



## Test of Antidiabetic Effect of Taro Leaf Extract (*Colocasia esculenta* L.) on Zebrafish (*Danio rerio*)

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### Abstract

Diabetes mellitus (DM) is a common metabolic disorder defined as chronic hyperglycemia. In addition to the symptoms associated with hyperglycemia itself such as thirst, polyuria and weight loss, it can also cause acute hyperglycemia emergencies that are potentially life threatening. One of the traditional plants that has potential as an antidiabetic drug is the taro plant (*Colocasia esculenta* L.) because it contains chemical compounds such as alkaloids, flavonoids, saponins, tannins and polyphenols which are known to have antidiabetic effects. This study aims to determine the effectiveness and what dose of Taro Leaf Extract gives the best effect of reducing blood glucose levels in zebrafish. This study used zebrafish (*Danio rerio*) induced with alloxan and glucose to raise blood glucose levels. The 20 test animals used were divided into 6 groups, namely group 1 without treatment (normal), group 2 control (-) alloxan induction 0.1% + glucose 1%, group 3 control (+) metformin, group 4 taro leaf extract 200 mg, group 5 taro leaf extract 300 mg, group 6 taro leaf extract 400 mg. Then glucose levels were measured using a glucometer. Data analysis was carried out statistical tests. The results showed that a dose of 400 mg/2L had the ability to reduce blood glucose levels that were not significantly different from normal zebrafish glucose levels.

**Keywords:** Antidiabetic, blood glucose levels, taro leaves, zebrafish

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## 1 Introduction

Diabetes mellitus (DM) is a common metabolic disorder defined as chronic hyperglycemia. In addition to the symptoms associated with hyperglycemia itself such as thirst, polyuria and weight loss, it can also lead to potentially life-threatening acute hyperglycemia emergencies. It is a major cause of morbidity and premature death due to long-term complications such as cardiovascular disease, blindness, kidney failure, amputation and stroke. Blood sugar levels of DM patients must be well controlled to reduce the risk of complications [1].

Diabetes mellitus is caused by disruptions in insulin, glucagon, and other hormone production, resulting in improper glucose and fat metabolism. This is frequently associated with insulin resistance, particularly in patients with type 2 diabetes. [1].

Worldwide, there were 463 million cases of DM in 2019, with 4.2 million deaths. The country with the fifth highest number of people with diabetes in the world is Indonesia. According to Riskesda 2018, DM was found to be 2% more common in Indonesians under the age of 15. The prevalence of DM by gender was 1.78% for females and 1.21% for males. For the average by age group, the most prominent data is in the 55-64 years age group with a percentage of 6.3%. Meanwhile, according to the International Diabetes Federation (IDF) report, it is estimated that there will be around 19.5 million Indonesians aged 20-79 years suffering from diabetes mellitus in 2021[1].

Unlike other diseases, diabetes mellitus requires lifelong treatment and its treatment for the most part can provide a near-normal life. Although there are many oral drugs and insulin to treat DM, they are inadequate and ineffective, especially for long-term treatment, adverse reactions and loss of efficacy may occur. There are many time-tested antidiabetic plants that are very safe and effective when taken appropriately along with lifestyle changes that

include physical activity and mental stress reduction [1].

Hypoglycemia is a common side effect of various diabetic treatments, including insulin, sulfonylureas, and meglitinides, and it is a major barrier to achieving adequate glycemic control. Level 1 hypoglycemia (hypoglycemia alert value; 70 mg/dL [3.9 mmol/L]) may not cause symptoms but is sufficiently low that it should be treated with a fast-acting carbohydrate and may require a dose adjustment of glucose-lowering therapy, level 2 (clinically significant hypoglycemia; 54 mg/dL [3.0 mmol/L]) is sufficiently low to indicate serious, clinically important hypoglycemia, and level 3 (severe hypoglycemia) which is associated with cognitive impairment requiring external assistance for recovery and can be life threatening [1].

The use of medicinal plants in Indonesia for health purposes has been known and practiced by ancestors and passed down from generation to generation [4]. The use of traditional medicine is increasing along with the increase in population, healthy lifestyle and cases of degenerative diseases [5]. As many as 21.4% of the Indonesian population reported using traditional medicine for self-treatment of health problems. The consumption of traditional medicine is increasing by 5.4% every year in Indonesia. Medicinal plants that are widely used as herbal antidiabetics include brotowali, soursop leaves, cinnamon, coriander, mengkudu, bitter melon, salam, sambiloto, tapak dara and temulawak [6].

