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Antidiabetic Activity of Methoxy Bergenin Isolated from Ethanol Extract of Raru Stem Bark (*Vatica pauciflora* Blume) in Alloxan Induced Diabetic Wistar Rats

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This paper describes the potential of methoxy bergenin isolated from ethanol extract of the raru stem bark (*Vatica pauciflora* Blume) to be antidiabetics. In the experiment, each of the alloxan induced wistar rats group is given a dose of 65 mg/200 g BB/2 mL, 130 mg/200 g BB/2 mL and 195 mg/200 g BB/2 mL of methoxy bergenin. On the day of 14 and 21 of treatment, the blood sugar levels of wistar rats are measured by a glucometer. The ANOVA result showed there are the different effects of methoxy bergenin on the blood sugar levels of wistar rats significantly. The dose of 195 mg/200 g BB/2 mL on day 21, give the best effect of a decrease in the blood sugar levels. The t_{test} results showed the wistar rats's body weight are also decreased significantly after 21 days of treatment. Based on these the methoxy bergenin has a potential as an antidibetics which resembles acarbose.

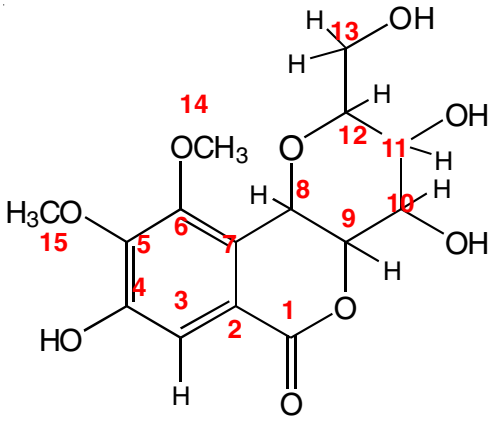
Keywords: Blood glucose, Body weight, Flavonoid.

INTRODUCTION

Indonesian peoples are still using traditional herbal medicine in particular those in the region that has a variety of plants [1]. One of the herbs used are raru trees, which is the plants of forests with many types of diversity. The extract of rarustem bark of type of *Cotylelobium* sp. has been investigated by Gunawan [2] and found it could inhibit α-glucosidase enzyme *in vitro*. The raru stem bark of the types of *Vatica pauciflora* Blume are consumed by the Bataks's peoples in North Sumatra region, which is believed to be a drug that can lower the blood sugar levels, namely diabetes mellitus. But it remains to be investigated because of there are concerns other than to provide therapeutic effects, it will be able to cause the intoxication [3].

The previous researchers who have evaluated the drug ingredients from plants in general says there are flavonoids in every plant. Markham [4] states that the flavonoid compounds in the form of glycosides found in all parts of the tall plants such as flowers, leaves, fruits, wood, roots and bark. Several flavonoid compounds extracted from crops has been examined and given in experimental animals, such as extracts of *Selaginella tamariscina* (Beauv) spring [5], extracts of Terminalia, fruit [6], extracts *Alternanthera ficodia* linn [7] and extracts of *Polygonatum odoratum* [8]. They expressed the decrease in the blood sugar levels of the wistar rats significantly, as the result of enzymatic reaction with glucose flavonoid that inhibits the enzyme α-glucosidase. In this case they use acarbose as a comparison as an oral drug lowering blood sugar, which is competitive and reversible in human gut [9].

The raru stem bark extract has been isolated by Riris *et al.* [10] and found that the flavonoid compound has the activity as α-glucosidase enzyme inhibitors *in vitro*. The results of the chemical structure elucidation based on spectral data UV, FT IR, RMI 1D, ^{1}H, ^{13}C NMR, RMI 2D (COSY, HMQC, HMBC) and mass spectra (HR-MS), it is designated as methoxy bergenin ($C_{15}H_{18}O_9$)with a chemical structure as given below.



Chemical structure of methoxy bergenin ($C_{15}H_{18}O_9$)

Marinova *et al.* [11] suggest the flavonoid is also included phenol, which is beneficial for preventing heart disease, reducing inflamation, antidiabetic and antioxidant. Diabetes mellitus is characterized by hyperglukemia, glucose urea and a lack of insulin secretion by the β-cells of the pancreas, is the result of disruption of the metabolism of carbohydrates, fats and proteins [12]. At the time of insulin deficiency as a result of damage to the β-cells of pancreas, the glucose transporters are not raised so that the increase in the blood sugar and do not get into the network. This resulted in cells starved of energy. Therefore it happens gluconeogenesis cause fat and protein reserves decreased to weight loss [13]. It has also been investigated by Demoz *et al.* [14] and found that there is a relationship of diabetic effects and weight gain. In this regard, the paper describes diabetic activity of methoxy bergenin of raru stem bark extracts in wistar rats induced by alloxan.

