

# Antihyperglycemic Activity of Ethanolic Herb Extract of Ceplukan (*Physalis angulata* L.) in Diabetic Hypercholesterolemia in Male Hamsters

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Abstract: Diabetes mellitus is a disease characterized by hyperglycemia as well as progressive changes to the pancreatic beta cell structure. This study was conducted to determine the antihyperglycemic activity of ceplukan herb extract (*Physalis angulata* L.) in alloxan-induced male Syrian hamsters and high cholesterol feed. The study used 24 hamsters divided into six groups. Group I were given a standard diet and regular drinking water, Group II were given metformin dose 61,66 mg/kg body weight (BW), Group III were alloxan-induced and high cholesterol feed, Groups IV, V and VI were given extract dose 60, 120 and 240 mg/kg BW respectively. The animals were induced alloxan monohydrate as well as were given high cholesterol feed during treatment. Blood sampling was performed on the 29th and 44th day using a clinical spectrophotometer. The results show significant differences between treatment groups ( $\alpha < 0,05$ ), followed by Tukey test. In conclusion, the preparation of herbal extract test ceplukan at doses of 120 and 240 mg/kg BW could reduce blood glucose levels by 50.84% and 43.41% which is equivalent to metformin dose of 61.66 mg/kg BW with a percentage of 53.12%.

## 1 INTRODUCTION

Diabetes mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia and abnormalities in carbohydrate, fat, and protein, resulting from insulin secretion abnormalities, insulin work or both (Dipiro, 2014). Diabetes mellitus is divided into two types, namely type I DM (5%-10% of cases), which is caused by damaged pancreatic  $\beta$  cells suffered by hereditary or genetic and type II DM (90% of cases) caused by insulin resistance due to increased lipolysis, free fatty acid production or increased glucose production in the liver (Dipiro, 2015).

DM in Indonesia is a serious threat to health development, can cause blindness, kidney failure, diabetic feet (gangrene) so it must be amputated, heart disease and stroke (Depkes, 2013). The *World Health Organization* (WHO) Global Status Report on NCD in 2010 stated that 60% of the cause of death of all ages in the world is due to non-communicable diseases and DM is ranked 6th as the cause of death. Approximately 1.3 million people die from diabetes and 4% die before the age of 70

years. In 2030, it is estimated that DM will be ranked 7th as the cause of death worldwide and that Indonesia will have 21.3 million people with DM (Depkes, 2013). This condition will make Indonesia on the 4<sup>th</sup> position after the United States, China and India as countries that have the largest diabetics with the largest population in the world (Depkes, 2012).

One of the medicinal plants that can be used as an antidiabetic agent is *ceplukan* (*Physalis angulata* L.). In West Java, ceplukan has been used as diabetes drug (Sutjiatmo *et al.* 2011). Chemical compounds of this plant include alkaloids, flavonoids, saponins, fisalin, sterols/terpenes and citric acid (Depkes, 1995). Traditionally, this plant has been used to treat boils, and diabetes (Depkes, 1995).

Previous research has shown that the ethanol extract of ceplukan leaves at 100 mg/kg BW can reduce blood glucose levels in mice with a percentage of 56.34% (Kasali, 2016). In isolation research from ethanol extract of ceplukan fruits at 25 mg/kg BW and 50 mg/kg BW for 15 days can decrease rat blood glucose level to 38,75% and 27,55% respectively (Raju, 2015). Another study has

reported that the fraction of chloroform herb ceplukan at a dose of 0,5 mg/20g BW, 1 mg/20g BW and 2 mg/20g BW can reduce blood glucose levels in mice and the dose 2 mg/20g BW can reduce blood glucose levels of mice equally to glibenclamid 0,013 mg/20g BW (Sunaryo *et al.* 2012).

## 2 MATERIALS AND METHODS

### 2.1 Materials

The ingredients used are ceplukan herb (*Physalis angulata* L.). Determination was done at "Herbarium Bogoriense" Botani section, Pusat Penelitian Biologi - LIPI Cibinong.

Alloxan monohydrate from Sigma-Aldrich Co., USA. Metformin HCL from Sohan Healthcare Pvt Ltd, Maharashtra. High cholesterol feed, glucose kit reagent was using commercial kits purchased from Human Diagnostics Worldwide Germany.

### 2.2 Animal Subjects

The experiment used twenty-four male Syrian hamsters (*Mesocricetus auratus*), aged 3–4 months and weighed around 80 g. The animals were housed under standard environmental conditions. The study protocol was approved by the Health Research Ethics Committee of Universitas Muhammadiyah Prof. Dr. Hamka, West Jakarta, Indonesian. The reference number for approval was 02/18.05/005.

The animal subjects were acclimatized in a cage for approximately one week to adapt to the new environment. The animals were divided into 6 groups consisting of 4 hamsters.

