Hypoglycemic Activity of Extract Leaf and Root Plant of Jombang (Taraxacum Officinale) in Alloxan-Induced Diabetic Wistar Male Rats

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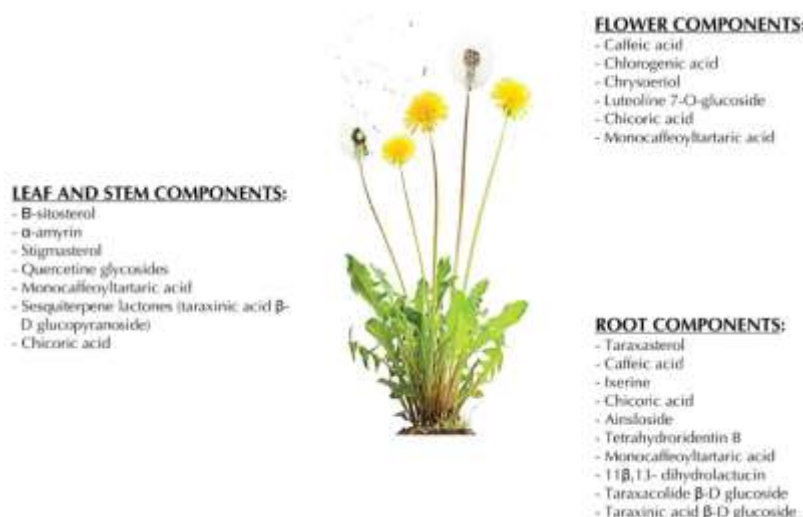*donn.ricky@unai.edu*

ABSTRACT

Diabetes mellitus (DM) is a serious health problem worldwide, World Health Organization (WHO) declared Indonesia takes the fourth place in the DM problem. To overcome this problem, a synthesis drug and medicines have been produced. But production from this medicine has side effects and require a lot of money for it, while many try to find a solution to this disease. There are plants that can help in lowering blood glucose and that is Jombang as we known as dandelion. Because of this plant ability, this research is made. The purpose of this study is to obtain accurate data for lowering white rat's blood glucose from 1 g extract of leaf and root as determined volume is 0.4 ml, 0.6 ml, and 0.8 ml from leaf extract each contains 0.65 g, 0.97 g, and 1.29 g and root extract each contains 0.77 g, 1.15 g, and 1.54 g weight wet of herbs. This study uses True Experimental Design, 22 male rats are divided into five groups: Negative Control (Group I), Positive Control (group II), Experiment I (group III): Rats were induced by alloxan and given extracted jombang leaves orally as much as 0.4 ml (0.65 gram), 0.6 ml (0.97 gram), and 0.8 ml (1.29gram), Experiment II (group IV): Rats were induced by alloxan and given extracted jombang roots orally as much as 0.4 ml (0.77 grams), 0.6 ml (1.15 grams) and 0.8 ml (1.54 grams) and Experiment III (Group v): Rats were induced by alloxan and given extracted as much mixture of leaves and roots of Jombang 0.4 ml (0.71 gram), 0.6 ml (1.06 gram) and 0.8 ml (1.42 gram). Treatment started from day 6 to day 15 and administered twice a day every 12-hour. The measurement of data was collected on day 6, 9, 12, and 15. The data obtained is analyzed with logistic regression, ANOVA multivariate followed by Duncan's multiple range test. The result showed that the volume of group leaf extract, robot extract and the mixture of leaf and root extract 0.4 ml (0.65 g), 0.6 ml (0.97 g), and 0.8 ml (1.29 ml) can reducing blood glucose levels in alloxan-induced rats at the end of the Study. The best volume of extract in lowering blood glucose is 0.4 ml (1.42 g) mixture of leaf and root extracts of Jombang.

Keywords: Hypoglycemic, jombang, leaf extract, root extract, blood glucose

INTRODUCTION

Jombang or known as Jombang is a plant of the genus *Taraxacum* and a member of the Asteraceae family. It has been used as a medicinal herb for a long Time that grows naturally in

Europe, North and South America and Asia. Figure 1 shows an image of the Jombang plant including its various components that may be used for formulations. (Honek et al., 2011).

