The Effectiveness of X Herbal Medicine On The Reducing Blood Sugar In A Diabetic Mice Model

Purwaeni[1*], Nurbaiti Fentiani Siahaan[2], Meilinda Windy Astuty[3]

[1]Bachelor of Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Universitas Islam Bandung, Indonesia
[2]Bachelor of Pharmacy Study Program, Faculty of Pharmacy, Institut Kesehatan Rajawali, Indonesia
[*] Corresponding Author:
Email: purwaenieni@gmail.com

Abstract.

The prevalence of Diabetes Mellitus in Indonesia is estimated to reach 21.3 million people by 2030. Treatment with oral antidiabetic drugs carries the risk of myocardial infarction and congestive heart failure, while modern therapy of NIDDM has adverse side effects. WHO recommends researching traditional methods, especially plants with hypoglycemic effects. Efficacy claims in herbal medicines, especially herbal medicines, are rarely reported, so it is necessary to test antidiabetic activity to ensure their use. To determine the antidiabetic activity of herbal medicine product X on blood sugar levels in aloxane-induced mice (Mus Musculus), determine the most effective dose, and determine the decrease in GDP after the administration of herbal medicine X on days 7 and 14. Experimental research uses male mice (Mus Musculus) as test animals. Blood glucose levels were measured before and after treatment, with a negative control of CMC-Na of 0.5% and a positive control of glibenclamide of 0.65mg/Kg BB. The optimal dose of herbal product X is determined by a ratio of doses of 52 mg/KgBB, 104 mg/KgBB, and 156 mg/KgBB for 7 to 14 days. Herbal Medicine X at doses of 52mg/KgBB, 104mg/KgBB, and 156mg/KgBB succeeded in reducing aloxan-induced blood sugar levels in mice. The dose of 156mg/KgBB was found to be the optimal dose based on the Mann-Whitney test with Sig<0.05. The most significant decline on day 14 was based on the Kruskal Wallis test with Sig<0.05. Herbal Medicine X was effective in lowering blood sugar levels in mice, with an optimal dose of 156mg/Kg BB and a significant decrease on day 14.

Keywords: *Antidiabetic activity test; Herbal Medicine X; Mice (Mus Musculus); Aloxan and Blood sugar levels.*

I. INTRODUCTION

Diabetes is a metabolic disease characterized by high blood sugar levels (hyperglycemia). This disease is caused by various medical conditions, including: impaired insulin secretion and action function, impaired metabolism of carbohydrates, fats and proteins. The effects felt by diabetics in the long term are retinophaty, nephrophaty, neurophaty and other complications. Diabetics also tend to have an increased risk of other diseases such as heart disease, obesity, cataracts, erectile dysfunction, liver damage, and some liver diseases. Based on a report by the International Diabetes Federation (IDF) in 2019, data was obtained on 463 million people living with diabetes. This number is expected to increase to 578 million people, or 700 people by 2045, with an increase rate of 51% [1], [2]. Epidemiologically, it is estimated that the number of people with Diabetes Mellitus (DM) in Indonesia will reach 21.3 million people by 2030. Based on the results of the 2018 Basic Health Research (Riskesdas), data was obtained that the proportion of causes of death due to DM in the age group of 45-54 years in urban areas ranked 1st at 1.9% and in rural areas, ranked 2nd at 1.0%. Of the 25,274 respondents over 15 years old, 30.8% experienced Impaired Glucose Tolerance (TGT), namely glucose levels reaching 140-200 mg/dL after 14 hours of fasting and being given 75 grams of oral glucose. As many as 1.5% experienced diagnosed DM, while 4.2% experienced undiagnosed DM. Both DM and TGT are more common in women than men, and are more common in groups with low levels of education and social status. The age group with the most DM sufferers is 55-64 years old, which is 6.3%. Some of the things related to the risk of DM are obesity, hypertension, lack of physical activity, and the intake of vegetables and fruits is less than 5 servings per day [3], [4].

Modern therapy for insulin Non-dependent Diabetes Mellitus (NIDDM) involves a cascade of treatment. It starts with dietary modifications before progressing to oral antidiabetics and then insulin. The use of existing therapies such as sulfonylureas and biguanids is limited by their pharmacokinetic properties, the rate of secondary failure and the side effects that accompany them are quite significant such as adverse effects on the spinal cord . Based on a study in the United Kingdom, DM patients, both men and women,

who take Oral Antidiabetic Drugs (OAD) from different groups have a risk of myocardial infarction and congestive heart failure. Meanwhile, in previous studies, elderly DM patients who used OAD were more at risk of experiencing congestive heart failure [4]. The treatment and maintenance of DM's health has required a very large amount of money every year. With the increasing number of patented drugs for DM patients, medical costs are getting more expensive and unaffordable, especially for patients in developing countries such as Indonesia. The World Health Organization (WHO) diabetes commission recommends traditional methods for diabetes treatment to be further researched. Plants with hypoglycemic effects can provide a beneficial source for new components of oral antidiabetic drugs. Currently more than 400 traditional medicinal plants have been reported to be used for alternative and complementary treatment of diabetes, although only a few have been scientifically studied for their efficacy [4].Traditional medicinal herbs are generally made from natural ingredients of medicinal plants such as roots, tubers, rhizomes, wood, tree bark, seeds, leaves, sap or from medicinal plant extracts.

