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Submission date: 31-Oct-2018 10:07AM (UTC+0700)
Submission ID: 619532542
File name: In_vivo_anti-diabetic_activity.pdf (655.48K)
Word count: 6102
Character count: 28337

Available online at www.sciencedirect.com

ScienceDirect

Procedia Computer Science 135 (2018) 632–642

Procedia Computer Science

www.elsevier.com/locate/procedia

3rd International Conference on Computer Science and Computational Intelligence 2018

In vivo anti-diabetic activity test of ethanol extract of the leaves of Cassia Siamea Lamk

Heruna Tanty[a]*, Syarifah Diana Permai[b], Herena Pudjihastuti[b]

[a]*Department of Mathematics, School of Computer Science, Bina Nusantara University, Jakarta 11480*
[b]*Department of Statistics, School of Computer Science, Bina Nusantara University, Jakarta 11480*

Abstract

Cassia siamea Lamk has been used as a traditional medicine in Indonesia including to treat diabetes mellitus (DM). This study aimed to investigate in vivo anti-diabetic activities of the ethanol extract, ethyl acetate fraction and n-hexane fraction of Cassia siamea Lamk in alloxan-induced mice. The study concluded that: (1) the ethanol extract and n-hexane fraction of Cassia siamea Lamk (Juar) leaves have anti-diabetic activity in Webster albino mice induced with alloxan, (2) The extract of Cassia siamea Lamk leaves, the fractions of 500 mg ethyl acetate and 500 mg n-Hexana of Cassia siamea Lamk provided better performances in lowering blood glucose levels compared to Ethanol extracts both 500 mg and 1000 mg. In the form of ethyl acetate and n-hexane fraction at a dose of 150 mg/kg BW provided the highest anti-diabetic activity compared to the other test groups that are able to decrease blood sugar level by 10.25% and 9.98% respectively. Its effect is equivalent to glibenclamide at a dose of 0.65 mg/kg BW which can lower blood sugar levels by 9.27%. Thus Cassia siamea Lamk leaf is very potential as an alternative drug antidiabetes mellitus, and (3) the 1000 mg Ethanol extract, 150 mg Ethyl acetate and 150 mg n-Hexana had no difference effects in lowering blood glucose levels compared to the anti-diabetic chemical drug glibenclamide.

*Keywords:*Cassia siamea Lamk; In Vivo anti-diabetic test; Anova

* Corresponding author.
E-mail address: herunatanty@yahoo.com

10.1016/j.procs.2018.08.223

1. Introduction

The people having Diabetes Mellitus (DM) in Indonesia continues to increase every year. According to the Ministry of Health of the Republic of Indonesia, by 2030, about 21.3 million people in Indonesia are estimated to have diabetes. About 16.7% of patients with DM are under the age of 40 years, 46.5% are at the age of 40-59 years, and the rest at age above 60 years [1].

DM is a disease caused by high levels of glucose in the blood as a result of defects in insulin secretion. The elevated blood glucose is the risk factor that can cause various disorders in some organs such as liver, heart, kidneys, eyes, nerves, and sexual function [2]. Diabetes Mellitus can be managed by taking chemical drugs continuously for long periods of time. However, the use of chemical drugs can produces negative side effects to other organs. Hence, the search for alternative source of medicine from medicinal plants has continued.

Juar (Cassia siamea Lamk) is a plant from the fabaceae family. A qualitative analysis showed that the leaves of Cassia siamea contains a secondary metabolic compound such as antrona, flavona, triterpenoide, alkaloids and casiadinine [3], isoquinolone Siaminine A, Siaminine B and Siaminine C [4], new bischromone and chrobisiamone A [5] and 5-acetylmethyl-7-hydroxy-2-methyl chromone [6]. The Cassia siamea leaves are believed able to heal various diseases such as malaria, diarrhea, hives, anti-diabetic and anti-inflammatory [7]. In vitro studies showed that the extract of ethanol of Juar leaves in the fractions of ethyl acetate and n-hexane can inhibit the enzyme of alpha glucosidase about 52.319%. This suggests that Cassia Siamea is very potential as an alternative drug for anti-diabetic [8]. This study was conducted to continue the anti-diabetic test that has been performed by in vitro.