EXPERIMENTAL

The raru stem bark (*Vatica pauciflora* Blume) were taken from the forest is chopped and dried first, then crushed and extracted with a solvent of *n*-hexane, ethyl acetate, ethanol and water. Furthermore, the extraction of ethanol was concentrated and purified by chromatography to obtain methoxy bergenin [15].

Animals preparation: A total of 25 adult wistar rats, age about 3 months, body weight about 100 g, in a healthy condition adapted to the conditions of the laboratory for 7 days. Furthermore, the blood sugar levels of them were measured to make sure that are in the normal range, which is 50-125 mg/dl. And then, they were induced by alloxanin at dose of 150 mg/KgBW *via* intra-peritoneal injection [15]. It was done to damage the insulin producing β-cells in the pancreas [16] and then they were placed in five groups, namely K_0, K_1, K_2, K_3 and K_4, which each ofgroup has five tails.

Procedure: The treatment given to each of the five groups are K_0 only given rations and water, the K_1 given acarbose at a dose of 0.126 mg/200 g BW/2 mL (as a positive control), K_2 = given methoxy bergenin at a dose of 65 mg/200 g BW/2 mL, K_3 = methoxy bergenin given at a dose of 130 mg/200 g BW/2 mL and K_4 = methoxy bergenin given at a dose of 195 mg/200 gBW/2 mL. The blood sugar levels of all of the experimental groups were measured (mg/dl) by using glucometers accompanied glucostrip [17], namely when after-induced alloxan, after 14 days of treatment and after 21 days of treatment. The wistar rats' body weight were also weighed after 21 days of treatment (the end of the experiment).

The data of the blood sugar levels of each of the experimental group were analyzed by ANOVA to compare the average of the reduction of the blood sugar levels among wistar rats fed a methoxy bergenin, at the 0.05 significance level. The t-test was also used to see the comparison of the average onf the blood sugar levels between the groups of after 14 days of treatment and 21 days of treatment and to see the difference of the body weight of wistar rats after 21 days of treatment (end of the experiment) to the normal weight. In this case the SPSS is used as the analysis tools.

RESULTS AND DISCUSSION

The average of the blood sugar levels of the wistar rats after inducing the alloxan are above of the normal limit (141, 440 mg/dl > (50-125 mg/dl). Based on this, hiperglekimia has occurred on the wistar rats.The analysis results of the blood sugar levels of the each of the experimental group (after 14 days and 21 days of treatment) were obtained (Table-1).

The test results of the diversity of the blood sugar levels of the two treatment groups of 14 days and 21 days, indicating the range of the blood sugar levels in both groups of wistar rats were identical. This is indicated by Levene Statistic = 6.956, Sig. = 001 (the group of14 days) and Levene Statistic = 6.580, Sig. = 0.002 (the group of 21 days).

The average difference test results of the blood sugar levels of the wistar rats between the groups of 14 days of treatment and the group of 21 days of treatment (Table-2), showed that they are different significantly, F = 553.275; Sig. = 0.00 (groups of 14 days) and F = 619.146; Sig. = 0.00 (groups of 21 days).

Based on these, the administration of the methoxy bergenin in the different of time periods gave a different effect on the blood sugar levels of the wistar rats. According to the multiple

TABLE-1
WISTARS' BLOOD GLUCOSE LEVEL AFTER 14 DAYS AND 21 DAYS OF TREATMENT (mg/dl), n = 5

Group	Treatment	Day 14th		Day 21st	
		Mean	Std. Deviation	Mean	Std. Deviation
K_0	Untreated	115,6000	0,54772	106,8000	1,30384
K_1	Acarbose	61,6000	1,67332	50,4000	0,89443
K_2	65 mg/200 g BW/2 mL	90,2000	3,76829	77,0000	4,00000
K_3	130 mg/200 mg BW/2 mL	75,2000	1,64317	62,6000	0,89443
K_4	195 mg/200 mg BW/2 mL	68,8000	0,83666	56,2000	1,09545

TABLE-2
ANOVA OF BLOOD GLUCOSE LEVEL AFTER 14 DAYS AND 21 DAYS OF TREATMENT

		Sum of Squares	Df	Mean Square	F	Sig.
Blood glucose, 14 days	Between groups	9162,240	4	2290,560	553,275	,000
	Within groups	82,800	20	4,140		
	Total	9245,040	24			
Blood glucose, 21 days	Between groups	10154,000	4	2538,500	619,146	,000
	Within groups	82,000	20	4,100		
	Total	10236,000	24			

comparison of the average of the blood sugar levels among each of the treatment group showed that the blood sugar levels are different significantly, either after 14 days or after 21 days of treatment (Table-3).