The antidiabetic activity of plants is obtained due to the presence of secondary metabolite compounds of phenolic groups, flavonoids, alkaloids, and steroids in traditional plants [5], [6].In recent years, the use of complementary medicine has increased in almost every country. From 2012 to 2017 in the United States, the use of complementary medicine increased from 35.1% to 38.3%. The consumption of herbal medicine, acupuncture, and reflexology is developing into the most popular alternative medicine. reports that 85% of the world's population uses herbal medicine in their lives. However, complementary treatment methods have a wide range of effects and can vary among their users around the world. The wide and vassive effects make it difficult to conduct clinical trials for standardization purposes and difficult to determine the user's perspective [7], [8], [9], [10].The side effects of drugs on conventional medicine (chemical drugs) and the individual's belief that natural products have far fewer side effects than conventional drugs lead to the high use of herbal medicines. in many DM patients. Individuals with DM prefer herbal supplements as a form of complementary treatment. According to a recent study in diabetic patients, the rate of use of herbal supplements is generally between 17% and 71% Studies conducted in Indonesia show this figure to be 85% [8], [11], [12].Antidiabetic activity tests for herbal medicine products are rarely reported. For this reason, this research reported the antidiabetic activity of the X herbal medicine product produced by the Traditional Medicine Small Business (UKOT), in order to help provide preclinical efficacy claims and increase the category of this herbal medicine product from herbal medicine or traditional medicine to standardized herbal medicine if needed.

II. METHODS

This section of the report gives a detailed account of the procedure that was followed in completing the experiment(s) discussed in the report. Such an account is very important, not only so that the reader has a clear understanding of the experiment, but a well written Materials and Methods section also serves as a set of instructions for anyone desiring to replicate the study in the future. List the major tools or specific tools used in the study including the brand, type, and specifications. The tools which commonly used in laboratory such as glassware, napkins, scalpels, etc., should not be included. Specific such as self-designed tools, or modifications should be shown the schematic/drawing/photograph. The genes, strains, age, and average body weight of the test animals should be reported in this section. The plant samples should be mentioned the origin and its authentication.

Type of Research

This research is an experimental research, namely research to control, manipulate, and observe the research subject. With the research design of the timer series control group design, which is an experimental research with repeated measurements based on the course of time [13]. In this study, measurements were carried out every 7 days for 14 days, with three treatment groups and two control groups, where the treatment group included herbal medicine products X with doses of 52mg/Kg BB, 104mg/Kg BB, and 156mg/Kg. As well as the CMC-Na negative control group 0.5%, and the glibenclamide positive control group 0.65mg/Kg BB.

Phytochemical Filtration

Phytochemical screening was carried out to determine the secondary metabolite group contained in the extract sample of the finished herbal medicine X containing sambiloto extract (*Andrographis paniculata* (Burm.F.) Nees), duet bark extract (*Syzygium Cumini*), dutch teak leaf extract (*Guazuma ulmifolia* Lam.), and life graft leaf extract (*Gynura procumbens*). The secondary metabolites measured were alkaloids, flavonoids, tannins, saponins, quinones, steroids, triterpenoids and phenolics.

Specific and Non-Specific Standardization

Meanwhile, the standardization of extracts is carried out specifically and non-specifically. The specific parameters of the extract carried out in this study were the identification of organoleptis including the shape, color, and smell that became the specific characteristics of the extract. Meanwhile, specific standardization is through the determination of several standard parameters of herbal medicines based on the Indonesia Herbal Pharmacopoeia (FHI). These parameters include total ash content, water-soluble ash content, acid-insoluble ash content, ethanol-soluble juice content, water-soluble juice content, and water content [14], [15].

Test Animal Setup

The animal used is a male mouse (*Mus Musculus*) obtained from Pharm Mita Jl. Cemp. III, Citeureup, North Cimahi District, Cimahi City, West Java, 2-3 months old with a weight of about 20-30 grams. The mice used must be healthy and have never undergone any treatment. Before conducting the study, the animals tested to be used in the study must first be adapted for a week to the same environmental conditions, food and drinks. Test animals are fasted for 8-12 hours before use. The mice used were 25 heads, divided into 5 treatment groups where each group consisted of 5 male mice. Then each was weighed and each of the 5 test animals was kept in 1 cage.