FLOWER COMPONENTS:
- Caffeic acid
- Chlorogenic acid
- Chrysoeriol
- Luteoline 7-O-glucoside
- Chicoric acid
- Monocaffeoyltartaric acid

LEAF AND STEM COMPONENTS:
- β-sitosterol
- α-amyrin
- Stigmasterol
- Quercetine glycosides
- Monocaffeoyltartaric acid
- Sesquiterpene lactones (taraxinic acid β-D glucopyranoside)
- Chicoric acid

ROOT COMPONENTS:
- Taraxasterol
- Caffeic acid
- Ixerine
- Chicoric acid
- Ainsloside
- Tetrahydroridentin B
- Monocaffeoyltartaric acid
- 11β,13- dihydrolactucin
- Taraxacolide β-D glucoside
- Taraxinic acid β-D glucoside

Figure 1. Image of Jombang and some components present at the level of flowers, stems, and leaves.

Roots of this herb are firstly considered for enhancing digestion and alto are generally used for as liver tonic by stimulating bile production and removing toxins and re-establishing hydration and electrolyte balance. Jombang leaves could be used successfully as digestive stimulant and diuretic. Moreover, Jombang leaves were reported to improve growth and productive performance of poultry. Several beneficial effects have been reported on intestinal mucosa regarding architecture of villi, villus height/ crypt depth ratio as well as cellular infiltration. Jombang has many properties like immunomodulator, digestive stimulant, prebiotic, insulin stimulant, anti-inflammation, antiangiogenic, antineoplastic and demulcent. Furthermore, Jombang treats indigestion and hepatitis B infection as well as enhances metabolise androgenic hormones. Because of the scarcity of in vivo studies on Jombang, further investigations are needed to confirm its benefits and efficacy. (Qureshi et al., 2017).

The bioactive components in Jombang havedemonstrated a series of anti-diabetic effects, which are due to the pharmacological actions ofcomponents such as sesquiterpene lactones, triterpenes/phytosterols (taraxasterol), phenols, flavonoids,and phenolic acids (Schützet et al., 2006). A possible explanation for the effects and mechanisms of Jombang on Type 2 Diabetes Mellitus couldbe its interaction with factors involved in the metabolic syndrome (lipid metabolism, glucose metabolism, protein metabolism, α- and β-cells dysfunction) (Grundy et al., 2004). The mechanisms by which plant-derived compoundsmanifest their anti-diabetic properties are (Mir et al., 2015) :

1. Inhibition of renal glucose reabsorption.
2. Reduction of the activity of carbohydrate enzymes (αamylase with β-galactosidase andα-glucosidase).
3. Reduction of dietary blood glucose (whichstimulates hepatic glycolysis and glycogenesis).
4. Inhibition of potassium channel flow.

METHODS

Tools

The tools used in this study are measuring cups, scales, spatula, beaker, oral sonde, syringe 1 ml, flat flask glass, Water baths, vaporizer cups, blood glucose checks, and inspection trips blood glucose.

Ingredients

The ingredients used in the study are aquades, leaves and roots of Jombang and carboxyl metyl cellulose (CMC). The animals used in this study were white rats strain Wistar that will be taken blood to be tested for blood glucose levels.

Research Preparation

Sterilization of Tools and Materials

Sterilization is very important to reduce or kill bacteria attached to the equipment used so as not to interfere with the resultan from research. the sterilization process using an autoclave at a temperature of 120°C.

Preparation of Wistar Strain Rats

Rats aged 2 months and over were used as many as 22 animals.The rats were acclimatized for 4 days and the rats were grouped accordingly randomly divided into 5 groups where 2 rats in each control group and 6 rats in each treatment group.The treatments taken are:

1. Two rats as positive control group, 2 rats as negative control group, 6 rats for experimental roots group, 6 rats for experimental leaves group, 6 rats for an experimental mixture of root and leaf extracts grup.
2. During the acclimation process, rats are accustomed to holding the tail so that the process of inducing alloxan and taking blood can well done. Provision of drinking orally is done so rats used to be given the results of extracts of roots and leaves of jombang.

Jombang Leaf and Root Preparation

Plant selection is made based on large size, The selected not based on age because the plants taken are wild plants from around the UNAI campus, after carefully selected plants were dug uap so that the roots do not break. The morphology of the selected plants is, diameter roots 0.5 cm at the base, leaf length 10 cm from the base, plants flowering.