### 2.3 Extraction

One kilogram of ceplukan dried powder (Research Institute for Spices and Medicinal Plants/BALITTRO, Bogor, Indonesia) was macerated with 70% ethanol for 6 hours and was stirred occasionally to reveal the active ingredients. The mixture was allowed to stand for 18 hours. The maceration result was then separated by filtration. The reproduction process was repeated three times. The maceration result was concentrated with a vacuum rotary evaporator until it became a viscous extract (Department of Health RI, 2008). The concentrated result was put in a water bath at 40–50°C for one day to obtain an extract with a constant weight.

### 2.4 Phytochemical Screening

The phytochemical screening of ceplukan herb extract included an examination of alkaloids using Bouchardat reagents, testing flavonoids with methanol, concentrated HCL and magnesium powder. Saponin test were performed with foam formation, tannin test with FeCl<sub>3</sub> 1% reagent, and Steroid testing with ethanol, concentrated H<sub>2</sub>SO<sub>4</sub> were done if there is a red or purple color change indicating the presence of triterpenoids and the green color indicates steroid presence (Depkes, 1995). Extract yield was determined by calculating the dry extract weight obtained from the weight of dry powder before extraction.

### 2.5 Category and Animals Subject Treatment

The experiment was done with a complete randomized design, using 24 white male, hamsters divided into six groups consist of 4 rats.

- a. Group I: The group was given a standard diet and regular drinking water.
- b. Group II: Positive Control, was given alloxan monohydrate, high cholesterol feed and comparative preparations.
- c. Group III: Negative Control, was given alloxan monohydrate, high cholesterol feed and Na-CMC 0,5%.
- d. Group IV: was given alloxan monohydrate, high cholesterol feed and ethanolic herb extract of ceplukan dose I.
- e. Group V: was given alloxan monohydrate, high cholesterol feed and ethanolic herb extract of ceplukan dose II.
- f. Group VI: was given alloxan monohydrate, high cholesterol feed and ethanolic herb extract of ceplukan dose III.

### 2.6 Method of Glucose Levels Measurement

Blood sampling was taken on Day 29 and Day 44; hamsters were first anaesthetized using ketamine dose 10 mg/kg BW (Lacy *et al.* 2009). Blood collection was performed at the eye orbital sinuses after the animals were previously preoccupied for 12 hours. The blood was taken as much as one mL, and was collected in a microtube. The blood was centrifuged at 4000 rpm for 15 minutes to obtain the serum (Suharmiati, 2003). Serum was taken ten µL, was mixed with enzyme reagent (glucose reagent kit) 1000 µL, then was homogenized using vortex

Table 1: Data extraction results

No.	Type	Result
1.	Ceplukan Fresh	15 kg
2.	Ceplukan Dry	1,5 kg
3.	Ceplukan Powder	1,1 kg
4.	Macerate of Powder	1 kg
5.	Extract	158,86 g
6.	Extract Yield	15,886 %

for 1 minute and was incubated for 5 minutes at 37°C blood glucose level were tested with a clinical photometer (Human, 2012).

## 2.7 Data Analysis

The data using statistical analysis show that the decrease in percentage data from the initial and late glucose levels, baseline levels are levels after alloxan induction and high cholesterol feed while the final content levels after treatment. The data were tested for normality and homogeneity and were then analyzed with one-way ANOVA test with 95% significance level ( $\alpha = 0,05$ ). If there is a significant difference, the data were then analyzed with the Tukey test (Santosa, 2010).

## 3 RESULTS AND DISCUSSION

Based on the results of the determination, indicating that the plant is *Physalis angulata* L. included in Solanaceae. Data extraction of ceplukan herb such as weigh ceplukan fresh and dry, weigh of ceplukan extract and extract yield are given in Table 1.

Parts of ceplukan that used is the herbaceous part. A report by The Department of Health (1995) stated that chemical compounds found in ceplukan include alkaloids, flavonoids, saponins, fisalin, sterols/terpene and citric acid. Alkaloids, flavonoids, saponins, and steroids could be obtained by phytochemical screening. (Table 2). The results of extract quality characteristics showed specific smell, thick form and had a drying loss of 9.59% can be seen in table 3.

Metformin was used as a comparative drug in this study because it is a first-line antidiabetic drug against DM2 patients with a history of obesity. In this study, the test animals not only being treatment to become hyperglycemia, but also induced to be hypercholesterolemia. Then, induction of alloxan will damage cells  $\beta$  Langerhans pancreatic cells to inhibit the production of insulin. Therefore

Table 2: Results of Phytochemical Extract Screening

No.	Chemical compound	Extract
1.	Alkaloid	+
2.	Saponin	+
3.	Tanin	-
4.	Flavonoid	+
5.	Steroid	+
6.	Triterpenoid	-

Note: “+” (positive), “-“ (negative)

metformin has a mechanism of increasing peripheral tissue sensitivity of insulin (Suharmiati, 2003) and it was chosen as a comparative drug preparation in this study because the mechanism was aligned with the alloxan to correct the damage caused by alloxan.