Antidiabetic Activity Test

The antidiabetic test method was carried out on aloxan-induced diabetic mice. The mice were fasted for 8-12 hours, while still given a drink, the mice were induced alokasan 0.65mg/Kg BB mice interperitoneally. Glucose levels were observed on the third day to find out which experimental animals had developed diabetes mellitus. Mice with blood sugar levels above 200mg/dl were used in the study. The mice were weighed and grouped into 5 groups (2 control groups and 3 test groups) where each group consisted of 5 mouse test animals. Then, mice with diabetes were given treatment. The herbal medicinal products X and glibenclamide were suspended in 0.5% CMC-Na and administered orally in mice. The negative control group was given 0.5% CMC-Na once a day. The positive control group was given a suspension of glibenclamide at a dose of 0.65mg/Kg BB once a day in mice. Test Group 1 was given a suspension of herbal medicine product X with a dose of 52mg/Kg BB three times a day, Test Group II was given a suspension of herbal medicine product X with a dose of 104mg/Kg BB three times a day. Group III was given a suspension of herbal medicine product X with a dose of 156mg/Kg BB three times a day.The treatment was carried out for 14 days. Glucose level measurement was carried out on day 0 taken from the tail (intravenously) and days 7 and 14 after treatment. Furthermore, glucose levels are measured using a glucometer (Easy Touch® GCU).

Data Analysis

The data obtained is then processed statistically using the SPSS application. The data obtained were carried out normality tests and homogeneity tests. The normality test was carried out using the Shapiro wilk method while the homogeneity test was carried out using the Levene method. If the data obtained is normally distributed and has homogeneous variants. Furthermore, data analysis was carried out using the one-way variant analysis method (ANOVA) followed by the smallest real difference test (BNT). If the data obtained is not normally distributed or has non-homogeneous variants, then data analysis is carried out using the Kruskal-Wallis method followed by the Mann-Whitney test [16].

Materials

The tools used in this study are animal scales, analytical balances (YHG YH-1002R), sonde (Obsisi Medica), glass (Pyrex), gloves, droppipettes (Onemed), stirring rods (Pyrex), stirrer (Pro'skit PT-5205U), cotton (Selection), 1ml syringe (Onemed), handscoon (Onemed), mouse box, measuring cup (Pyrex), funnel

(Pyrex), hot plate (79-1), stamper and mortar (Onemed), clamp (Pudak scientific), test tube rack (Pudak scientific), test tubes (Pyrex), Cutter (Taco), surgical scissors (Gold Cross), alcohol swab (Onemed), tissue (Jolly), blood lancet (Easy Touch®), glucometer (Easy Touch® GCU Meter), Check strip (EasyTouch® GCU) and data analysis software (IBM SPSS Statistics 27).The ingredients used in this study are herbal medicine products X, mice, glibenclamide (Indo Farma), aloxan monohydrate (A Aldrich), CMC-Na 0.5% (Sabmedika), NaCl 0.9% (Onemed), aquadest (Chem Mart), H2SO4 (Polylab), Mayer Reagent (Chemical Nitrate), Dragendroff Reagent (Chemical Nitra), Hydrochloric Acid (Emsure), Chloroform (Emsure), HCL concentrate (Chem Mart), Mg metal (Smart-Lab), Libermann-Burchard (Chemical Nitrate), FeCl3 1% (Aloin), FeCl 0.1% (Aloin), Ethanol 96% (JK-Care), NaOH 10% (Pudak scientific).

III. RESULT AND DISCUSSION

Herbal medicine products produced by small traditional medicine enterprises (UKOT) have received registration from BPOM RI and have been commercialized. This X herbal medicine product is included in the category of herbal medicine in the classification of herbal medicines in Indonesia based on the provisions issued by BPOM RI where the efficacy claims are still based on empirical data and have not been proven through preclinical trials and clinical trials. One of the X herbal medicine products containing sambiloto extract (*Andrographis paniculata* (Burm.F.) Nees), duet bark extract (*Syzygium Cumini*), dutch teak leaf extract (*Guazuma ulmifolia* Lam.), and life graft leaf extract (*Gynura procumbens*) have been tested *in vivo* which is one of the stages of pre-clinical trials in proving the efficacy of this herbal medicine product based on the claims registered. The results of phytochemical filtration of herbal medicine X obtained showed the presence of alkaloids, flavonoids, saponins, triterpenoids, steroids, tannins, quinones, and phenolics. All of these compounds have antidiabetic effects, according to research on sambiloto leaves by Aprillia & Safitri [17], which reported that*Andrographis paniculata* (Burm.F.) Nees contains flavonoid compounds, alkaloids, saponins and tannins. The study by Aini et al. [19] on jamblang bark (*Syzygium cumini*) showed that its methanol extract contains phytochemical compounds such as alkaloids, terpenoids, saponins, flavonoids, phenolics, and tannins, which have antidiabetic properties. Meanwhile, the study by leaves Puspitasari [21] on Dutch teak leaves (*Guazuma ulmifolia* Lam.) reported the presence of alkaloids, flavonoids, saponins, and tannins.