Making Jombang Leaf and Root Extracts

The simplicia that has been taken is cleaned, dried under the Light the sun but covered with a cloth then cut as smooth as possible and done as follows:

1. Simplisia macerated with 95% ethanol for 3 x 24 hours os liquid extracts and pulp are found.
2. The extracts obtained are then stored in sterile bottles and closed tightly.
3. After that, the extract is evaporated and diluted using CMC in a ratio of 1: 1 (1 gram of dry extract: 100 ml retail CMC).
4. After obtaining the results of dilution, the contents of each dry extracts of leaves and roots were calculated to see the weight of wet simplicia used.

Research procedure

A total of 22 rats were randomly divided into 5 groups as follows:

1. Negative Control (Group I): Rats are not induced by alloxan and are not given the ethanol extract of roots and leaves of Jombang
2. Positive Control (group II): Rats are induced by alloxan and given 1 ml CMC
3. Experiment I (group III): Rats were induced by alloxan and given extracted jombang leaves orally as much as 0.4 ml (0.65 gram), 0.6 ml (0.97 gram), and 0.8 ml (1.29gram).
4. Experiment II (group IV): Rats were induced by alloxan and given extracted jombang roots orally as much as 0.4 ml (0.77 grams), 0.6 ml (1.15 grams) and 0.8 ml (1.54 grams).
5. Experiment III (Group v): Rats were induced by alloxan and given extracted as much mixture of leaves and roots of Jombang 0.4 ml (0.71 gram), 0.6 ml (1.06 gram) and 0.8 ml (1.42 gram)

After being treated, rat blood was taken and then examined using a glucose test kits. The data obtained were analyzed with statistics.

Test for Reducing Blood Glucose Levels in Rats

Testing for blood glucose levels aims to determine whether there si the effect of root, leaf extract, and mixture of roots and leaves on ras which has been influenced by alloxan substances. The testing steps are:

1. The tip of the rat's tail is cut ± 0.3 mm, then the blood is dripped Tinto the strip. Before the rat's tail is cut, the rat is sure to be quiet without it pressure.
2. All data obtained are recorded
3. All the steps above are carried out on all rats.

Data analysis

In this study, data were analyzed using logistic regression Ana Multivariate variance analysis (ANAVA) and Duncan test.

RESULTS AND DISCUSSION

Extraction

In this study the stages of plant extraction Ana testing of plant extracts to reduce blood glucose levels of white rats which is induced by alloxan. Results from extraction of roots and leaves of Jombang obtained by maceration using ethanol 95% and solven concentration using a water bath.

Table 1. Jombang Plant Extraction

SOURCE	LEAF	ROOTS
Weight of Wet Simplisia	500 g	500 g
Weight of Dry Simplisia	58 g	64 g
Weight of Dry Extract	3.1 g	2.6 g

From the results of concentration, obtained dried extracts of jombang leaves as much as 3.1 grams and dry extracts of jombang roots as much as 2.6 grams, where the weight of the wet simplicia used is, for a volume of 0.4 ml, 0.6 ml, and 0.8 ml leaf extract containing 0.65 grams, 0.97, respectively grams, and 1.29 grams then extract the roots 0.77 grams, 1.15 grams, and 1.54 gram.

Blood Glucose Test Results

In this study extracts from jombang leaves and roots were obtained concentrated insoluble in water, so emulsifiers are catalyst that is CMC-Na. These emulsifiers are dissolved in distilled water and 1% suspension is obtained which is then used to dissolve the extract with a ratio of 1: 1 which is 1 gram of extract: 100 ml CMC. After a suspension was made from alloxan, the rats were induced subcutaneously as much as 67 ml / kg body weight. Measurement of rat blood glucose level done after being fasted for 16 hours, 2 hours after fasting, day 6, day 9, day 12, day 15, and fasting 24 hours after day 15. Results Observation of rat blood glucose from day 1 to day 15 can be seen at table 2 below shows the average blood glucose level of rats for 10 days of treatment.

