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RESEARCH ARTICLE

The Impact of Moringa Oleifera and Folic Acid on Reducing Blood Glucose Levels in Alloxan-Induced Rattus Norvegicus

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ABSTRACT

Moringa leaf (Moringa oleifera) is a plant with antidiabetic activity. This study aims to determine the effectiveness of giving Moringa Oleifera and Folic Acid on blood glucose levels in female rats. Moringa Oleifera on blood glucose levels in rats after induced alloxan 120mg / kgBB. This study used alloxan as an inducer of diabetes mellitus in female rat test animals. 15 female rats were divided into 5 treatment groups. Group I as a Negative Group (N), was given standard feed and Na-CMC, Positive Group (P) alloxan induction, group III treatment I (P1) alloxan induction and given EDK (MO), Group IV Treatment II (P2) alloxan induction and given Folic Acid (AF), Group V Treatment III (P3) alloxan induction and given MO and AF. The results showed that the combination of MO and AF could reduce blood glucose levels in female rats induced by alloxan. The results showed that the administration of Moringa Leaf Extract (MLE) and Folic Acid (AF) had a significant effect in reducing blood glucose levels of female rats $p<0.05$. The average decrease in rats' blood glucose levels in group P1 amounted to 151.00g/dl, P2 amounted to 374.33g/dl, and group P3 amounted to 82.67g/dl. The decrease in blood glucose levels is due to the presence of biochemical compounds that are thought to have a hyperglycemic effect.

INTRODUCTION

Blood glucose is the sugar (glucose) concentration in humans' circulatory systems. Glucose is the primary energy substrate for the body's cells, particularly the brain and muscles(Klonoff et al., 2017). Upon consumption of carbohydrate-containing food, the human body metabolizes the carbs into glucose, which is then absorbed into the circulatory system(Sami et al., 2017). A balanced glucose level is very important for maintaining good cognitive function in humans, both those who are healthy and those with certain diseases(Sebastian et al., 2023).

In 2019, the International Diabetes Federation (IDF) reported that the worldwide prevalence of diabetes mellitus had reached 463 million individuals aged 20-70. Projections indicate a further growth of 576 million by 2030 and 700 million by 2045 (Suharniayanti et al., 2022).

Indonesia is a country famous for its highly nutritious plants used by the community as medicine so that they can be used to help treat various diseases including diabetes mellitus. Medical treatment

for diabetes is still considered expensive, so people are starting to look for alternative treatments based on traditional ingredients (Ikrima et al., 2019).

Moringa leaves (Moringa oleifera L.) belong to the Moringaceae species. Moringa leaves (Moringa oleifera L.) contain active compounds of alkaloids, flavonoids, and tannins, as well as moringa leaves that are efficacious against lowering blood glucose levels, the active compounds in moringa leaves can secrete insulin which works by stimulating beta cells of the pancreas (Bobaya et al., 2023)

Based on this description, it is known that the Moringa plant, especially the leaves and fruits of Moringa (Moringa oleifera L.) can be an alternative choice for the treatment of diabetes mellitus (Surya, et al., 2020).

Based on research in 2020, that Moringa Leaf Extract can reduce blood sugar levels in mice at a dose of 14mg/20grBB mice. Because Moringa Leaf Extract contains secondary metabolites such as alkaloids, flavonoids, tannins, saponins and polyphenols as well as moringa leaves and fruit itself which is an alternative to lowering blood glucose (Pitriya, et al., 2017).

In this study, researchers used rats (Rattus novergicus) induced with alloxan compounds to obtain experimental diabetic conditions in a short time. Alloxan compounds are cyclicurea derivatives that have high effectiveness against diabetogenic agents to induce experimental diabetic conditions. Alloxan compounds can cause damage to pancreatic B cells by forming ROS structures that interfere with the induction or secretion of insulin. Based on the explanation above, researchers are interested in conducting research on how effective*Moringa* leaf extract (*Moringa olifera*) is in reducing blood glucose levels in rats (*Rattus novergicus*).