Finally, the study by Agustira et al. [23] on life graft leaves (*Gynura procumbens*) showed the presence of alkaloid compounds and steroids/triterpenoids that play an active role in lowering blood sugar levels.Standardization of traditional medicine is something that needs to be considered in the context of developing traditional medicine into standardized herbal medicine and phytopharmaceuticals. Specific and non-specific parameters are a standardization process carried out to ensure the quality of the extract. The specific parameters of the extract carried out in this study were organoleptis identification including shape, color, smell, and taste which are the specific characteristics of the extract in the preparation of herbal medicine product X [24].The preliminary test of aloxan is an effort to increase blood sugar levels by inducing mice to use aloxan. On day 0 blood sugar is measured before induction, then after such induction, blood sugar levels are controlled on day 3 to ensure that aloxan at that dose can cause damage to the pancreas. The results of preliminary tests showed that all mice induced by aloxan with a dose of 150 mg/KgBB intraperitonally experienced hyperglycemia which was characterized by an increase in fasting blood sugar levels of >200 mg/dL, namely the average GDP level of group 1 was 232.8 mg/dL, group 2 was 234 mg/dL, group 3 was 237 mg/dL, group 4 was 234 mg/dL and finally group 5 was 215 mg/dL without causing death in mouse test animals. Therefore, an intraperitonial dose of 150mg/KgBB of aloxan was applied to this study.Administration of aloxan at a dose of 150 mg/kgBB via the intraperitoneal route has been carried out in preliminary trials, which have been shown to cause hyperglycemic on day 3 after induction.

This test was carried out on 25 mice (*Mus Musculus*) aged 2-3 months with a weight of about 20-30 grams. The test mice were grouped into 5 groups consisting of 2 control groups and 3 test dose groups. Before induction, mice are acclimatized for 7 days.The antidiabetic activity test of herbal medicine product preparations X on aloxan-induced diabetic mice was carried out for 14 days. During the 14 days, all mice

received treatment according to their respective test groups. Mice in the negative control group were given a suspension treatment in CMC-Na 0.5% for 14 days. The results of observation of blood sugar levels of mice on day 0, day 7, and day 14 were seen in (Figure 1). The mice in the positive control group were given a suspension treatment of glibenclamide in CMC-Na 0.5% with a dose of 0.65mg/Kg BB for 14 days. The results of the observation of blood sugar levels of mice on day 0, day 7, and day 14 were seen on (Figure 2). Mice in the Dose I group were given a suspension treatment of herbal medicine X in CMC-Na 0.5% with a dose of 52mg/Kg BB for 14 days. The results of the observation of blood sugar levels of mice on day 0, day 7, and day 14 were seen on (Figure 3). Mice in the Dose II group were given a suspension treatment of herbal medicine X in CMC-Na 0.5% with a dose of 104mg/Kg BB for 14 days. The results of the observation of blood sugar levels of mice on day 0, day 7, and day 14 were seen on (Figure 3). Mice in the Dose III group were given a suspension treatment of herbal medicine X in CMC-Na 0.5% with a dose of 156mg/Kg BB for 14 days. The results of the observation of blood sugar levels of mice on day 0, day 7, and day 14 were seen on (Figure 4).

