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*Full Length Research Paper*

# Effects of anti-hyperglicemic of yacon leaf (*Smallanthus sonchifolius*) ethanolic extracts using zebrafish as a model

**Zuhelmi Aziz, Nanda Tamara, Rahmi Three Wahyuni, Inawati and Syamsudin Abdillah***

Department of Pharmacology, Faculty of Pharmacy, Pancasila University, South Jakarta 12640, Indonesia.

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**This study aims to investigate the anti-hyperglicemic effects of yacon leaf (*Smallanthus sonchifolius*) using zebrafish. In this study, zebrafish is used as a model, with alloxan compounds to induce hyperglicemic. The induction stage consists of 3 phases: in the first the zebra was induced at 0.3% for 30 min, in the second stage, it was transferred to a 3% D-glucose solution for 30 min, and in the last, immersion was carried out in a solution containing yacon leaf extract with doses of 20, 30 and 40 mg/ml for 3 h in 3 consecutive days.The results showed that yacon leaf extract in doses of 20, 30 and 40 mg were able to reduce blood glucose levels in zebrafish induced by alloxan.The results indicated that 95% ethanolic extract has an antidiabetic effect.**

**Key words:** Yacon leaf (*Smallanthus sonchifolius*), zebrafish, alloxan.

## INTRODUCTION

Diabetes mellitus affects a large percentage of the world's population, significantly altering their quality of life (Lawrence et al., 2008). The definition of diabetes mellitus is a metabolic disease caused by a lack of insulin production or inability of the body to respond to insulin (Stumwoll et al., 2005).

The World Health Organization (WHO) predicts an increase in the number of populations living with diabetes mellitus, considering it a global health threat (WHO, 2016). In Indonesia, it is estimated to rise from 8.4 million in 2000 to 21.3 million by 2030, with a staggering increase of 2-3 times that by 2035 (Wild et al., 2004). Meanwhile, the International Diabetes Federation (IDF, 2015) predicts an increase of 9.1 million in 2014 with an expected 14.1 million by 2035 (IDF, 2015).A natural ingredient found in Indonesia and capable of reducing blood glucose levels is the yacon (*Smallanthus sonchifolius*) plant. In a study conducted by Genta et al. (2010), it was found that a decrease in blood glucose levels from the extract was caused by caffeonylquinic, caffeic and chlorogenic compounds. In addition, enhydrin and sesquiterpenic lactone in the leaves are effective for reducing post-prandial glucose and is useful in the treatment of animal diabetes (minimum dose: 0.8 mg / kg body weight). According to research conducted by Dou et al. regarding chemical compounds from *Smallanthus sonchifolius*, a structure of new compounds were identified that are as efficacious as diabetes agents,

*Corresponding author. E-mail: syamsudin.abdillah@gmail.com.

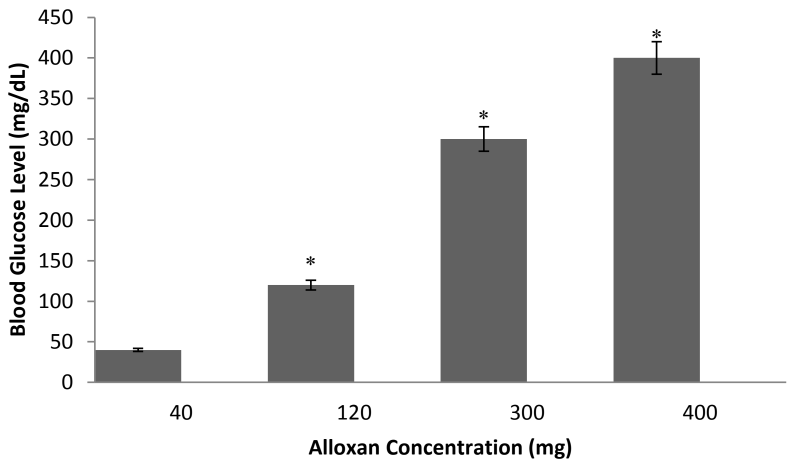


**Figure 1.** Blood glucose levels on alloxan solution concentration. The zebrafish were exposed to various alloxan concentrations in half saline solution; 40 mg/100 ml (40 mg), 120 mg/100 ml (120mg), 300 mg/100 ml (300 mg), or 400 mg/100 ml (400 mg) for 30 min, moved to a 1% water glucose solution in water for 30 min, and then exposed to water for 1 h. After induction, blood glucose levels were measured; *p < 0.05.

namely ent-Kaurane-3β,16β, 17,19-tertol, ent-Kaurane-16 β, 17,18,19-tertol, ent Kaurane-3β, 16β, 17-triol, ent-Kaurane-16β, 17-diol-19-oic acid, ent-Kauran-6β, 17,18-triol and 1-Pentacosanol (Dou et al., 2010).