Table 2. Average Rats Blood Glucose Levels for 15 Days Research

Group	Fasting 16 hours	After 120 Minutes	Day 6	Day 9	Day 12	Day 15	Fasting 24 hours
Control -	91	391.5	496	571.5	565.5	574	573.5
Control +	83.5	164.5	111	106.5	111.5	109.5	109.5
K1	83	397	520	439	314.5	251.5	499
K2	87.5	437	531.5	399	169	97.5	516
K3	79	286.5	352	237	180.5	103.5	332.5
K4	77	387.5	347.5	237.5	164	105.5	346
K5	77	287.5	591.5	376	182.5	111	507
K6	78	332.5	408	130	96.5	96	321.5
K7	58.5	358	512	200.5	110	93	218
K8	91	323	391.5	249	214.5	122.5	253.5
K9	85.5	227.5	158.5	200.5	133	115.5	221.5

Information :

Control (+): Rats induced alloxan and given 1 ml of CMC-Na

Control (-): Rats were not treated

K 1: Rats were given 0.4 ml (0.65 gram) leaf extract

K 2: Rats were given 0.6 ml (0.97 gram) leaf extract

K 3: Rats were given 0.8 ml (1.29 gram) leaf extract

K 4: Rats were given 0.4 ml (0.77 gram) root extract

K 5: Rats were given 0.6 ml (1.15 gram) root extract

K 6: Rats were given 0.8 ml (1.54 gram) root extract

K 7: Rats were given a mixture of 0.4 ml (0.71 gram)

K 8 : Rats were given a mixture of 0.6 ml (1.06 gram)

K 9 : Rats were given a mixture of 0.8 ml (1.42 gram)

From Table 2, alloxan-induced (+) control was given treatment 1 ml of CMC-Na to see whether there is an influence of CMC-Na in reduced rat blood glucose levels, while control (-) was not induced alloxan is used to see whether there is an influence of water and food pellets given causes the rat's blood glucose level to rise or fall, K1
up to K9 are the treatment groups used for viewing decrease in blood glucose levels.

Regression Analysis

Table 3 shows the result of regression analysis of leaf extracts Jombang to see the relationship between treatment time and glucose content rat blood.

Table 3. Summary of Regression Analysis for Glucose Decrease Blood by Jombang Leaf Extract

TIME	R	R Square	Adjusted R-square	Std. Error of the Estimate
K 1	0.99	0.99	0.98	0.05
K 2	0.98	0.97	0.95	0.17
K 3	0.99	0.98	0.97	0.09

K 1: Rats were given 0.4 ml (0.65 gram) leaf extract

K 2: Rats were given 0.6 ml (0.97 gram) leaf extract

K 3: Rats were given 0.8 ml (1.29 gram) leaf extract

From the table 3 we get the results of the calculation of the correlation between time Ana rat blood glucose levels with rat blood glucose levels for K4 = 1.00, K5 = 1.00, and K6 = 0.87, this means the relationship between treatment time Ana a decrease in blood glucose levels is very strong and a positive correlation is also obtained this means this relationship is

unidirectional. Means if the treatment time and decrease blood glucose levels are increased the blood glucose levels will be increasingly decreased.

To see the strong effect of treatment time on glucose level then rat blood R squared from the Table 3 was tested, for K4, K5, and K6 the results are 100%, 99%, and 87% and the rest of the results are 0%, 1%, and 13% is another factor in the decrease in rat blood glucose levels. The relationship between variables time of treatment with decreased levels blood glucose, analysis can be seen in table 4.

Table 4. Analysis of Variants of Reducing Blood Glucose by Jombang Leaf Extracts

| Time	Sum of Squares	Df	Mean Square	F	Sig
Regression					
K1	0.32	1	0.32	140.52	0.01
K2	1.77	1	1.77	62.96	0.02
K3	0.78	1	0.78	105.61	0.01
Residual					
K1	0.00	2	0.00		
K2	0.06	2	0.03		
K3	0.02	2	0.01		
TOTAL					
K1	0.32	3			
K2	1.82	3			
K3	0.79	3			