RESEARCH METHODS

Design Research

This study used the Laboratory Experimental research method with Post Test Only Control Design Group Design. Post Test Only Control Group Design is a design where the samples are randomly grouped into two groups, namely the treatment group and the control group.

Research Ethics

This research was conducted at the Biopharmaca Laboratory, Faculty of Pharmacy, Hasanuddin University on June 01, 2024. Hasanuddin University on June 01, 2024 - July 01, 2024 and has obtained approval recommendation from the Ethics Commission with approval recommendation number 773/UN4.17.8/KP.06.07/2024

Tools and Materials

The tools used in this study are glucometer (Nesco), rat cage, digital kris scales, oral sonde, glass jar, measuring cup, stirring rod, cool box.

The materials used are chaff, feed, drink, cotton, handscoon, mask, EDTA tube, capillary tube, Underpad, 3 cc spoit, Nesco strip, diethyl ether, Folic Acid, MO capsule, Alloxan, Na-CMC, Sterile Aquadest, Wet and dry tissue, Markers, Female mice.

Grouping of Test Animals

Animals used in this test amounted to 20 heads, which were divided into 5 treatment groups with each group totaling 4 heads (3 treatment tails and 1 reserve tail) each group was given standard feed and drink. Group 1 (Negative Control), Group 2 (Positive Control), Group 3 (Moringa leaf extract), Group 4 (Folic Acid), Group (Moringa leaf extract and folic acid). Each rat has a size of 150-250 grams, 2-3 months old, acclimatized in laboratory conditions for one week or 7 (seven) days and habituated

with adequate food and drink. During acclimatization, the body weight of the rats was weighed regularly. The rats used were healthy and did not have a large weight loss of 10%.

Sample Size

Determination of the sample size was carried out using the Federer formula

$$(t-1)\,(n-1)\geq 15$$

Description:

t = Treatment group

n = number of samples for 1 treatment group

$$(t-1)\,(n-1) \geq 15$$
$$(5-1)\,(3n-1) \geq 15$$
$$4\,(2n) \geq 15$$
$$4n\,(2n) \geq 15$$
$$8n \geq 15$$
$$n \geq 15+8$$
$$n \geq 23/8$$
$$n \geq 2{,}875 \rightarrow 3$$

The samples used in each research group were 3 heads and the number of groups used was 5 groups. Thus, this study used 15 female white rats from the existing population.

To anticipate the possibility of the selected sample experiencing dropout:

$$N = \frac{n}{(1 - f)}$$

Description:

N = number of correlation samples

n = initial sample size

f = estimated proportion of dropouts at 10% (f = 0.1)

$$N=n/((1-f))$$
$$= 3/((1-0{,}1))$$
$$= 3/0{,}9$$
$$= 3{,}333 = 4$$

Based on Frederer's formula, the number of rats to be used after rounding to 4 heads per group with a total of 20 rats used in this study.

Research sample

The samples of this study were female rats selected by simple random sampling from a population of rats that met certain criteria. The selected rats had to be 2-4 months old, weighing around 150-250 grams, and in good health (active and not deformed). Exclusion criteria included inactive rats,

rats that died during the adaptation period, and rats that had lost weight. In other words, only rats that meet all inclusion criteria and none of the exclusion criteria will be sampled in this study.

Treatment of rat test animals

Before the experiment, the rats were acclimatized for a week to adapt to the new environment. Afterwards, the rats were divided into groups: control group (CMC-treated), positive group (alloxan-treated), and treatment group (alloxan-treated and various treatment combinations). After alloxan induction, the rats were observed for several weeks, and blood was taken periodically to measure blood glucose levels. The purpose of taking blood was to monitor the progression of the diabetic condition in the rats and the effectiveness of the various treatments given.

Data Analysis

The study analyzed blood glucose level data using computer software, including the Shapiro-Wilk Test for normality, Oneway Anova Test with Post Hoc Bonferroni for normal distribution and variance, and LSD analysis for significant differences between treatment groups. The mean and median data were displayed for normal distribution and different variances for normal distribution.