The main objectives of this study including: (1) to test whether Cassia Siamea Lamk extracts have an effect on lowering blood glucose levels, (2) to examine whether 4 types of extracts i.e. Ethanol 500 mg/kgBW, Ethanol 1000 mg/kgBW, Ethyl acetate 150 mg/kgBW and n-Hexana 150 mg/kgBW have a different effect on lowering blood glucose levels, and (3) to find out which extract gives the best performance in lowering blood glucose levels. The tests were performed using statistical analysis including descriptive analysis, ANOVA and the Tukey Honest Significance Difference test.

There are 4 types of extracts that will be compared, because there are more than two types of extract that will be compared, then the method that can be used is one way ANOVA. There are several studies of extract test in in vivo activity using one way ANOVA method, such as Ethanol extract of Aloe vera for anti-diabetic test [9], Ethanol extract of Tithonia diversifolia (Hemls.) A. Gray for anti-hyperglycemic test [10], Water extract of Pandan Wangi leaf to decrease blood glucose levels [11] and Extract of Actinidia columnikta for anti-diabetic test [12].

ANOVA can only test whether there are differences between 4 types of extracts. But to investigate differences among the various extracts cannot provide by ANOVA. One method that can be used is t-test between each of the pairs of extracts. However it is not good approach because of repeated statistical tests on the same data. This will cause a higher probability of making a Type I error. There are several alternatives to the multiple comparison analysis tests that can be used among Bonferroni, Sheffèe, Tukey, Newman-Keuls and Dunnett. Each method has strengths and limitations. Based on the comparison of different multiple comparison analysis tests by McHugh [13], this research used Tukey Honest Significance Difference (HSD) method because it can be avoid the probability of making a Type I error.

2. One Way ANOVA

The completely randomized design is a set of independent samples from a set of several populations. Analysis of variance (ANOVA) is one of statistical method that can be used to compare the means of more than two groups / levels. But there are assumptions to use the analysis of variance: (1) the response variable have to normally distributed for each group, (2) the population variances of response variable in each group are the same, (3) the observations must be independent of one another [14]. Suppose that *k* groups, the general form of hypothesis testing to compare population means is

H_0 : $\mu_1 = \mu_2 = \mu_3 = \ldots = \mu_k$

H_1 : at least one of $\mu_i \neq \mu_j$ for $i \neq j$

Table 1. ANOVA table for a completely randomized design

Source of Variation	Degree of Freedom	Sum of Square	Mean Square	F test
Treatments	$k-1$	SSTR	MSTR	F
Error	n_T-k	SSE	MSE	
Total	n_T-1	SST		

Calculate total sum of square (SST), sum square of treatments (SSTR) and sum square of error (SSE). The $\bar{x}_i$ is represent the mean sample i (i = 1, 2, ..., k) and $\bar{\bar{x}}$ is the grand mean [15].

$$SSTR = \sum_{i=1}^{k} n_i \left(\bar{x}_i - \bar{\bar{x}}\right)^2 \quad (1)$$

$$SSE = \sum_{i=1}^{k}\sum_{j=1}^{n_i} n_i \left(x_{ij} - \bar{x}_i\right)^2 \quad (2)$$

$$SST = \sum_{i=1}^{k}\sum_{j=1}^{n_i} n_i \left(x_{ij} - \bar{\bar{x}}\right)^2 \quad (3)$$

The mean sum of square for treatments (MSTR) and mean sum of square for error (MSE) as follows

$$MSTR = \frac{SSTR}{k-1} \quad (4)$$

$$MSE = \frac{SSE}{n_T-k} \quad (5)$$

The F test statistic used to test the equality of k population means. Reject H_0 if the F test is larger than F distribution with $k-1$ numerator degree of freedom and n_T-k denominator degree of freedom at α level of significance [16]. The F test statistic is

$$\boldsymbol{F = \frac{MSTR}{MSE}} \quad (6)$$

3. Tukey's Honest Significance Difference (HSD) Test

If the null hyphotesis in analysis of variance is rejected, then the next step is discover which ones have been shown to be different. There are several methods that can be used, one of them is Tukey's Honest Significance Difference (HSD) test. This research use HSD test because this method can be avoid the probability of making a Type I error [13]. Tukey's HSD test follow the distribution of studentized range or q statistic [17].