K 1: Rats were given 0.4 ml (0.65 gram) leaf extract

K 2: Rats were given 0.6 ml (0.97 gram) leaf extract

K 3: Rats were given 0.8 ml (1.29 gram) leaf extract

Based on the results of the count on the previous page, obtained figures of K1 = 0.01, K2 = 0.02, and K3 = 0.01 where the numbers 0.01, 0.02, and 0.01 <0.05. This shows that there is an effect of jombang leaf extract with a volume of 0.4 ml (0.65 gram), 0.6 ml (0.97 gram), and 0.8 ml (1.29 gram) on the decrease in blood glucose levels of alloxan-induced rats.
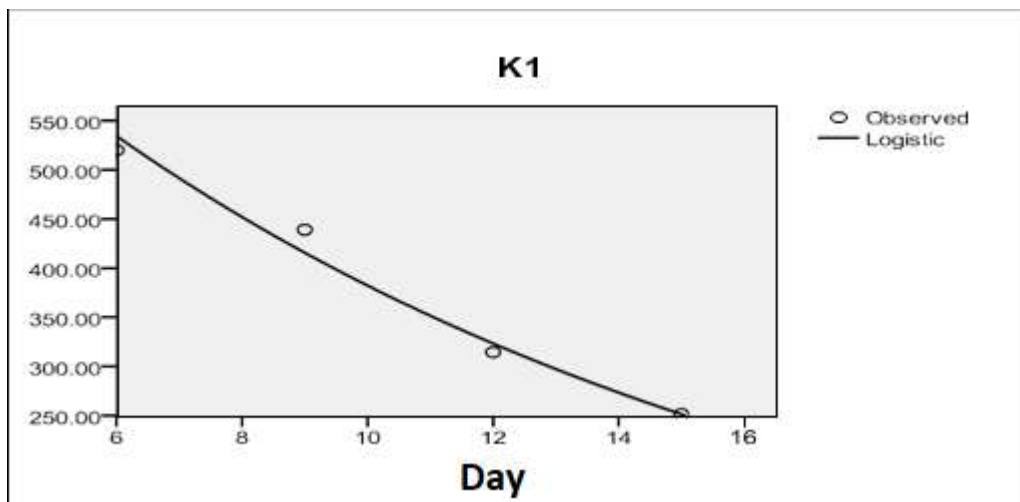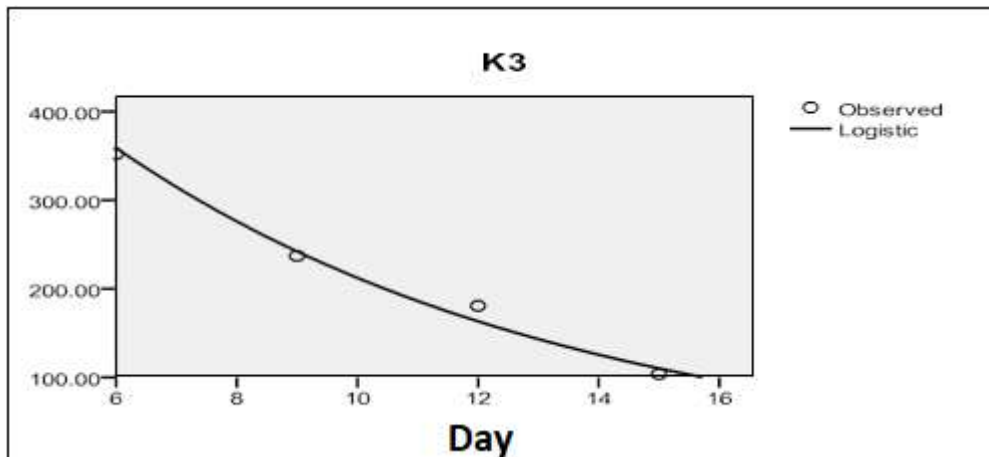
Figure 1. Graph of Decrease in Blood Glucose by Jombang Leaf Extract Volume 0.4 ml (0.65 gram)