The yacon leaves contain fructooligosaccharide, carbohydrates, and flavonoids which can reduce glucose levels in blood and effectively transport components of phenol and caffeonylquinic which inhibit glucosidase (Djamil et al., 2014). Baroni et al. (2008) found that the crude extract of yacon leaves significantly reduced blood glucose levels of diabetic rats in the experimental model induced by STZ. Most of the available models are rodent-based, which have drawbacks in that they are labour intensive and because of ethical issues, only small groups of animals can be used. To overcome these limitations, the zebrafish (Danio rerio) has been increasingly used to study diabetes and its related diseases, chosen because of the high similarities in organ physiology and metabolism between zebrafish and mammals (Zhang et al., 2017).

Zebrafish is an excellent model when compared to rodents in understanding the conditions and pathology of vertebrates (Zhang et al., 2018). Used in previous studies and induced by alloxan monohydrate at a dose of 300 mg and glucose 1%, considerable damage to the pancreatic Langerhans cells, causing diabetes was observed (Shin, 2012). This study aims to determine the effect of 95% ethanolic extracts of yacon leaves (*Smallanthus sonchifolius*) on the production of insulin using the zebrafish model. The development of zebrafish-based antidiabetes compound screening is founded on the discovery of marked similarities, which justify the use of the fish to screen antidiabetic compounds.

## MATERIALS AND METHODS

### Animal experimentals

The experimental animals used in this study were black striped zebrafish weighing180-220 mg, obtained from Bogor Agricultural University, Bogor, West Java and were used after ethical approval. 100 zebrafish were divided into 10 tails per aquarium used during acclimatization and were fed at 4% per tail daily (Kinkel and Prince, 2009). Fish were fed twice daily, at 10 am with gelatin containing shrimp as described below and at 4 pm with Zeigler Adult Diet (Aquatic Habitats). Aerators were installed, to provide oxygen, and to prevent stress and death. Specific conditions were necessary, including approximately 10 h of darkness for rest periods, made by covering a tank with a trash bag. However, zebrafish activities required approximately 14 h of bright lighting, with an optimum temperature of 28.5 (Zhang et al., 2017). After acclimatization, they were chosen on the basis of activity levels and weighed (220-260 mg). The zebrafish were divided into 6 groups, with each consisting of 10 randomly picked tails, and given treatment for 3 days. Group 1 is a normal control, without any treatment, Group 2 a negative control with 3% alloxan monohydrate, hyperglycemic, D-glucose, and treated water induced. Group 3 is a positive control and was given liquid containing metformin. Positive control was comparison; Group 4 with the administration of 20 mg dosage of yacon leaves ethanolic extracts; Group 5, with the administration of 30 mg dosage of ethanolic extracts; Group 6, with the administration of 40 mg dosage of ethanolic extracts.

The zebrafish were exposed to various alloxan concentration in half saline solution; 40 mg/100 ml (40 mg), 120 mg/100 ml (120 mg), 300 mg/100 ml (300 mg) and 400 mg/100 ml (400 mg) for 3 days. After induction, blood glucose levels were measured as shown in Figure 1. To optimize alloxan levels on the zebrafish, alloxan doses of 40, 120, 300 and 400 mg were used. 300 mg / 100 ml dosage was the most optimal as blood glucose levels had