H_0 : $\mu_i = \mu_j$ for $i \neq j$
H_1 : $\mu_i \neq \mu_j$ for $i \neq j$

$$HSD = q_{k,df,\alpha}\sqrt{\frac{MSE}{n}} \quad (7)$$

Where $q_{k,df,\alpha}$ is the critical value with k levels, df (degree of freedom) = $n_T - k$ and α level of significance. MSE is mean square of error from ANOVA table and n is number of sample for each population. In order to evaluate the

means difference between populations is take the absolute value of the difference between means and compare to HSD value. Then, H_0 is rejected if

$$|\bar{X}_i - \bar{X}_j| \geq HSD \quad (8)$$

4. Methodology

Samples of C.siamea leaves were collected from the village of Setu, in Cirebon district, West Java, Indonesia. The leaves were cleaned, dried for three weeks and then made into powder. About 454.99 gram of the dried and powdered leaves of C.siamea leaves were extracted with 4 liter of 90% ethanol for 24 hours. The extraction were conducted in 3 replications (3x4Lx24 hours). The filtrates were strained using filter paper and evaporated at 40°C using Rotary Evaporator to get the crude ethanol extract (15.1 gram). The extract were then fractionated using the mixture of 1 part of water and 9 part of n-hexane to obtain n-hexane fraction and water fraction. The water fraction was given ethyl acetate to obtain an ethyl acetate fraction. The n-hexane fraction and ethyl acetate fraction were then concentrated to 5.1 gram and 2.4 gram respectively [8].

The test were using animals induced with aloxan, since aloxan price is relatively cheaper compared to other diabetogen and it can make the animal experience DM condition in a short time. Some researchers tested in Vivo using white mice induced with alloxan [9 11], while S.Kumar performed in Vivo tests with white mice using another diabetogen, i.e. streptozotocin induction [18]. The animal used for experiment were 8-week old Swiss Webster albino mice with weights ranging from 20 to 30 gram. The animals were kept for 1 week in advance to adapt to the conditions of the cage before being treated. The mice were placed in a cage that is given a husk base to absorb dirt from mice. During the adaptation period, the mice were fed, watered and weighted daily. The mice are considered healthy when their weight increases or remains the same or decreases no more than 10%.

The mice used for experiment were 21 animals, which were divided into 7 groups, containing 3 animals in each group. Before treatment, the mice fasted for 18 hours (ad libitum). Blood samples were drawn from each mouse's tail for measuring the first blood glucose levels (baseline). All mice then were treated intra peritoneally with 250 mg/kg BW of alloxan, to elevate their blood glucose levels. After 48 hours being treated, blood samples were drawn from each mice for measuring their blood glucose levels. If the blood glucose levels of mice > 200 mg/dL then the mice are considered to have hyperglycemia. Furthermore, the alloxan-induced groups of diabetic miceorally received the following treatment schedule for 7 days:

- Group 1 served as normal control, received 2% PGA suspension without induction
- Group 2 served as negative control, received alloxan induction in 2% PGA
- Group 3 served as positive control, received 0.65 mg/kg BW glibenclamide in 2% PGA suspension
- Group 4 served as the test group, received ethanol extract at a dose of 500 mg/kg BW in 2% PGA suspension
- Group 5 served as the test group, received ethanol extract at a dose of 1000 mg/kg BW in 2% PGA suspension
- Group 6 served as the test group, received the ethyl acetate fraction at a dose of 150 mg/kg BW in 2% PGA suspension
- Group 7 served as the test group, received the n-hexane fraction at a dose of 150 mg/kg BW in 2% PGA suspension

Blood glucose level were measured every day for 8 days after treatment, using amperometric method that utilizes enzymatic glucose dehydrogenase reaction with Glucometer instrument. The relative blood glucose level can be calculated by the following formula:

$$\text{Relative blood glucose level} = \frac{\text{blood glucose level at time t}}{\text{intial blood glucose level}} \times 100\% \quad (9)$$

5. Results and Discussions

The result of blood glucose measurements from each group are in the following Table.

Table 2. Blood glucose levels of each group during the examination of anti-diabetic activity of extracts of Cassia Siamea leaves fraction