Taro plant (*Colocasia esculenta* L.) which is a type of plant that is well known to the public as a food ingredient both as a staple food and as an additional food is one of the traditional plants that has the potential for antidiabetic drugs. The utilization of taro fronds and leaves has long been used by the community to make vegetables and traditional medicine [2]. Alkaloids, flavonoids, steroids, tannins, and saponins are some of the secondary metabolites found in taro leaf stalks (*Colocasia esculenta* L.). Flavonoid

secondary metabolites have various properties, including antiviral, anticancer, anti-inflammatory, antioxidant, antihepatotoxic, and antidiabetic [3].

According to previous research, proving that taro leaves (*Colocasia esculenta* L.) contain chemical compounds such as alkaloids, flavonoids, saponins, tannins and polyphenols which are known to have antidiabetic effects [1].

The use of animal models in the world of research is something that has long been recognized. Animal models in DM can be applied to rodents (rodents) such as rats, mice because they have similarities in anatomy, physiology with humans and have a relatively short life span and are easy to handle. Rodent tests have disadvantages such as adequate infrastructure and the need for reliable labor. So it can be considered using other alternative test animals, namely using Zebrafish (*Danio rerio*) test animals. Based on this, research was conducted to determine the effect of giving ethanol extract of taro leaves and different graded doses of ethanol extract of taro leaves on zebrafish [1].

## 2 Experimental section

The type of research used in this study is experimental research post test only control group design. After being given the test compound, the test animals only measured blood sugar levels once, and the negative control group was used as a comparison using zebrafish test animals.

The stages of this study included extracting taro leaves, inducing hyperglycemia, exposure to test compounds, measuring blood glucose levels, and analyzing the findings.

Taro leaves were extracted by the masesari method using 70% ethanol solvent. Then the extract was evaporated until a thick extract was obtained.

Zebrafish were adapted for 1 week in an aquarium. Zebrafish were fasted for 24 hours before induction. Induction was done by giving 0.1% alloxan for 10 minutes followed by giving 1% glucose for 10 minutes. Induction was carried out for 5 days.

Zebrafish test animals were divided into 6 groups. Each treatment consisted of 3 zebrafish (*Danio rerio*), namely the untreated group (normal), negative control group, positive control group and treatment group consisting of

200 mg/2L taro leaf extract, 300 mg/2L, and 400 mg/2L. Zebrafish then measured blood glucose levels by decapitation on the tail of zebrafish. Furthermore, blood glucose levels were checked using a glucometer. The data obtained were analyzed using statistical tests.

## 3 Results and Discussion

This study aims to determine the effectiveness of taro leaf extract (*Colocasia esculenta* L.) on reducing blood glucose levels in alloxan-induced zebrafish.

Taro leaves (*Colocasia esculenta* L.) contain more phenolic compounds and flavonoid compounds than the stems and tubers. Taro leaf powder is produced in the form of green powder, distinctive odor and tasteless. Taro leaves (*Colocasia esculenta* L.) which were previously sorted wet then sorted dry so as to produce dry simplisia. Taro leaf powder is extracted with the aim of attracting and separating various chemical components contained in natural materials using 70% ethanol solvent. Ethanol is used as a solvent because it is polar, universal and volatile. Ethanol is used as a solution because it is polar, common and unpredictable. Ethanol is able to extract great polarity from non-polar compounds into polar compounds. Ethanol is also a solvent because it is a safe solvent. Because flavonoid compounds are usually in the form of polar glycosides, they must be dissolved in polar solvents. Since 70% ethanol is a polar solvent, its degree of polarity is higher than 96% ethanol. This is another reason for choosing 70% ethanol solvent.

In this research used extraction by maceration. The maceration method was chosen because it is a direct extraction technique that does not require complicated tools, is simple, and is suitable for samples that cannot withstand heat so that the metabolites in Taro Leaves (*Colocasia esculenta* L.) remain stable, especially compounds that are easily damaged at hot temperatures.

The resulting extract changed color to blackish green. The color change is caused by the occurrence of an oxidation reaction in the process of evaporating the filtrate into a thick extract. The yield of the thick extract obtained was 18.17% (Table 1). The size of the yield value indicates the extraction process's efficiency. The

extraction process's efficiency is controlled by the type of solvent used as a solvent, the size of the simplicia particles, the extraction method, and the extraction time.