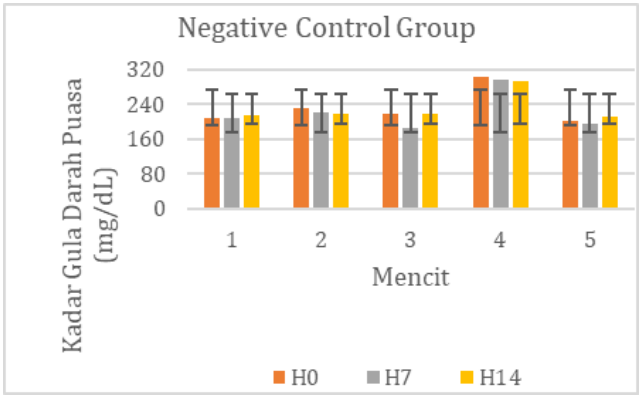

Fig 1. Fasting blood sugar level graph of the negative control group

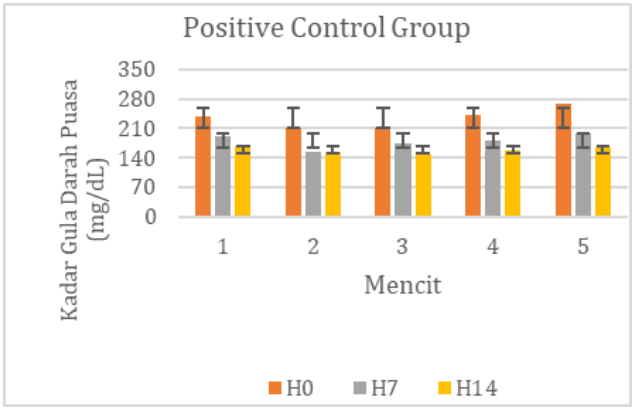

Fig 2. Fasting blood sugar level graph of the positive control group

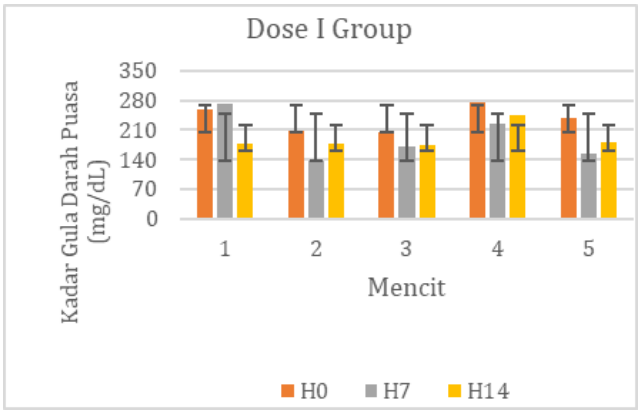

Fig 3. Chart of fasting blood sugar levels in dose I group

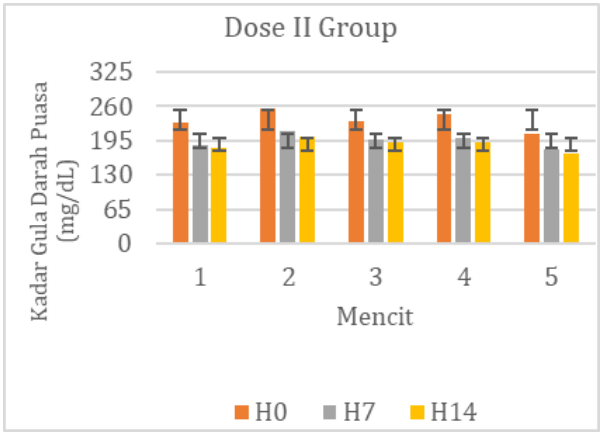

Fig 4. Fasting blood sugar level graph of dose II group

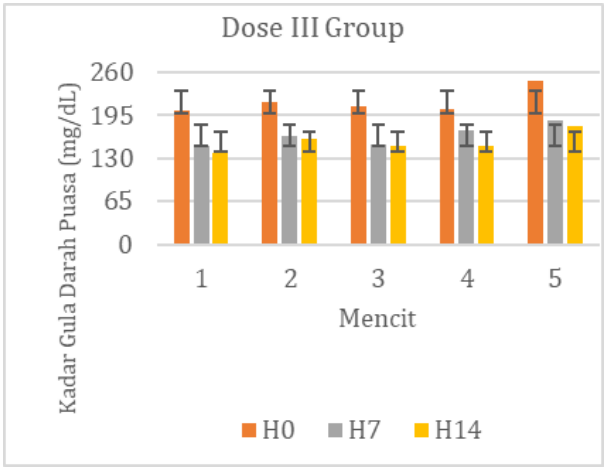

Fig 5. Fasting blood sugar level graph for the III dose group

In the group of diabetic mice treated with CMC-Na 0.5%, unstable data were obtained and did not show a significant decrease. This is because CMC-Na has no antidiabetic activity or has no effect on the variables tested. [25]. In the group of diabetic mice treated with Glibenclamide 0.65mg/Kg BB, stable and meaningful reduction data were obtained. This is because Glibenkalmide can inhibit cell death β the pancreas by increasing the release of insulin from the pancreas [26].In the group of diabetic mice treated with Herbal Medicine X (52mg/Kg BB), unstable data were obtained. This is thought to be because doses that are too small may be below the threshold of effectiveness necessary to produce consistent biological effects. In the case of low doses, the active substance may not be enough to provide the desired effect stably [27]. In the group of diabetic mice treated with Herbal Medicine X (104mg/Kg BB) showed stable decline data every week so that the second dose of herbal medicine preparation X with a dose of 104mg/KgBB was quite effective in being used as an antihyperglycemic drug.In the group of diabetic mice treated with Herbal Medicine X (156mg/Kg BB), it showed stable and largest decrease data every week so that dose III in herbal medicine preparation X with a dose of 156mg/KgBB was most effectively used as an antihyperglycemic drug. All test groups showed the effects of lowering and increasing fasting blood sugar levels (Table 1) and (Figure 6).