Group	No	Blood Glucose Levels at day								
		D0	D1 (Diabetes)	D2	D3	D4	D5	D6	D7	D8
Group 1 (Normal)	1	80	77	64	100	85	95	90	88	87
	2	101	68	68	101	80	80	100	80	92
	3	84	80	61	84	78	83	86	98	73
	$\bar{x}$	88.33	75.00	64.33	95.00	81.00	86.00	92.00	88.67	84.00
Group 2 (Negative)	1	77	325	331	328	326	320	315	309	303
	2	78	327	366	355	345	339	334	327	320
	3	80	274	283	298	313	304	296	274	252
	$\bar{x}$	78.33	308.67	326.67	327.00	328.00	321.00	315.00	303.33	291.67
Group3 (Positive)	1	90	471	457	448	440	415	391	343	296
	2	96	387	383	348	313	276	240	230	220
	3	100	488	476	435	395	342	290	259	229
	$\bar{x}$	95.33	448.67	438.67	410.33	382.67	344.33	307.00	277.33	248.33
Group 4 ethanol extract 500 mg/kg of weight	1	101	269	418	288	262	238	180	168	157
	2	78	292	415	314	261	231	222	204	202
	3	98	263	379	219	190	164	157	147	145
	$\bar{x}$	92.33	274.67	404.00	273.67	237.67	211.00	186.33	173.00	168.00
Group 5 ethanol extract 1000 mg/kg of weight	1	98	266	242	240	232	212	197	154	126
	2	83	264	248	238	225	220	206	181	140
	3	78	273	257	232	220	185	183	166	143
	$\bar{x}$	86.33	267.67	249.00	236.67	225.67	205.67	195.33	167.00	136.33
Group 6 ethyl acetate fraction 150 mg/kg of weight	1	90	385	323	310	300	300	273	223	163
	2	93	381	303	302	261	256	211	191	165
	3	87	370	357	329	261	223	219	210	204
	$\bar{x}$	90.00	378.67	327.67	313.67	274.00	259.67	234.33	208.00	177.33

Group	No	Blood Glucose Levels at day								
		D0	D1 (Diabetes)	D2	D3	D4	D5	D6	D7	D8
Group 7 n-hexane fraction 150 mg/kg of weight	1	90	322	305	299	283	272	210	190	147
	2	96	366	363	249	226	180	156	128	118
	3	101	348	340	258	228	218	207	193	163
	$\bar{x}$	95.67	345.33	336.00	268.67	245.67	223.33	191.00	170.33	142.67

The blood glucose level of mice was observed before alloxan induction (D0) and after the mice had diabetes (D1) and continued by giving the 8 days suspension. Table 2 above explained that the blood glucose levels of all treatment groups before alloxan induction (H0) ranged from 78.33 ± 2.87 to 95.67 ± 7.78 mg/dL. This indicates that all test animals before alloxan induction had normal blood glucose levels, ranging from 62-175 mg/dL. Furthermore, at day 1 (D1) the mice intraperitoneally were induced with alloxan at a dose of 250 mg/kgBW

After alloxan induction, blood glucose levels of mice were monitored for 3 days to see the effect of hyperglycemia. After 3 days of induction, there would be mice that have hyperglycemia and mice that was still in normal condition. As for mice who were still in normal condition would be re-induced with the same dose of alloxan.

The observation showed that the blood glucose levels of the mice with hyperglycaemia ranged between 267.67 ± 3.86- 438.67 ± 40.12 mg/dL. The hyperglycemia mice were then divided at random into 6 groups, consisting of 3 animals in each group.

Mice with hyperglycemia were then orally treated according to their group for 8 days. Group 3, as a positive control group was treated with glibenclamide at a dose of 0.65 mg/kgBW. Glibenclamide is insoluble in water so it is suspended with PGA agent. Group 2 as negative control was only induced by alloxan, it used to determine the decrease in blood glucose levels from normal circumstances. The measurement of blood glucose level was performed on day 1 (after mouse became diabetic) until day 8 (Table 2).

The relative blood glucose level and its percentage of reduction were calculated based on the data in Table 2. The relative blood glucose levels of each group are presented in Table 3 below

Table 3. The relative blood glucose levels of each group

Group	The relative blood glucose levels of each group (%)								
	H0	H1	H2	H3	H4	H5	H6	H7	H8
K Normal	100.00	86.27	73.32	108.33	92.77	98.92	104.63	101.96	95.58
K(-)	100.00	394.60	417.62	417.87	418.98	410.07	402.43	387.68	372.92
K(+)	100.00	471.49	460.91	431.76	403.31	363.54	324.81	293.23	262.35
Extract ethanol 500	100.00	303.02	444.22	303.73	262.63	233.05	207.68	192.63	187.46
Extract ethanol 1000	100.00	313.17	291.74	276.36	263.29	239.52	227.94	196.01	160.19
Fraction EtOAc 150	100.00	420.91	365.01	349.11	304.66	288.31	260.65	231.51	197.67
Fraction n-Heksan 150	100.00	361.19	351.22	282.35	258.53	235.19	200.26	178.51	149.21