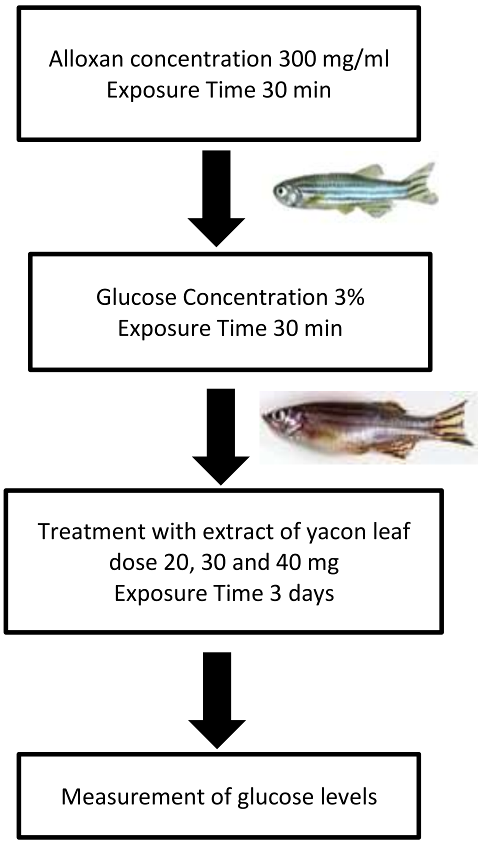


**Figure 2.** Process for antidiabetic assay with Zebrafishmodel.

reached 300.45-378.34 mg / dl and all exposed zebrafishes did not die.The dosage of 400 mg / 100 ml blood glucose levels could reach levels of 400.34-456.67 mg / dl, and therefore, more than 50% of the Zebrafish died. The diabetic zebrafish model induced with only water glucose is required for a longer time compared to the model induced with alloxan.

Changes in the body weight before and after diabetogenic induction is shown in Figure 3. The zebrafish were also weighed to determine the amount of feed to be offered daily. This is used to maintain control variables and identify the differences between the administration of metformin and 95% ethanol extract of yacon leaves.

### Antidiabetic activation test

The selection of the dosage of yacon leaf extract was carried out after optimization; a dose of 20, 30 and 40 mg was given for 3 days after the hyperglycemic induction, and the zebrafish blood glucose levels were measured (Figure 2).

### Statistic analysis

Glucose level data obtained from each treatment group was tested for normality and homogeneity. If the data are normally distributed and homogeneous, parametric statistical tests were carried out using one-way analysis of variance (ANOVA). If the results showed a significant difference, the analysis was then followed by the Tukey Post Hoc test to see the variations in each group at a significance level of 0.05%.

## RESULTS AND DISCUSSION

The data before the acclimatization initially showed non-uniform results; however, afterwards they gained weight and were broadly uniform. This may be a result of food factors, given everyday for 7 days. After the induced hyperglycemic, the zebrafish lost weight. This is because weight loss is one of the typical symptoms of metabolic disorders, experienced by patients with diabetes mellitus, despite having very good appetite (Zhang et al., 2017). The zebrafish lost weight because their energy needs were not met from glucose metabolism because of the fat and protein overhaul. This condition is caused by pancreatic β cell damage after being induced by alloxan, leading to a decrease in the production of in the body, causing glucose to not binding to insulin to produce energy (Zhang et al., 2018; Capiotti et al., 2014).

Figure 4 highlights the blood glucose levels of the normal, diabetic group, positive controls, and administration of yacon leaf extract in 20, 30 and 40 mg dosages. It can be seen that there is a decrease in blood glucose levels by to day 3 in the positive control with yacon leaf extract dosages of 20, 30, and 40 mg. The measurement of blood glucose level of the yacon leaf ethanol extract shows that the higher the dose of ethanol extract, the higher the rate at which blood glucose levels are decreasing in zebrafish.

In the positive control group, metformin had a better decrease in activity because it can reduce more blood glucose levels of zebrafish than the 3 groups of dosages of 95% ethanolic extracts. In another study, the antidiabetic effects of yacon leaf extract were investigated using rat animals induced with streptozotocin. Animal models type 1 diabetes mellitus was caused by the loss of pancreatic β- cells resulting in reduced insulin production (Kinkel and Prince, 2009). An alloxan-induced acute hyperglycemia zebrafish model of type 1 diabetes lead to the generation of a destroyed pancreas. This model is a mild acute hyperglycemia model for short-term experimentation; therefore, further research is needed to develop a permanent model (Kinkel and Prince, 2009).

