyperglycemia [6]. The results of the examination of the average glucose levels are presented in Table 2.

The administration of torch ginger flower methanol extract showed a decrease in blood glucose percentage of 43.54% in the high-fat control group. The content of secondary metabolites with antioxidant and antihyperglycemic properties contained in torch ginger flower extract is thought to help decrease blood glucose levels in obese Wistar rats by inhibiting the reaction of peroxides that damage pancreatic beta cells. The results of phytochemical screening showed that the torch ginger flower extract contains several secondary metabolites such as flavonoids, alkaloids, terpenoids, saponins, and tannins which act as antioxidants. Flavonoids can lower blood glucose by slowing down the action of GLUT2 (Glucose Transporter Isoform 2), a glucose transporter protein in the intestinal membrane, resulting in decreased blood glucose levels. Flavonoids are also able to rejuvenate damaged pancreatic  $\beta$ -cells so that they can overcome insulin deficiency [7].

### FFA levels of rat blood

In obesity, high free fatty acid values are often found. The high level of fatty acids in obesity is caused by the breakdown of triglycerides in fat tissue, especially in the high abdominal area. High levels of fatty acids in the blood result in insulin resistance, followed by an increase in the uptake of free fatty acids by muscle tissue. Under normal circumstances, muscles use glucose to produce energy, so the oxidation of fatty acids in the muscles increases. It interferes with glucose absorption by forces, so hyperglycemia occurs (high blood glucose). [8] whereas what happens in the liver, the liver will accommodate fatty acids, which will later be used as materials for the gluconeogenesis process and the production of VLDL (Very Low-Density Lipoprotein). The increased gluconeogenesis process causes fasting blood glucose also to increase. The results of the determination of the average free fatty acid content are shown in Table 2.

The administration of torch ginger flower methanol extract showed a decrease in the free fatty acid percentage of 26.60% in the high-fat control group. Compounds that act as antioxidants, such as flavonoids and phenolics, can reduce free fatty acid levels. Flavonoid compounds also have activity as anti-hyperlipidemia and anti-hyperglycemia. Antioxidants are needed to prevent oxidative stress due to an imbalance in the number of antioxidants in the body with the number of free radicals present. Free radicals can oxidize lipids and are very reactive, interfering with fat metabolism. Fatty acids cannot be used as fuel for the body's metabolism when fatty acid oxidation is decreased. Antioxidants that are easily oxidized cause antioxidants to be oxidized so that they can protect molecules in cells from damage. The supported research [9] about the potential of torch ginger as an antioxidant against the activity of superoxide dismutase (SOD), glutathione (GSH), and fatty liver of obese rats showed that torch ginger stem and flower extracts were able to increase the concentration of SOD and GSH and seen from liver histology results also showed a decrease in fatty liver of obese rats.

### Triglyceride levels

Triglycerides are a type of fat that is carried in the blood and stored in adipose tissue. High triglycerides generally indicate that the number of calories consumed is more than the number of calories burned during activity. Under certain conditions such as obesity, triglyceride levels can rise to more than 200mg/dL and even reach more than 500 mg/dL, which is called hypertriglyceridemia [10].

The administration of torch ginger flower methanol extract showed a 57.39% reduction in triglyceride levels in the high-fat control group. Reducing triglyceride levels by extracts of torch ginger stems and flowers can occur due to the role of antioxidant compounds, one of which is flavonoids. Flavonoids play a role in reducing triglyceride levels by increasing the work of the lipoprotein lipase enzyme [11]. This statement is supported by research [12] which states that increasing the enzyme lipoprotein lipase causes VLDL (Very Low-Density Lipoprotein) triglyceride carriers to be hydrolyzed into fatty acids and glycerol. The free fatty acids will be absorbed in muscles, and other tissues, which are then oxidized to produce energy, and adipose tissue will store them as energy reserves in the body. Another study stated that the antioxidant content (saponins and tannins) in *P. indica* leaf extract was thought to be able to reduce triglyceride levels in sucrose-induced rats. The pancreatic lipase enzyme plays a role in the hydrolysis of free fatty acids, where tannin compounds have an inhibitory effect on this enzyme. Saponins can bind fat in the intestinal lumen and make complex compounds that are insoluble and not easily absorbed by the intestinal mucosa. In addition, saponins can increase the production and secretion of bile and facilitate lipid metabolism so that there is a decrease in triglyceride levels.

**Conclusion**

Oral administration of torch ginger flower methanol extract at a dose of 100 mg/kg BW to Obese Wistar rats fed a high-fat diet reduced free fatty acids, blood glucose, and triglycerides in obese Wistar rats.

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