Feeding for the hypercholesterolemic hamsters was performed for 44 days to maintain the stability of cholesterol. On Day 22, alloxan was induced intraperitoneally with a dose of 180 mg/kg BW. Before blood sample collection, the hamsters were fasted for 12 hours to avoid the effect of increased glucose level after meal. Measurement of baseline blood glucose level of the test animals were performed on Day 29 to determine whether the induced alloxan had successfully increased the blood glucose level. After the level of glucose and cholesterol were stated to increase pathologically (the level of complication was achieved), then the extract was orally administered by using feeding tube. The extract was given every morning for 14 days. On Day 44, the final blood glucose level was checked to see whether there is a decrease or not.

The data of the percentage of decrease of blood glucose level was examined by normal distribution test  $\{(\alpha = 0,202) > 0,05\}$  and homogeneity test  $\{(\alpha = 0,104) > 0,05\}$  which show that the data is normally distributed and homogeneous. One-way ANOVA test was run to test whether the mean of % decrease in the blood glucose level is the same or significantly different. The result  $\{(\alpha = 0,000) < 0,05\}$  shows that there are significant differences between all groups. Then the one-way ANOVA results were further examined with the Tukey test to

Table 3: Extract characteristics

No.	Type of Test	Extract
1.	Organoleptic	
	Smell	Specific
	Color	Brown Green
	Form	Thick, bitter
2.	Flavors	9,59%

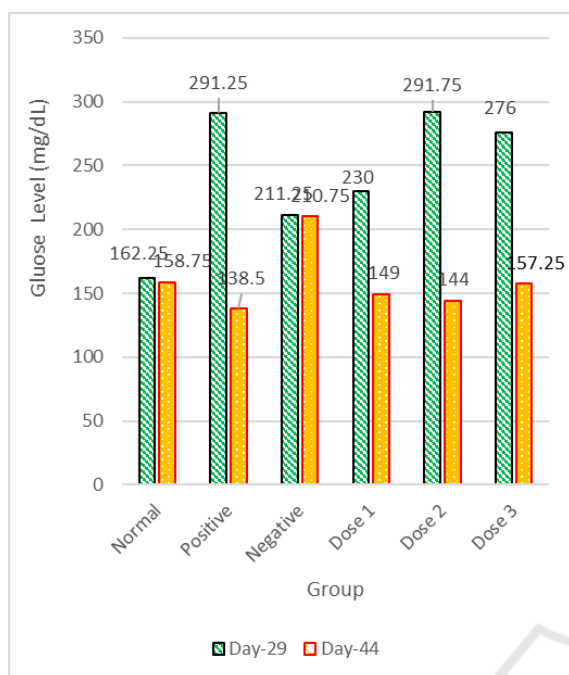


Figure 1: The average percentage decrease in blood glucose levels in the administration of ceplukan herb extract

know which group has, or has no significant difference between normal control, negative control, positive control, Dose 1, Dose 2 and Dose 3.

The-ability to decrease blood glucose is related to the biological activity of compounds in ceplukan plants. The compounds contained are flavonoids, alkaloids, steroids, and saponins. The chemical content of steroids can stimulate the release of insulin from the pancreas to lower blood glucose levels (Sediarso *et al.* 2008). Insulin will then work to increase the transport of glucose from the blood into cells by increasing the permeability of the cell membrane to glucose. Once inside the cell, glucose will then be used to generate energy. The liver and muscles will convert glucose into glycogen and will then be stored there. This conversion causes the blood glucose level in the body decreases slowly (Gustina, 2012).

Figure 1 shows the plasma glucose levels of the experimental animals. The highest decrease in blood glucose was shown by Dose 2 (120 mg/kg BW) which was 50,84% compared to Dose 3 (240 mg/kg BW) which was 43,41% and Dose 1 (60 mg/kg BW) which was 34,74%. All pharmacologic responses must have the maximum effect (Emax), regardless of the high concentration that occurs, a point is reached when adding the concentration does not

increase the response (Katzung *et al.* 2014). This occurs in Dose 2 that was smaller than Dose 3, but Dose 2 gave a greater decrease effect compared to Dose 3, the maximum effect occurred in Dose 2; although Dose 3 twice higher. Therefore, this study shows that Dose 2 (120 mg/kg BW) has a better activity in lowering blood glucose by 50,84%.

## 4 CONCLUSION

It can be concluded that ceplukan extract can lower blood glucose levels in male hamsters with hypercholesterolemia and hyperglycemia. Dose 2 (120 mg/kg BW) has a better activity in lowering blood glucose by 50,84% and equivalent to metformin dose 61,66 mg/kg BW which was 53,12%.

## FUTURE RECOMMENDATION

The next stage of the research may focus on the content of plant compounds and fractionation phase as a decrease in blood glucose levels.

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