Table 1. Average Fasting Blood Sugar Levels of All Test Groups

Group	Blood Sugar Levels (mg/dL)		
	H0	H7	H14
KN (CMC-Na 0.5%)	232.8 ± 40.91	220.8 ± 43.94	231 ± 34.18
KP (Glibenclamide 0.65mg/Kg BB)	234 ± 23.95	180 ± 16.66	158 ± 7.87
KD I (Herbal Medicine X 52mg/Kg BB)	237 ± 31.81	191.6 ± 55.77	190.6 ± 31.03
KD II (Herbal Medicine X 104mg/Kg BB)	234 ± 18.33	195 ± 13.78	187.2 ± 12.60
KD III (Herbal Medicine X 156mg/Kg BB)	215 ± 17.86	164.6 ± 16.30	154.4 ± 15.14

Description:
KN : Negative Group
KP : Positive Group
KD I : Dose I Group
KD II : Dose II Group
KD III : Dose III Group
H0 : Blood sugar levels after induction of aloxane before administration of test preparations
H7 : Blood sugar levels after 7 days of administration of the test preparation
H14 : Blood sugar levels after 14 days of administration of the test preparation

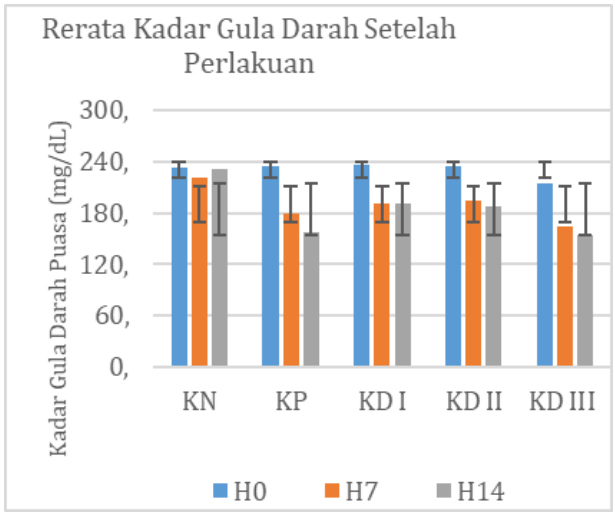

Fig 5. Graph of fasting blood sugar levels of all test groups

In this study, changes in blood sugar levels were observed after the administration of test preparations in diabetic mice. Aloxan is used as a diabetogenic compound to cause hyperglycemic conditions in mice [28]. Mice have been shown to be sensitive to the diabetogenic effects of aloxants. Fasting blood sugar levels in mice above 200 mg/dL were used in the study. The method used in checking blood glucose levels is an enzymatic method using a glucometer. This method was chosen because it is the most commonly used and simple method because it requires only a small number of blood samples, relatively short measurement time, high accuracy and precision measurement results, and very small possibility of contamination [25].From the results of the antidiabetic activity test, the average fasting blood sugar level on days 7 and 14 was obtained where the negative control test group experienced a decrease and increase in KDP levels every week while the positive control group and the dose group experienced a decrease in KDP, but the dose of 156 mg/KgBB showed the best decrease in glucose levels compared to the comparators (Table 1) and (Figure 6).The results of the fasting blood sugar level examination on day 0 (after aloxane induction), 7 and 14 that have been obtained are then statistically analyzed with the SPSS version 27.0 program. Statistical analysis was used to analyze and compare the blood glucose levels of test animals in the control group and the test dose group (Tables 2 and 3).

Table 2. Statistical Results of Testing H7 Fasting Blood Sugar Levels on the Kruskal Wallis Test

Group	**n**	**Median (min-max)**	**n**	***p***
Negative Control Group	5	207 (186-296)	220.8±43.94	0,081
Positive Control Group	5	182 (155-198)	180±16.66	
Dose I Group	5	170 (137-272)	191.6±55.77	
Dose II Group	5	197 (178-214)	195±13.78	
Dose III Group	5	164 (148-188)	164.6±16.30	

Remarks: * : There is a meaningful difference ($p < 0.05$)
H7 : Blood sugar levels after 7 days of administration of the test preparation

Based on Table 2, it was shown that there was no significant difference in fasting blood sugar levels in the five groups on the 7th day with ($p > 0.081$).

Table 3. Statistical Results of Testing H14 Fasting Blood Sugar Levels in the Kruskal Wallis Test

Group	n	Median (min-max)	n	*p*
Negative Control Group	5	217 (212-292)	231±34.18	0,001*
Positive Control Group	5	157 (147-167)	158±7.87	
Dose I Group	5	176 (175-246)	190.6±31.03	
Dose II Group	5	191 (170-203)	187.2±12.60	
Dose III Group	5	149 (138-178)	154.4±15.14	

Remarks: * : There is a meaningful difference ($p < 0.05$)

H14 : Blood sugar levels after 14 days of administration of the test preparation

Based on Table 3, it was shown that there was a significant difference in fasting blood sugar levels in the five groups on the 14th day with (p > 0.001). The analysis continued with the Mann-Whitney test.