Figure 1. shows that on the 6th day it is the peak of the rat blood glucose level and after being given a volume of jombang leaf extract 0.4 ml (0.65 grams) 2 times a day every 12 hours saw a decrease on days 9 to 15.
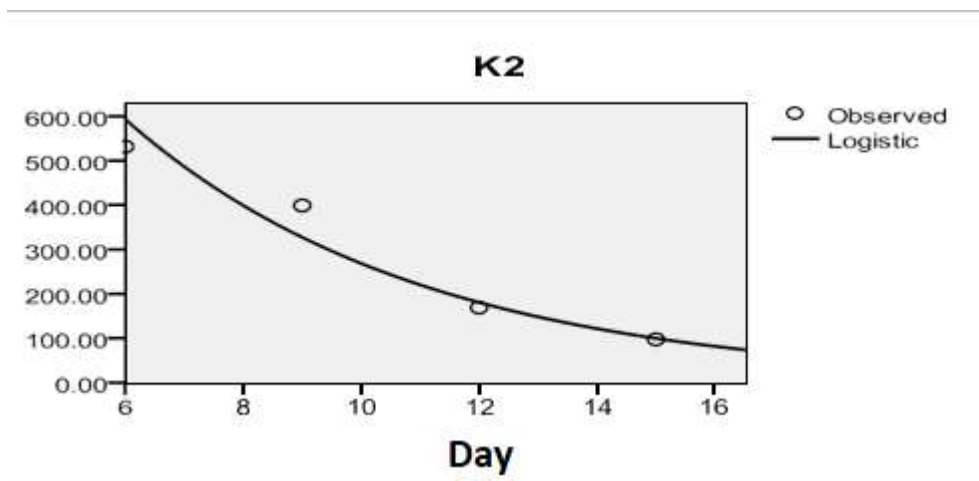
Figure 2. Graph of Decrease in Blood Glucose by Jombang Leaf Extract Volume 0.6 ml (0.97 gram)

Figure 2. shows a decrease in blood glucose levels starting on day 9, that is after being given extracts for 3 days from day 6 and after being given continuous treatment for 6 days, seen on day 15 the rat's blood glucose has reached normal.

Figure 3. Graph of Reducing Blood Glucose by Jombang Leaf Extract Volume 0.8 ml (1.29 grams)

From Figure 3. it shows that there is a decrease in blood glucose from day 9 to day 15, where on the 15th day the blood glucose levels of rats have reached normal, from this picture also seen a decrease in blood glucose levels is regular.

Table 5. Analysis of the Reducing Coefficient of Blood Glucose by Jombang Leaf Extract

Time	Unstandardized Coefficients		Standardized Coefficients	T	Sig
	B	Std. Error	Beta		
Group					
K 1	1.09	0.01	2.70	141.53	0.00
K 2	1.22	0.03	2.68	40.03	0.00
K 3	1.14	0.02	2.64	78.16	0.00
Constant					
K 1	0.00	0.00		12.84	0.00
K 2	0.00	0.00		3.63	0.07
K 3	0.001	0.00		7.09	0.02

K 1: Rats were given 0.4 ml (0.65 gram) leaf extract

K 2: Rats were given 0.6 ml (0.97 gram) leaf extract

K 3: Rats were given 0.8 ml (1.29 gram) leaf extract

From table 5, the difference in the average treatment time for decreasing rat blood glucose levels for K1 = 1.09, K2 = 1.22, and K3 = 1.14 seen from the coefficient analysis is significant where K1 = 0.00, K2 = 0.00, and K3 = 0.00. This means that the difference in treatment time for a decrease in rat blood glucose levels during 10 days of regular treatment is well.

Table 6. Summary of Regression Analysis of Decreased Blood Glucose by Jombang Root Extract

TIME	R	R Square	Adjusted R-square	Std. Error of the Estimate
K 4	1.00	1.00	1.00	0.03
K 5	1.00	0.99	0.99	0.08
K6	0.87	0.76	0.64	0.41

K 4: Rats were given 0.4 ml (0.77 gram) root extract

K 5: Rats were given 0.6 ml (1.15 gram) root extract

K 6: Rats were given 0.8 ml (1.54 gram) root extract

From the table 6, the correlation between time and blood glucose levels and rat blood glucose levels obtained for K4 = 1.00, K5 = 1.00, and K6 = 0.87, this means that the relationship between treatment time and reduction in blood glucose levels is very strong and is obtained also this positive correlation means this relationship is unidirectional. Means that if the treatment time and blood glucose levels are increased the blood glucose levels will decrease.

Table 7. Analysis of Variants of Decreased Blood Glucose by Jombang Root Extract

Time	Sum of Squares	Df	Mean Square	F	Sig
Regression					
K 4	0.78	1	0.78	1249.87	0.00
K 5	1.63	1	1.65	258.46	0.00
K 6	1.07	1	1.08	6.34	0.13
Residual					
K 4	0.00	2	0.00		
K 5	0.01	2	0.01		

| K 6	0.34	2	0.17
TOTAL			
K 4	0.78	3	
K 5	1.66	3	
K 6	1.42	3	

K 4: Rats were given 0.4 ml (0.77 gram) root extract

K 5: Rats were given 0.6 ml (1.15 gram) root extract

K 6: Rats were given 0.8 ml (1.54 gram) root extract

Based on the analysis of variance on Table 7, obtained K4 = 0.00, K5 = 0.00, and K6 = 0.13, for K4 and K5 the results of the analysis are 0.00 and $0.00 < 0.05$ and for K6 the results of the analysis are $0.13 > 0.05$. Means from this analysis K1 and K2 are significant and K6 is not significant.