This study uses zebrafish (*Danio rerio*) test animals because the zebrafish pancreas has the same function as mammals, namely having endocrine and exocrine functions that are connected to the ductal system to the digestive system as found in mammals. Zebrafish can regenerate pancreatic  $\beta$ -cells throughout its life. This is due to the adenosine signal that encourages  $\beta$ -cell regeneration in zebrafish.

Zebrafish were induced to increase blood glucose levels using inducers in the form of 0.1% alloxan and 1% glucose in all treatment groups. Giving alloxan aims to damage pancreatic  $\beta$  cells so that insulin production decreases which causes hyperglycemia and giving glucose to maintain blood glucose levels more stable. The zebrafish used were zebrafish aged 4-6 months or adult zebrafish, reared for at least one week prior to the experiment. Zebrafish were divided

into 6 groups and each group consisted of three zebrafish. Group one was without treatment (normal), group two as negative control was given 0.1% alloxan solution and 1% glucose, group three as positive control was induced with 0.1% alloxan + 1% glucose + 100 $\mu$ M metformin, group four was given taro leaf extract 200 mg, group five was given taro leaf extract 300 mg and group six was given taro leaf extract 400 mg.

Table 2 shows the observation data of blood glucose levels in zebrafish from the six treatment groups. Normal blood glucose levels of zebrafish are 50-75 mg/dL. From the data, it can be seen that the average blood glucose level of zebrafish was 54mg/dL before induction. Zebrafish blood glucose levels after alloxan and glucose induction ranged from an average of 130mg/dL. This shows that alloxan has damaged zebrafish pancreatic  $\beta$ -cells. Giving 1% glucose after alloxan induction aims to prevent the occurrence of hypoglycemia phase in zebrafish.

Table 1 Taro Leaf Extract Weight (*Colocasia esculenta* L.)

Sample	Type Of Solvent	Extraction Method	Dry Sample Weight	Extract Weight	Yield
Daun Talas ( <i>Colocasia esculenta</i> L.)	Ethanol 70 %	Maceration	100 g	50,37 g	18,17 %

Table 2 Results of Measuring Blood Glucose Levels in Zebrafish Induced by Alloxan

Zebrafish	Normal (without treatment)	Control (-) Induction Alloxan dan Glucose	Control (+) Metformin	Taro Leaf Extract 200 mg	Taro Leaf Extract 300 mg	Taro Leaf Extract 400 mg
1	54	140	43	79	112	59
2	43	112	65	90	124	59
3	65	138	65	88	145	38
Average	54 $\pm$ 11	130 $\pm$ 15,6	57,6 $\pm$ 12,7	85,6 $\pm$ 5,8	127 $\pm$ 16,7	52 $\pm$ 12,1

Table 3 P Value of Statistical Test Results

Group	1	2	3	4	5	6
1	-	0,001	0,637	0,050	0,050	0,825
2	0,001	-	0,046	0,050	1,000	0,046
3	0,637	0,046	-	0,046	0,046	0,261
4	0,050	0,050	0,046	-	0,050	0,268
5	0,050	1,000	0,046	0,050	-	0,046
6	0,825	0,046	0,261	0,268	0,046	-

Information :

Group 1: Normal (no treatment)