Table 4. Statistical Results of Testing H14 Fasting Blood Sugar Levels on the Mann-Whitney Test

Group	Comparison	*p*
Negative Control Group	Positive Control Group	0,009*
	Dose I Group	0,075
	Dose II Group	0.009*
	Dose III Group	0.009*
Positive Control Group	Dose I Group	0.0009*
	Dose II Group	0.009*
	Dose III Group	0,602
Dose I Group	Dose II Group	0,528

Remarks: * : There is a meaningful difference ($p < 0.05$)

H14 : Blood sugar levels after 14 days of administration of the test preparation

Based on Table 4.16 of the Mann-Whitney test, it was shown that there was a significant difference between the positive control group, dose II, and dose III compared to the negative control group (CMC-Na 0.5%). In addition, the dose III group showed no significant difference with the positive control group (Glibenclamide). Therefore, the dose III group (Herbal Medicine X 156 mg/kg BB) is considered the best dose in lowering blood sugar levels in *aloxan-induced* male mice (*Mus Musculus*).

IV. CONCLUSION

Based on the research that has been conducted on the antidiabetic activity test of herbal medicine product X on blood sugar levels in aloxan-induced mice (Mus Musculus), it can be concluded that on the 7th day there was no significant difference in fasting blood sugar levels between the negative control group, positive control, dose I, dose II and dose III based on the Kruskal-Wallis test (p = 0.081). This shows that herbal medicine X has not been able to significantly lower blood sugar levels on day 7. Meanwhile, on the 14th day there was a significant difference in fasting blood sugar levels between the negative control group, positive control, dose I, dose II and dose III based on the Kruskal-Wallis test with (p = 0.001). This means that herbal medicine X is able to significantly lower blood sugar levels on the 14th day. Furthermore, dose III (Herbal Medicine X 156mg/KgBB) was found to be the optimal dose because the fasting blood sugar level of the positive group (Glibenclamide 0.65 mg/Kg BB) was no different from dose III (Herbal Medicine X 156mg/KgBB) based on the Mann-Whitnay Test (p = 0.602).

V. ACKNOWLEDGMENTS

The author would like to thank all parties who have helped both morally and materially.

REFERENCES

[1] World Health Organization, “Classification of Diabetes Mellitus 2019,” Geneva, 2019.

[2] International Diabetes Federation (IDF), *IDF Diabetes Atlas (9th ed.)*. Brussels, Belgium: International Diabetes Federation, 2019.

[3] International Diabetes Federation (IDF), *IDF Diabetes Atlas (10th ed.)*. Brussels, Belgium: International Diabetes Federation, 2021.

[4] P. Sukardiman, H. Poerwono, and L. Arifianti, “Pengembangan Obat Herbal Fitofarmaka Antidiabetes dari Campuran Ekstrak Herba Sambiloto (Andrographis Paniculata Nees) dan Ekstrak Biji Mahoni (Swietenia Mahagoni Jacq),” Lembaga Penelitian Universitas Airlangga, Surabaya, 2018.

[5] A. Dalimunthe, "Interaksi Sambiloto (andrographis paniculata)," ***J. Ilm.,*** 2009.

[6] C. P. H. Anugrahini and A. S. Wahyuni, "Narrative Review: Aktivitas Antidiabetes Tanaman Tradisional Di Pulau Jawa," ***Pharmacon J. Farm. Indones.,*** pp. 120–131, 2021.

[7] M. Holt, S. N. Baile, and M. Friswe, "Consumption Behaviour among Patients with Diabetes to Complementary and Alternative Medicine in Amerika: Systematic Review," ***Inter J Basic Clin Stud,*** vol. 2, pp. 16–30, 2013.

[8] N. N. Rahmat, D. Hartono, and N. Laili, "Persepsi dan Perilaku Konsumsi Obat Herbal Penderita Diabetes Mellitus Tipe 2 di Desa Pesisir Kecamatan Gending Kabupaten Probolinggo," ***J. Nurs. Care Biomol***., vol. 5, no. 1, pp. 41–48, 2020, doi: 10.32700/jnc.v5i1.178.

[9] D. F. Hamzah, "Analisis Penggunaan Obat Herbal Pasien Diabetes Mellitus Tipe Ii Di Kota Langsa," ***JUMANTIK (Jurnal Ilm. Penelit. Kesehatan),*** vol. 4, no. 2, pp. 168–177, 2019, doi: 10.30829/jumantik.v4i2.5057.

[10] H. Jangid, S. Chaturvedi, and M. P. Khinchi, "An Overview on Diabetis Mellitus," ***Asian J. Pharm. Res. Dev.,*** vol. 5, no. 3, pp. 1–11, 2017.

[11] L. K. Nguma, "Health Seeking and Health Related Behaviour for Type 2 Diabetes Mellitus among Adults in an Urban Community in Tanzania," University of Otago, 2010.

[12] L. Lopez, W. Chang, and V. Maro, "Complementary medicine practices among diabetics in Northern Tanzania: An ethnomedical survey," *Complement. Altern. Med.*, vol. 1, no. 1, p. 282, 2017.

[13] S.Saryono,*Metodologi Penelitian Kesehatan: penuntun praktis bagi pemula*.Yogyakarta: Mitra Cendikia Press, 2011.