By looking at the provision of the methoxy bergenin in the different doses at the different times, it appears that the blood sugar levels was different significantly. It is demonstrated by the value of F = 207.101, Sig. = 0.000 (based on the period of treatment) and F = 197.264, Sig. = 0.00 (based dosing). But there is no interaction between duration of treatment with doses of bergeninmethoxy given, F = 0.051, Sig. = 0.951 (Table-4).

The decrease of the blood sugar levels of the alloxan induced wistar rats after administration of the different doses of methoxy bergenin in treatment for 14 days and 21 days shown in Fig. 1.

Fig. 1 indicated that the deterioration of the wistar rats' blood sugar levels are higher visible at the higher doses, both on after 14 days or after 21 days of treatment. Based on the analysis results, the administration of methoxy bergenin at a dose of 195 mg/200 g BW/2 L, either after 14 days of treatment and after 21 days of treatment is approaching the level of the blood sugar levels by administering acarbose, as shown in Table-5. It indicates the function of methoxy bergenin is approaching antidiabetic drug functions.

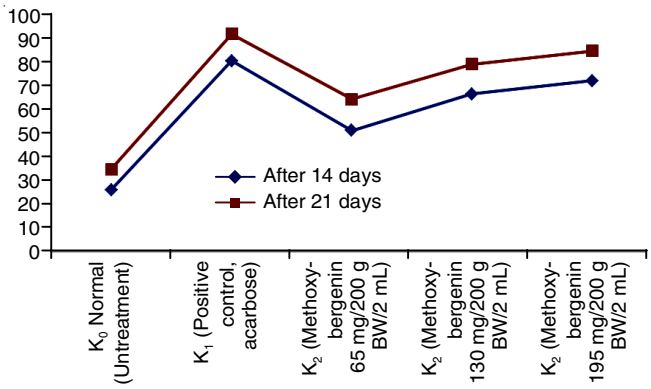


Fig. 1. Graph of the wistars' blood sugar levels decrease

The results of the analysis of the weight measurement of wistar rats after 21 days of administration of the methoxy-bergeninis given in Table-6.

The average difference test results of the body weight loss of the wistar rats'showed the body weight decreased significantly, which is compared to the initial weight after giving methoxy bergenin after 21 days of the treatment (end of the experiment) *i.e.*, t = 2.383, Sig. = 0.025 (Table-7).

The results of the alloxan injection at a dose of 150 mg/kg by intra-peritoneal injection, giving the effect of hyperglycemia

TABLE-3
MULTIPLE COMPARISON OF WISTARS' BLOOD GLUCOSE LEVEL AVARAGE

(I) Group of treatment	(J) Group of treatment	Day 14th		Day 21st	
		Mean difference (I-J)	Sig.	Mean difference (I-J)	Sig.
K_0	K_1	54,00000*	,000	56,40000*	,000
	K_2	25,40000*	,000	29,80000*	,000
	K_3	40,40000*	,000	44,20000*	,000
	K_4	46,80000*	,000	50,60000*	,000
K_1	K_0	-54,00000*	,000	-56,40000*	,000
	K_2	-28,60000*	,000	-26,60000*	,000
	K_3	-13,60000*	,000	-12,20000*	,000
	K_4	-7,20000*	,000	-5,80000*	,000
K_2	K_0	-25,40000*	,000	-29,80000*	,000
	K_1	28,60000*	,000	26,60000*	,000
	K_3	15,00000*	,000	14,40000*	,000
K_3	K_0	-40,40000*	,000	-44,20000*	,000
	K_1	13,60000*	,000	12,20000*	,000
	K_2	-15,00000*	,000	-14,40000*	,000
	K_4	6,40000*	,000	6,40000*	,000
K_4	K_0	-46,80000*	,000	-50,60000*	,000
	K_1	7,20000*	,000	5,80000*	,000
	K_2	-21,40000*	,000	-20,80000*	,000
	K_3	-6,40000*	,000	-6,40000*	,000

Description: K_0 = Untreatment; K_1 = Acarbose; K_2 = 65 mg/200 g BW/2 mL; K_3 = 130 mg/200 mg BW/2 mL; K_4 = 195mg/200 mg BW/2 mL