RESULTS

The ability of Moringa fruit extract (MO) and Folic Acid in reducing blood glucose levels of rats can be seen from the average percentage reduction in each treatment group can be seen in Table 1.

Table 1. Average percentage reduction in each treatment group

Group		Glucose Level (g/dL) Mean±SD	P-Value*
Pretest	Positive	117.67±6.807	0.270
	Negative	102.33±1.528	
	MO capsules	128.00±36.592	
	AF	120.67±15.535	
	MO and AF capsules	97.00±6.557	
Alloxan Induction	Positive	102.67±2.082	0.301
	Negative	148.67±11.015	
	MO capsules	525.67±638.080	
	AF	522.67±321.298	
	MO and AF capsules	126.00±19.698	
Posttest	Positive	95.67±24.786	0.113
	Negative	91.00±7.211	
	MO capsules	151.00±97.550	
	AF	374.33±286.678	
	MO and AF capsules	82.67±18.230	

Table 1 shows the distribution of glucose levels in the MO Capsule group pretest has the highest value of 128.00 ± 36.592 and the lowest value in the MO and AF group 97.00 ± 6.557. Furthermore, in the induction group, the highest value of glucose levels was in the MO Capsule group of 525.67 ± 638,080 and the lowest was the Positive group with a value of 102.67 ± 2,082. While in the posttest group that had the highest value was in the AF group at 374.33 ± 286.678 and the lowest value was the MO and AF capsule group at 82.67 ± 18.230. This shows that the Alloxan Induction group with a p-value of 0.301 has a significant value compared to the Postes group with a p-value of 0.113.

The data normality test (Kolmogorov-Smirnov Test and Sapiro-Wilk Test) is a normality test used to determine whether the data population is normally distributed or not. This test is usually used to

measure ordinal, interval, or ratio scale data. Glucose levels in the Alloxan Induction group and Postes group are greater than the P-Value> 0.05 so it can be concluded that the pretest group, Induction group and Postest group glucose levels are normally distributed. While the homogeneity test shows that the pretest group data probability value sig. 0.004, Alloxan Injection group probability value sig. 0.001 and posttest group probability value Sig. 0.048 which means the sig probability value> 0.05 indicates the data is not homogeneous.

Table 2. Results of *Kruskal-Wallis* Test Analysis of Glucose Levels (g/dl) between groups

Ranks				
	Group	N	Mean Rank	*P-Value**
Pretest	Positive	3	11.33	
	Negative	3	4.67	0.052
	MO capsules	3	10.17	
	AF	3	11.00	
	MO and AF capsules	3	2.83	
	Total	15		
Induction	Positive	3	2.00	
	Negative	3	8.83	0.034
	MO capsules	3	11.00	
	AF	3	12.33	
	MO and AF capsules	3	5.83	
	Total	15		
Posttest	Positive	3	6.83	0.672
	Negative	3	7.67	
	MO capsules	3	8.67	
	AF	3	11.00	
	MO and AF capsules	3	5.83	
	Total	15		

Based on table 2, the results of the Kruskal-Wallis test data out put of the Pretest group (0.052) means the probability value of Sig.>0.05, then Ho is accepted, the Alloxan Induction group (0.034) means the probability value of Sig.>0.05 then Ho is rejected, and the Postest group (0.672) means the probability value of Sig.>0.05, then Ho is accepted so it is stated that there is no difference or Ho is accepted and Ha is rejected so it can be concluded that there is no significant difference in glucose levels before treatment and after Alloxan induction treatment, administration of MO Capsules, AF and MO AND AFCapsules.

DISCUSSION

This study aims to determine the effectiveness of giving Moringa Oleifera and Folic Acid to female rat glucose levels in reducing blood glucose levels in alloxan-induced rats. Rat test animals were used in this study, because of easier handling, complete organs, metabolism and nutritional needs similar to humans. The rats used are female rats aged about 2- 3 months because they have stable hormonal activity and are easier to do research because it involves obstetrics. Before being treated, all groups of female rats were induced with alloxan. Alloxan is a pyrimidine oxygen derivative that can destroy pancreatic beta cells as insulin producers. Mice were divided into 5 treatment groups, group 1 was given 1% Na-CMC suspension as a negative control, group 2 was given alloxan, group 3 was given MO, group 4 was given AF and group 5 was given MO and AF.