Figure 1 shows the average relative glucose level profile of each group in graphical form. Table 3 and Figure 1 showed that during the test the blood glucose level of all the groups, except the normal group and the negative control

group, decreased gradually to near normal levels. While the normal and the negative control group still had hyperglycemia until the end of the test (day 8). The test group (group 4 to group 7) had a significant decrease in blood glucose levels, even lower than the positive control group treated with glibenclamide at a dose of 0.65 mg/kgBW. Group 4 that treated with Ethanol extract dose 500 mg / kg BW was still not able to lower blood glucose levels on day 2, while the other test groups have shown the effect of lowering blood glucose. However on the 3rd day, group 4 showed a very sharp decrease of blood glucose levels.

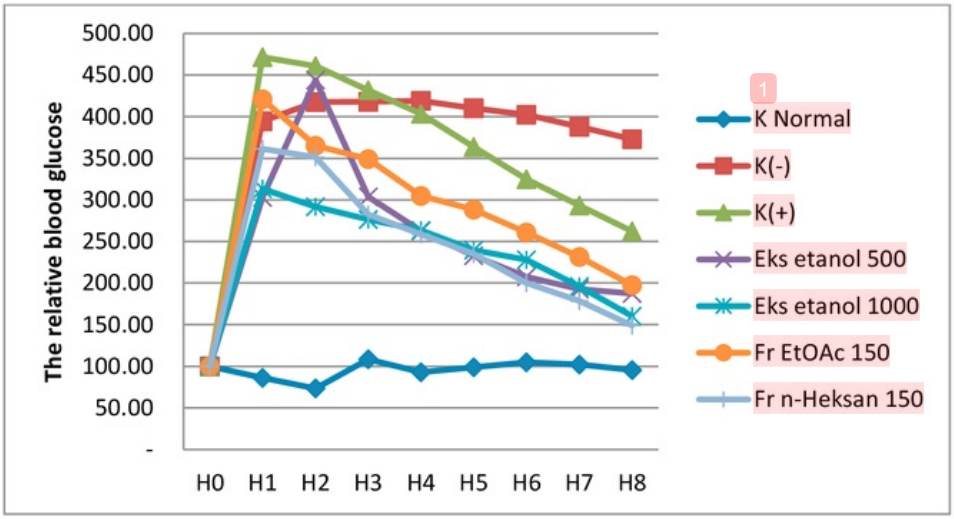

Fig. 1. The average relative glucose level profile of each test group

The percentage of decrease in blood glucose levels of each group can be seen in Figure 2 below:

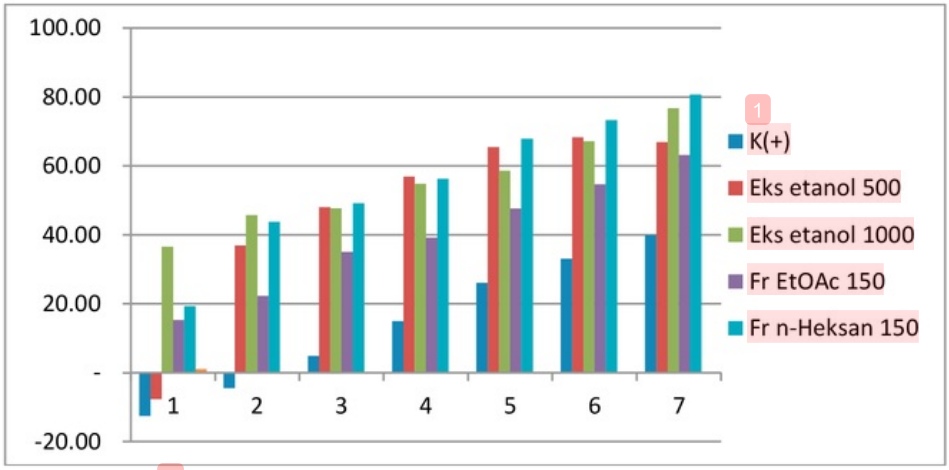

Fig. 2. The percentage of decrease in blood glucose levels of the test groups

The provision of ethanol extract at a dose of 1000 mg/kgBW may decrease blood glucose level, but was not better than at a dose of 500 mg/kgBW. Thus, it can be concluded that the increase of dose on ethanol extract was not showed differences in effect of decreasing of blood glucose levels. The ethanol extract at a dose of 500 mg/kg BW tended to have a faster effect on decreasing blood glucose level, shown in the third day (H3) of measurement.