[14] M. Ni'am, R. K. Saputri, and N. Februyani, "Standarisasi Parameter Spesifik dan Non-Spesifik Ekstrak Daun Binahong Merah (Anredera cordifolia) dengan Perbedaan Metode Ekstraksi," ***Indones. J. Heal. Sci.***, vol. 3, no. 2, pp. 431–437, 2023, doi: 10.54957/ijhs.v3i2a.564kalau.

[15] Kementerian Kesehatan Republik Indonesia,*Farmakope Herbal Indonesia:Suplemen 3*,1st ed. Jakarta: Direktorat Jenderal Pelayanan Kefarmasian dan Alat Kesehatan, Kementerian Kesehatan Republik Indonesia, 2013.

[16] M. S. Dahlan, *Statistik untuk Kedokteran dan Kesehatan: Deskriptif, Bivariat dan Multivariat, Dilengkapi dengan Menggunakan SPSS*. Jakarta: Salemba Medika, 2014.

[17] P. Aprillia and C. I. N. H. Safitri, "Uji Aktivitas Antidiabetes Kombinasi Ekstrak Herba Sambiloto dan Daun Sirih Hijau pada Mencit," *Pros. SNPBS (Seminar Nas. Pendidik. Biol. dan Saintek)*, 2020.

[18] Q. Aini, S. Suwarniati, and I. Mirza, "Phytochemical Screening of Methanol Extract of Jamblang (Syzygium cumini L.) As Antidiabetic," ***J. Biol. sains dan kependidikan,*** vol. 2, no. 2, 2022.

[19] Q. Aini, S. Suwarniati, and I. Mirza, "Phytochemical Screening of Methanol Extract of Jamblang (Syzygium cumini L.) As Antidiabetic," ***J. Biol. sains dan kependidikan,*** vol. 2, no. 2, 2022.

[20] R. Puspitasari, "Uji efek antidiabetes mellitus tipe II kombinasi madu (Mel depuratum) dan ekstrak daun jati belanda (Guazuma ulmifolia Lamk.) terhadap mencit jantan galur Swiss Webster," Universitas Al-Ghifari, 2021.

[21] R. Puspitasari, "Uji efek antidiabetes mellitus tipe II kombinasi madu (Mel depuratum) dan ekstrak daun jati belanda (Guazuma ulmifolia Lamk.) terhadap mencit jantan galur Swiss Webster," Universitas Al-Ghifari, 2021.

[22] A. Agustira, I. Darwis, and D. Isti angraini, "Tanaman sambung nyawa (Gynura procumbens) sebagai antihiperglikemi," ***Med. Prof. J. Lampung,*** vol. 9, no. 2, pp. 240–244, 2019, doi: 10.53089/medula.v9i2.265.

[23] A. Agustira, I. Darwis, and D. I. Angraini, "Tanaman sambung nyawa (Gynura procumbens) sebagai antihiperglikemi," ***Med. Prof. J. Lampung***, vol. 9, no. 2, 2019, doi: https://doi.org/10.24912/jssh.v1i1.24544213.

[24] E. R. Yuslianti, B. M. Bachtiar, D. F. Suniarti, and A. B. Sutjiatmo, "Natural Products Pharmaceutical Standardization Towards Phytopharmaca for Indonesian Traditional Medicine Development," ***Dentika Dent. J.,*** vol. 19, no. 2, pp. 179–185, 2016, doi: 10.32734/dentika.v19i2.463.

[25] M. Kenning, A. Puchert, and E. Salzsieder, "System accuracy evaluation of blood glucose monitoring systems: comparison of four devices for personal use pursuant to ISO and ADA guidelines," ***J. Diabetes Sci. Technol.,*** vol. 15, no. 5, pp. 1202–1203, 2021, doi: 10.1177/19322968211011185.

[26] I. K. D. I. Muliawan, "Efek Pemberian Kombinasi Jus Aloe Vera dan Glibenklamid terhadap Penurunan Kadar Glukosa Darah pada Model Tikus Diabetes yang Diinduksi dengan Streptozotosin dan Nikotinamid," *Intisari Sains Medis*, vol. 10, no. 2, pp. 527–531, 2019, doi: 10.15562/ism.v10i2.532.

[27] J. T. Barr, T. B. Tran, B. M. Rock, J. L. Wahlstrom, and U. P. Dahal, "Strain-Dependent Variability of Early Discovery Small Molecule Pharmacokinetics in Mice: Does Strain Matter?," *Drug Metab. Dispos.*, vol. 48, no. 8, pp. 613–621, 2020, doi: 10.1124/dmd.120.090621.

[28] F. T. B. Aminah and N. Qomariyah, "Efek Antihiperglikemik Ekstrak Daun Ceremai (Phyllanthus acidus) pada Mencit (Mus musculus) Diabetes Mellitus Tipe II," *LenteraBio Berk. Ilm. Biol.*, vol. 12, no. 3,pp.363–370, 2023.