Then the initial blood glucose levels of mice were measured before alloxan induction. To see the difference in the initial blood glucose levels of rats and after mice induced alloxan. Measurement of

the initial blood glucose levels of rats was done after the rats were fed first. Then the mice were induced alloxan and measured on the 3rd day after alloxan administration. Measurements were taken at the Makassar Public Health Laboratory Center.

The ability of Folic Acid and Moringa Oleifera to reduce blood glucose levels in rats is thought to have active substances alkaloids, flavonoids, and tannins. Alkaloids in MO are able to more efficiently repair free radical reactions that occur (Bobaya, et al., 2023). Flavanoids contained in EDK are thought to be able to increase antioxidant activity which can also regenerate pancreatic beta cells damaged by alloxan induction. Furthermore, flavonoids can also function as antioxidants that can prevent and capture free radicals in the body (Surya, et al., 2020).. Free radicals are one of the causes of diabetes. Tannin compounds contained in EDK can also increase glycogenesis so that blood glucose levels can drop faster because tannin compounds can help convert glucose into a form that is ready to be stored by tissue cells in this case, namely glycogen. The more tannin compounds contained in a plant, the more the activity of glycogenesis increases which can cause a faster decrease in blood glucose levels. Tannin can also capture and inhibit free radicals that cause diabestes mellitus because tannin is proven to have antioxidant effects (Pitriya, et al., 2017).. Folic acid, or vitamin B9, has been studied in the context of various aspects of health, including its relationship with blood glucose levels and diabetes. Although folic acid does not directly affect blood glucose levels as insulin or other antidiabetic drugs do, there are some indirect links that have been identified through several studies (Kooti, et al., 2016) The occurrence of a decrease in glucose is one sign of recovery from the increase in blood sugar after the administration of Moringa Oleifera (MO) capsules and Folic Acid (AF) at a dose of 150-200mg / kgBB in comparison with the negative control group which is only injected with Alloxan. This is because MO capsules have secondary compounds, namely Quertecin, Plavanoid, mirsetin, Kaemferol, kiercetin and high iron content of 28.39 grams (100 grams) and in AF content, namely Vitamin C, Vitamin B9, and Vitamin B12 which have hepatoprotector effects due to their high anti-oxidant content (Camillery and Blundell, 2024). However, those that are thought to have greater potential as anti-oxidants are flaphonoids and kiercetin because they have hydroxyl groups so that they can donate hydrogen atoms to free radicals so that free radicals become inactive (Prasetya et al., 2023).

The conclusion that can be drawn from the normality test and homogeneity test is that the data is normally distributed and does not have the same variance (not homogeneous), so for hypothesis testing using the Kruskal-Wallis nonparametric test. This test is to determine whether there are differences in sample groups that have received treatment. The test criteria are taken based on the probability value (sig) if> 0.05 then Ho is accepted and if the probability (sig) <0.05 then Ho is rejected. The Kruskal-Wallis Test is part of non-parametric statistics, which is used as an alternative to the One Way ANOVA test when one or all data distributions are not normally distributed or homogeneous.

CONCLUSIONS

The study found that the administration of Moringa Oleifera, Folic Acid, or a combination of both in female rats did not significantly reduce blood glucose levels, despite a decrease in glucose levels post-treatment.

ADVICE

Suggestions that can be given by the author for further research need to be carried out studies on humans directly to determine the hypoglycemic effect of moringa leaf extract. Studies need to be conducted to test the toxicity and effective doses of moringa leaf extract and folic acid that are safe for human consumption. Research is needed to determine how effective moringa leaves and folic acid are in reducing blood glucose levels in humans.

CONFLICT OF INTEREST

All authors state that they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Contributions from the authors: FA designed and executed the experiments; EW, MA, and SS produced the draft; and FA, EW, MA, SS, and ANU evaluated and finished the text. All authors reviewed and approved the final version of the text.

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