This suggests that there is an influence of jombang root extract with a volume of 0.4 ml (0.77 gram), 0.6 ml (1.15 gram), and 0.8 ml (1.54 gram) on the decrease in blood glucose levels of alloxan-induced rats, for a volume of 0.4 ml (0.77 gram) and 0.6 ml (1.15 grams) while in volume 0.8 ml (1.54 grams) there was no significant decrease in blood glucose levels.
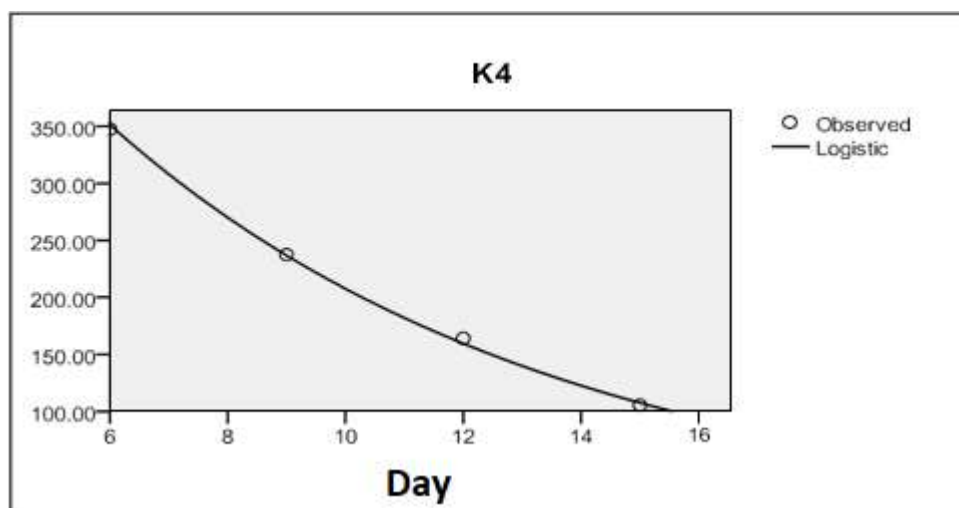
Figure 4. Graph of Decrease in Glucose Content by Jombang Root Extract Volume 0.4 ml (0.77 gram)

Figure 4. shows a 0.4 ml (0.77 gram) jombang root extract given to rats from day 6 to day 15. There was a decrease in blood glucose levels from day 9 to reach normal on day 15.
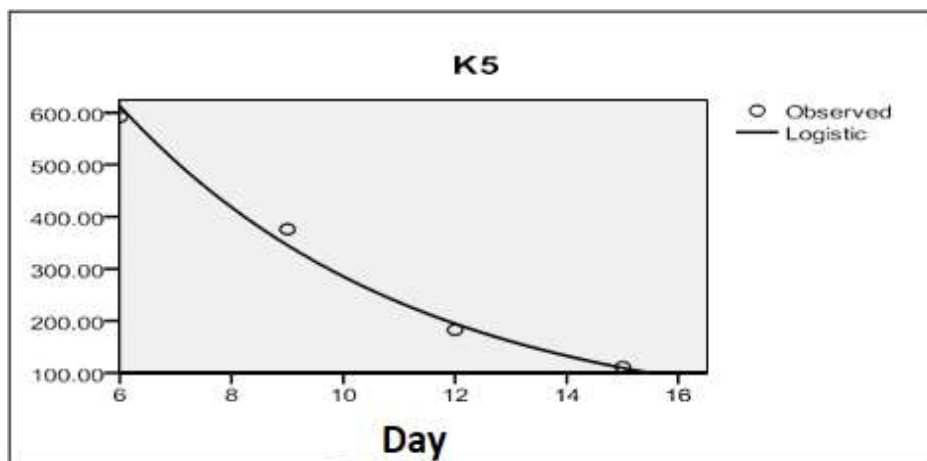
Figure 5. Graph of Reducing Blood Glucose by Jombang Root Extract Volume 0.6 ml (1.15 gram)

Figure 5. shows a decrease in blood glucose levels for a 0.6 ml (1.15 gram) jombang root extract given for 10 days, starting from the 6th day to the 15th day. A decrease in rat blood glucose is seen on the 9th day and continues until the day the last study, which was on day 15 rat blood glucose had reached normal.