The groups treated with n-hexane fraction and ethyl acetate fraction at a dose of 150 mg/kgBW, both showed almost similar decrease in blood glucose levels, although on day 2 to day 8, the n-hexane fraction showed smaller levels. The relative glucose level of the group 5 with ethanol extract test group 1000 mg/kg BW, the group 6 and 7 with n-hexane and ethyl acetate fractions on day 2 appear smaller compared to that of the group 4 with ethanol extract group 500 mg / kg BW, positive control group 3 and negative control group.

The formula to determine the best group with blood glucose level reduction is

$$P\ (\%) = \frac{\text{relative glucose level of K(-)- relative glucose level of K (test)}}{\text{relative glucose level of K(-)- relative glucose level of K (N)}} \times 100\% \quad (10)$$

where

P = percentage of relative blood glucose level reduction
K (-) = negative control group
K (test) = test group
K (N) = normal control group

Since the blood glucose levels at day 1 were different for each treatment group, the mean of difference in the reduction of blood glucose levels per group during the test day were calculated to conclude the highest decrease in glucose levels (Figure 3)

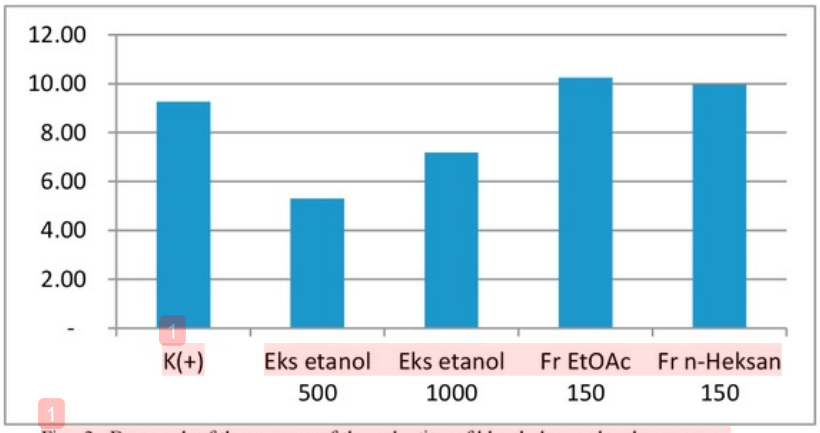

Fig. 3. Bar graph of the average of the reduction of blood glucose levels per group

Figure 3 showed that group 6 that was treated with ethyl acetate test fraction at a dose of 150 mg/kgBW, provided the highest reduction of blood glucose (i.e. 10.25%), while group 7, that was treated with n-hexane fraction at a dose of 150 mg/kgBW gave 9.98% reduction. Moreover, it showed that group 6 and 7 gave a decrease in the blood glucose almost equivalent compared to the positive control group (group 3) that was treated with glibenclamide at a dose of 0.65 mg/kgBW. While group 5, that was treated with ethanol extract at a dose of 1000 mg/kgBW gave a larger decrease rate (i.e. 7.18%) than group 4 that treated with ethanol extract at a dose of 500 mg/kgBW (i.e. 5.31%). It seems that the increasing dose of Ethanol extract will rise the anti-diabetic effect.

The results of in vivo test showed that the extract of Cassia siamea.Lamk leaves, in the form of ethyl acetate and n-hexane fraction at a dose of 150 mg/kgBW, were able to decrease blood sugar level by 10.25% and 9.98% respectively. It means that the anti-diabetic activity of C. siamea Lamk leaves extract is equivalent and even higher than the glibenclamide anti-diabetic drug at a dose of 0.65 mg/kgBW (i.e. 9.27%). Therefore it suggests that the extract of C. siamea.Lamk leaves can be used as an alternative medicine for people with diabetes mellitus (DM).

Furthermore, a statistical analysis was performed to test whether there was a difference in blood glucose levels before and after being treated with Cassia Siamea Lamk extract. If there is a difference then Cassia Siamea Lamk extract treatment is proven to lower blood glucose levels, instead it means that Cassia Siamea Lamk extract cannot be used to lower blood glucose levels. The following is a comparison test of blood glucose levels before and after treatment of Cassia Siamea Lamk extracts.