Based on the results, the hyperglycemic induction with 0.3% alloxan monohydrate using the immersion method with 3% D-glucose and aquadest, damaged the pancreatic β cells of the zebrafish, resulting in reduced insulin production in the body, and high glucose in the blood. Glucose in zebrafish bodies could not bind to insulin and glucose levels in normal controls do not change the condition of hyperglycemia from day 1 to 3. This is due to an increase in blood glucose levels by zebrafish treated with alloxan induction and glucose,

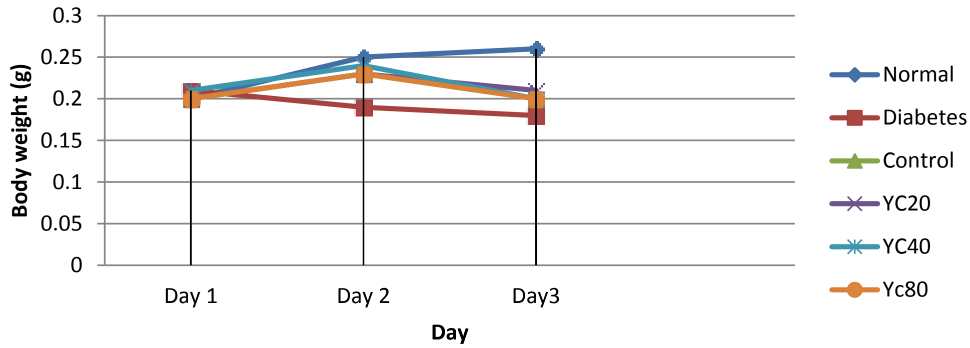


**Figure 3.** Body weight of the zebrafish before and after induction.

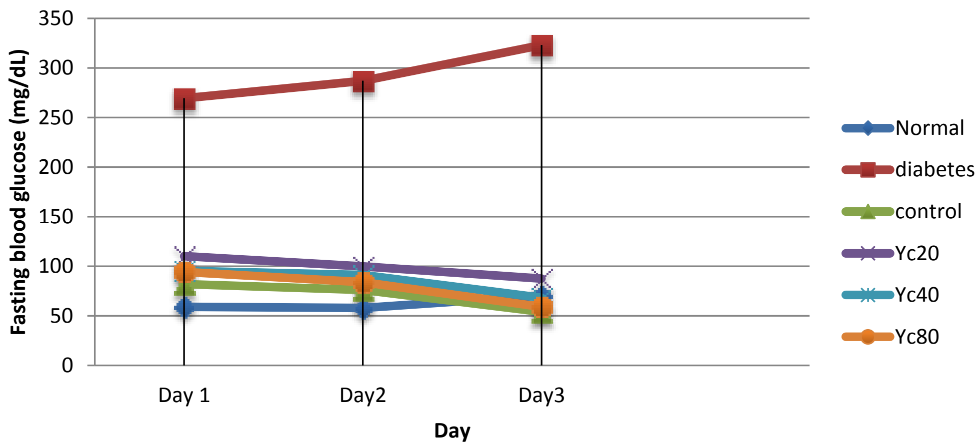


**Figure 4.** Average of blood glucose of treated zebrafish (Day 1, 2 and 3 treatment time with yacon dose 20 mg/100 ml (Yc20), yacon dose 40 mg/100 ml (Yc40), yacon dosen 80 mg/100 ml (Yc80) and with metformine (control), diabetes (negative) and normal).

resulting in an inability to regulate insulin. This led to the destruction of pancreatic β cells which could be seen by increased blood glucose levels of zebrafish. Overall, these data indicated that zebrafish could be induced to the develop mild and stable hyperglycemia and this model was appopriate for these experiments (WHO, 2016). A significant advantage of using zebrafish for diabetes research is that hyperglycemic can be induced by simply adding glucose to the fish water. In contrast, rodent models of diabetes typically require the injection of the toxic glucose analogue, streptozotocin or alloxan, which preferiantally kill pancreatic β cells (Dou et al., 2010).

## Conclusion

The results of this study indicated that yacon leaf ethanol extract could reduce blood glucose levels with a dosage of 40 mg. The research showed that an active compound isolated from yacon leaf extract had an antidiabetic effect.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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