Table 8. Analysis of the Reducing Coefficient of Blood Glucose by Jombang Root Extract

Time	Unstandardized Coefficients		Standardized Coefficients	T	Sig
	B	Std. Error	Beta		
Group					
K 4	1.14	0.00	2.72	268.72	0.00
K 5	1.21	0.01	2.71	83.99	0.00
K 6	1.17	0.07	2.39	16.29	0.00
Constant					
K 4	0.00	0.00		24.38	0.00
K 5	0.00	0.00		7.62	0.02
K 6	0.00	0.00		1.48	0.28

K 4: Rats were given 0.4 ml (0.77 gram) root extract

K 5: Rats were given 0.6 ml (1.15 gram) root extract

K 6: Rats were given 0.8 ml (1.54 gram) root extract

From table 8, it shows that the difference in treatment time for the reduction in rat blood glucose levels is K4 = 1.14, K5 = 1.21, K6 = 1.17 and analysis of this coefficient shows significant. This means that for 10 days the treatment carried out a decrease in rat blood glucose levels was well organized.

Analysis Variance

Multivariate analysis was performed to see the time difference in reducing blood glucose levels of rats from Groups K1, K2, K3, K4, K5, and K6 compared to K7, K8, and K9. The results of multivariate variant analysis can be seen in table 9.

Table 9. Analysis of Variance of Average Decreased Blood Glucose by Day in All Treatment Groups

Source	Dependent Variable	Sum of Square	Df	Mean Square	F	Sig.
Day						
	6	278819.78	8	34852.47	1.93	0.17
	9	176240.11	8	22030.01	2.61	0.09
	12	66806.00	8	8350.75	1.84	0.19
	15	39330.11	8	4916.26	11.63	0.00
Error						
	6	162236.50	9	18026.28		
	9	76027.50	9	8447.50		
	12	40930.50	9	4547.83		
	15	3799.00	9	422.11		
Total						
	6	3671091.00	18			
	9	1606377.00	18			
	12	651661.00	18			
	15	310066.00	18			

From the analysis of variance on Table 9, significant results were obtained only on the 15th day where 0.00 <0.05. Duncan multiple range test was then performed to see the difference in the time of the mixture of leaf extract and jombang roots with a volume of 0.4 ml (0.71 gram), 0.6 ml (1.06 gram), and 0.8 ml (1.42 gram) to reduce the blood glucose levels of alloxan-induced rats compared leaf extract and jombang root extract.

Table 10. Summary of Duncan Multiple Range Test

Group	N	Subset	
		1	2
K7	2	93.00	
K6	2	96.00	
K2	2	97.50	
K3	2	103.50	
K4	2	105.50	
K5	2	111.00	
K9	2	115.50	
K8	2	122.50	
K1	2		251.50

K 1: Rats were given 0.4 ml (0.65 gram) leaf extract

K 2: Rats were given 0.6 ml (0.97 gram) leaf extract

K 3: Rats were given 0.8 ml (1.29 gram) leaf extract

K 4: Rats were given 0.4 ml (0.77 gram) root extract

K 5: Rats were given 0.6 ml (1.15 gram) root extract

K 6: Rats were given 0.8 ml (1.54 gram) root extract

K 7: Rats were given a mixture of 0.4 ml (0.71 gram)

K 8 : Rats were given a mixture of 0.6 ml (1.06 gram)

K 9 : Rats were given a mixture of 0.8 ml (1.42 gram)

From table 10 shows that it was seen that all groups except K1 were in subset 1, meaning that there was no difference in the time to reduce the blood glucose levels of alloxan-induced rats for mixed extracts of leaves and jombang roots compared with leaf extracts and root extracts.

DISCUSSION

From the results of the study it was found that there was an effect of leaf extract and jombang roots in reducing blood glucose levels in alloxan-induced rats.

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