H_0 : $\mu_D = 0$
H_1 : $\mu_D \neq 0$

The test showed p-value = 2.385×10^{-7}. Because the p-value is less than $\alpha = 0.05$, it would be led to reject H_0. Thus, there was sufficient evidence to conclude that the mean of blood glucose levels differed between before and after being treated with the extracts or fractions of Cassia Siamea Lamk for 8 days. Based on these results, it can be concluded that the extracts or fractions of Cassia Siamea Lamk can be used as an alternative medicine to lower blood glucose levels.

Furthermore, analysis was conducted to find out what extracts/fractions had an effect on lowering blood glucose levels. This study used 4 types of extracts/fractions i.e. Ethanol 500 mg (group 4), Ethanol 1000 mg (group 5), Ethyl acetate 150 mg (group 6) and n-Hexana 150 mg (group 7). The one-way ANOVA was used to test whether there are differences in effect of the 4 extracts/fractions in lowering blood glucose levels. The data that be used in this research was fulfill ANOVA assumptions. Based on Levene's test for homogeneity of variance showed that p-value equal to 0.7311. It means variance of blood glucose levels in each group are same. This experiment used 21 animals, which were divided into 7 groups, containing 3 animals in each group, it means observations was independent of one another. Here are the results of ANOVA test

Table 4. ANOVA table

Source	df	Sum Square	Mean Square	F	p-value
Treatment	3	21582	7194	10.3	0.00403
Residuals	8	5589	699		
Total	11	27171			

H_0 : $\mu_4 = \mu_5 = \mu_6 = \mu_7$
H_1 : at least one of $\mu_i \neq \mu_j$ for $i \neq j$

Using the F test statistic on Table 4, showed p-value equal to 0.00403, which is less than $\alpha = 0.05$. Therefore, the H_0 is rejected which showed that there is at least a pair of extracts/fractions have a different mean of blood glucose level. To determine the best extract in lowering blood glucose levels, it is necessary to conduct further test using Tukey's Honest Significance Difference test.

Table 5. Tukey's Honest Significance Difference test for extracts of Cassia Siamea

	diff	lower	upper	p-value
Group 5 – Group 4	24.66667	-44.44201	93.77534	0.6755397
Group 6 – Group 4	94.66667	25.55799	163.77534	0.0100015
Group 7 – Group 4	96	26.89132	165.10867	0.0092342
Group 6 – Group 5	70	0.89132	139.10867	0.0471790
Group 7 – Group 5	71.33333	2.22466	140.44201	0.0432603
Group 7 – Group 6	1.33333	-67.77534	70.44201	0.9999078

H_0 : $\mu_i = \mu_j$
H_1 : $\mu_i \neq \mu_j$

When comparing group 4 that was treated with ethanol extract at a dose of 500 mg/kgBW and group 5 that was treated with ethanol extract at a dose of 1000 mg/kgBW, Table 5 showed that the p-value is 0.6755397. Since the p-value is larger than $\alpha = 0.05$, then H_0 cannot be rejected, which means that there is no difference in blood glucose levels between group 4 and group 5. Thus, it can be concluded that a dose of 500 mg and 1000 mg of Ethanol extract gives the same performance in lowering blood glucose levels, meaning that additional doses of ethanol extract have no effect on lowering the blood glucose level. Do the same thing for other groups, comparing group 4 that received ethanol extract at a dose of 500 mg/kg BW and group 6 that received Ethyl acetate fraction at a dose of 150 mg/kg BW, showed that the blood glucose levels of groups 4 and group 6 are significantly different. It concluded that 150 mg/kgBW Ethyl acetate fraction gave a better effect in lowering blood glucose level compare to 500 mg/kg BW

Ethanol extract. The result of p-value of comparing group 4 that received ethanol extract at a dose of 500 mg/kg BW and group 7 received n-Hexana fraction at a dose of 150 mg/kg BW is smaller than α=0.05. it can be concluded that the 150 mg/kgBW n-Hexana fraction provided a better effect in lowering blood glucose level compare to 500 mg/kg BW Ethanol extract.