TABLE-4
TESTS OF BETWEEN SUBJECTS EFFECTS ON WISTARS' BLOOD GLUCOSE AFTER TREATMENT

Source	Type III sum of squares	Df	Mean square	F	Sig.
Corrected model	3570,267[a]	5	714,053	120,346	,000
Intercept	154083,333	1	154083,333	25969,101	,000
Time	1228,800	1	1228,800	207,101	,000
Dose_of_Methoxy	2340,867	2	1170,433	197,264	,000
Time * Dose_of_Methoxy	,600	2	,300	,051	,951
Error	142,400	24	5,933		
Total	157796,000	30			
Corrected Total	3712,667	29			

a. R Squared = ,962 (Adjusted R Squared = ,954)

TABLE-5
WISTARS' BLOOD GLUCOSE LEVEL AFTER TREATMENT

Tukey B[a]	Group of treatment	N	Subset for alpha = 0.05				
			1	2	3	4	5
After 14 days	K_1 = Positive control, acarbose	5	61,6000				
	K_4 = 195 mg/200 mg BW/2 mL	5		68,8000			
	K_3 = 130 mg/200 mg BW/2 mL	5			75,2000		
	K_2 = 65 mg/200 g BW/2 mL	5				90,2000	
	K_0 = No treatment	5					115,6000
After 21 days	K_1 = Positive control, acarbose	5	50,4000				
	K_4 = 195 mg/200 mg BW/2 mL	5		56,2000			
	K_3 = 130 mg/200 mg BW/2 mL	5			62,6000		
	K_2 = 65 mg/200 g BW/2 mL	5				77,0000	
	K_0 = No treatment	5					106,8000

TABLE-6
WISTARS' BODY WEIGHT MESUREMENT

Mesurement of	Mean	N	Standard deviation	Standard error mean
Before treatment	101,3600	25	2,54755	,50951
After 21 days of treatment	96,1600	25	11,27933	2,25587

TABLE-7
EFFECT OF METHOXY BERGNIN ON WISTARS' BODY WEIGHT

	Paired differences					T	df	Sig. (2-tailed)
	Mean	Stadnard deviation	Stadnard error mean	95 % Confidence interval of the difference				
				Lower	Upper			
Wistars' body weight before treatment – Wistars' body weight after 21 days of treatment	5,20000	10,90871	2,18174	,69710	9,70290	2,383	24	,025

to the wistar rats. This occurs due to damage of β-cells of the pancreas so that insulin production is reduced, is consistent with experimental results [6,18].

The experiment showed the methoxy bergenin given to the alloxan induced wistar rats giving the effect of the decrease different of the blood sugar levels in the period of administration of 14 days and 21 days significantly. Duration of administration for 21 days is higher than in the period of 14 days on the variation of the doses administered. It indicates that the administration of the methoxy bergenin within a time period of relatively longer, resulting the blood sugar levels of the wistar rats getting controlled for the regeneration of pancreatic β-cell, which is according to the findings of Biscchoff [9].

The dose of 195 mg/200 g BW/2 mL of methoxy bergenin is giving the better effect than the other of dose, both within a 14-day administration and also 21 days. This suggests that the dose given methoxy bergenin higher, will lead to the greater resistance of the enzyme α-glucosidase, according to the findings of Nagappa *et al.* [19], Avijit, *et al.* [20], Zheng *et al.* [5], Shirwaikar *et al.* [21], Krief *et al.* [22], Chen *et al.* [23] and Subrahmanyam *et al.* [24].

The inhibition process is also associated with a decrease in body weight of rats. It has been found that the differences in body weight of rats in early before the induction of alloxan and after treatment for 21 days is significantly decreased. This weight loss is due to the impaired metabolism of carbohydrates, which resulted in gluconeogenesis or the use of glucose from the cell, such as the findings of Guyton and Hall [13] and Demoz *et al.* [14].

By observing the effects of the decrease of the blood sugar levels and the weight loss of the wistar rats, it appears that the methoxy bergenin works resembles oral medication of acarbose, which inhibits the enzyme α-glucosidase. Methoxy bergenin interfere with the process for the breakdown of carbohydrates into monosaccharides, so it can not be absorbed by the intestine. As a result of it is the blood glucose levels are not elevated in the time after eating foods that contain carbohydrates. Thus the pancreas is not stimulated to produce insulin, but decreased the hepatic glucose production and increased muscle and adipose tissue sensitivity to insulin.

Conclusion

The methoxy bergenin compound isolated from the raru stem bark (*Vatica pauciflora* Blume) ethanol extract is able to lower the blood sugar levels and the body weight of the wistar rats induced by alloxan. The compound has potential as an antidiabetic. Based on these findings, the methoxy bergenin can enrich the inventory of herbs as an antidiabetic drug, but it is still necessary to study the other bioactivity.

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