Group 2: Control (-) alloxan and glucose induced

Group 3: Control (+) metformin

Group 4: Taro Leaf Extract 200 mg

Group 5: Taro Leaf Extract 300 mg

Group 6: Taro Leaf Extract 400 mg

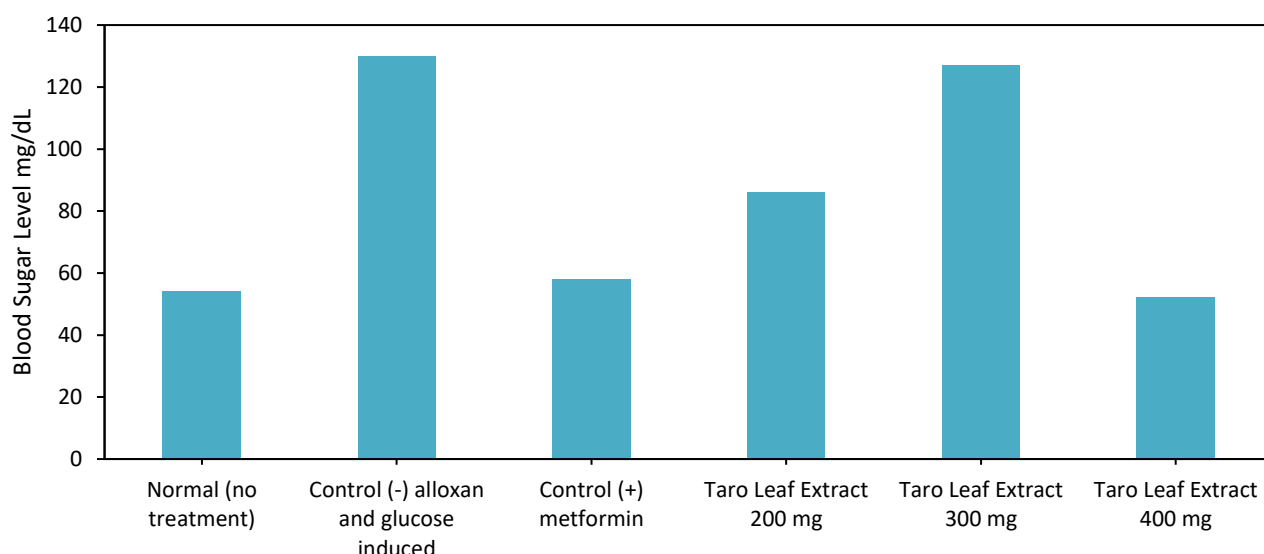


Figure 1 Average Zebrafish Blood Glucose Level Measurements

In the administration of 200 mg taro leaf extract obtained zebrafish blood glucose levels ranging from an average of 85.6 mg/dL, the administration of 300 mg taro leaf extract obtained zebrafish blood glucose levels ranging from an average of 127 mg/dL, the administration of 400 mg taro leaf extract obtained zebrafish blood glucose levels ranging from an average of 52 mg/dL, while in the positive control treatment with metformin obtained zebrafish blood glucose levels ranging from an average of 57.66 mg/dL.

The results showed that the zebrafish group given 200 mg of taro leaf extract, 400 mg, and the control group (+) which was given metformin caused zebrafish blood glucose levels to return to normal or not significantly different from normal zebrafish glucose levels (Table 3).

The zebrafish treatment group with 200 mg taro leaf extract was significantly different from the negative control, 300 mg taro leaf extract and not significantly different from the normal control, positive control and 400 mg taro leaf extract. This indicates that the zebrafish treatment group with 200 mg of Taro Leaf Extract has a comparable effect to the positive control as an antidiabetic.

The zebrafish treatment group with 300 mg of taro leaf extract was significantly different from the normal control, positive control, 200 mg and 400 mg of taro leaf extract and was not

significantly different from the negative control group. This indicates that the zebrafish treatment group with 300 mg of taro leaf extract has not given the maximum effect as antidiabetes.

The zebrafish treatment group with 400 mg taro leaf extract was significantly different from the negative control, 300 mg taro leaf extract and not significantly different from the normal control, positive control and 200 mg taro leaf extract. This indicates that the zebrafish treatment group with 400 mg of taro leaf extract has a comparable effect to the positive control as an antidiabetic (Table 3).

Based on the results of the study (Figure 1), it can be seen that taro leaf extract has an antidiabetic effect, where the most effective dose as antidiabetes in zebrafish test animals is a dose of 400 mg. The antidiabetic effect is due to the presence of bioactive compounds contained in taro leaf extract such as alkaloids, flavonoids, saponins, tannins and polyphenols.

#### 4 Conclusions

1. Taro leaf extract (*Colocasia esculenta* L.) is effective in reducing blood glucose levels in alloxan-induced zebrafish.
2. Of the treatment groups, taro leaf extract (*Colocasia esculenta* L.) at a dose of 400 mg is the most effective dose in reducing blood glucose levels.

## 5 Declarations

### 5.1 Author Contributions

The names of the authors listed in this journal contributed to this research.

### 5.2 Funding Statement

This research was not supported by any funding sources.

### 5.3 Conflicts of Interest

The authors declare no conflict of interest.

### 5.4 Ethic

No. 901/KEPK-PTKMS/I/2023 from Komisi Etik Penelitian Kesehatan Politeknik Kesehatan Makassar.

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