On the other hand, the blood glucose levels of group 5 and group 6 are significantly different, or it means that the 1000 mg/kg BW Ethanol extract and 150 mg/kg BW Ethyl acetate fraction provide a significantly different performance in lowering blood glucose levels. Since the difference of the value of the blood glucose level between group 7 and 4 is positive 70, it can be concluded that the Ethyl acetate fraction at a dose of 150 mg/kgBW provided a better effect in lowering blood glucose level compare to 1000 mg/kg BW Ethanol extract. The same result of comparing group 5 and group 7. The n-Hexana fraction at a dose of 150 mg/kg BW provided a better effect in lowering blood glucose level compare to Ethanol extract at a dose of 1000 mg/kg BW. But, in comparing group 6 that received Ethyl acetate at a dose of 150 mg/kg BW and group 7 that received n-Hexana at a dose of 150 mg/kg BW, the Tukey test gave p-value = 0.9999 which is larger than α=0.05. Hence, it showed that there is no sufficient evidence to reject H_0 and it suggested that the blood glucose levels of groups 6 and group 7 are not significantly different, or it means that Ethyl acetate fraction at a dose of 150 mg/kg BW and n-Hexana fraction at a dose of 150 mg/kg BW provide the same performance in lowering blood glucose levels.

Based on the results of the test, it showed that the 150 mg Ethyl Acetate fraction (group 6) and 150 mg n-Hexana fraction (group 7) are better in lowering blood glucose levels compared to 500 mg ethanol extract (group 4) and 1000 mg ethanol extract (group 5). However, Ethyl Acetate 150 mg (group 6) and n-Hexana 150 mg (group 7) extracts have no difference effect in lowering blood glucose levels. Therefore it can be concluded that Ethyl acetate 150 mg and n-Hexana 150 mg are two types of fractions which gives the best result in lowering blood glucose levels.

The analysis was continued to examine the differences in blood glucose levels between groups receiving Cassia Siamea Lamk extracts/fractions and K (+) group receiving anti-diabetic drugs i.e. glibenclamide at a dose of 0.65 mg/kgBW. The following are the results of the test using Tukey Honest Significance Difference test.

Table 6. Tukey Honest Significance Difference test between positive control and extracts of Cassia Siamea

	diff	lower	upper	p-value
Group 4 – Group 3	93.66667	5.425831	181.90750	0.0365162
Group 5 – Group 3	69	-19.240836	157.24084	0.1494212
Group 6 – Group 3	-1	-89.240836	87.24084	0.9999995
Group 7 – Group 3	-2.33333	-90.574169	85.90750	0.9999845

In comparing group 4 that received Ethanol 500 mg/kgBW and the positive control (group 3) that received glibenclamide at a dose of 0.65 mg/kg BW, the Tukey test gave p-value = 0.03651 which is smaller than α = 0.05. Hence, H_0 was rejected and it means that Ethanol 500 mg/kgBW provided different effect in lowering blood glucose levels compared to the anti-diabetic chemical drug glibenclamide. The Tukey tests in comparing group 5 to 3, and group 7 to 3 gave p-values that larger than α=0.05. Hence, H_0 was not rejected and it means that the Ethanol extract 1000 mg/kg BW and n-hexane fraction 150 mg provided no difference effect in lowering blood glucose levels compared to the anti-diabetic chemical drug glibenclamide.

6. CONCLUSION

The blood glucose levels after treated with the extracts or fractions of Cassia siamea Lamk were lower than before it received the extract or fractions. The ethanol extract and n-hexane fraction of Cassia siamea Lamk (Juar) leaves have anti-diabetic activity in Webster albino mice induced with alloxan. It means Cassia Siamea Lamk can be used as an alternative medicine to lower blood glucose levels. Based on the result of comparing 4 types of extracts/fractions i.e. Ethanol 500 mg (group 4), Ethanol 1000 mg (group 5), Ethyl acetate 150 mg (group 6) and n-Hexana 150 mg (group 7), it showed that Ethyl acetate 150 mg and n-Hexana 150 mg are two types of fractions which gives the best result in lowering blood glucose levels.

The extract of Cassia siamea Lamk leaves, in the form of ethyl acetate and n-hexane fraction at a dose of 150 mg/kg BW provided the highest anti-diabetic activity compared to the other test groups that are able to decrease blood sugar level by 10.25% and 9.98% respectively. Its effect is equivalent to glibenclamide at a dose of 0.65 mg / kg BW which can lower blood sugar levels by 9.27%. The Ethanol extract 1000 mg/kg BW and n-hexane fraction 150 mg provided no difference effect in lowering blood glucose levels compared to the anti-diabetic chemical drug glibenclamide. Thus C.siamea Lamk leaf is very potential as an alternative drug antidiabetes mellitus.

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