ANTIDIABETIC BIOACTIVITY TEST OF CHROMIUM(III) AND COPPER(II) COMPLEX COMPOUNDS ON MICE (*Mus musculus L.*)

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Abstract: The Chromium(III) complex compounds have been known to reduce glucose levels in people with type 2 diabetes. This study aims to test the antidiabetic bioactivity of mice from the synthesis of complex compounds of Cr(III) and Cu(II) with amino acid alanine. The antidiabetic testing was carried out in vivo using alloxan-induced mice. Antidiabetic test results are expressed in %GL (Glucose Lowering) for Cr-alanine dose of 50 µg at 29.79%, dose of 100 µg at 37.13%, and a dose of 200 µg at 59.19%. Whereas at Cu-alanin dose of 50 µg was 25.39%, dose of 100 µg was 33.87%, and the dose of 200 µg was 54.96%. Antidiabetic tests show that the Cr-alanine complex compound is more effective in reducing blood glucose levels in mice compared with Cu-alanine.

Keywords: Cr-alanine, Cu-alanine, antidiabetic, glucose, mice

INTRODUCTION

Diabetes mellitus is a disorder of blood glucose in the body, caused by the body's failure to produce insulin or available insulin but the insulin receptor is not active. The Data from the International Diabetes Federation (IDF) shows that diabetics among adults (aged 20-79 years) were 285 million (6.4%) in 2010, estimated to increase to 439 million (7.7%) in 2045. Indonesia is among the top 10 countries in the world with the highest number of diabetics reaching 10.3 million in 2017, this number is expected to increase to 16.7 million in 2045[1].

Diabetes mellitus (DM) is divided into two types, type 1 diabetes mellitus and type 2 diabetes mellitus. The type 1 diabetes mellitus is a condition where the body lacks insulin or insulin production is reduced, whereas type 2 diabetes mellitus is a condition where insulin is sufficient but insulin receptors are not active. The treatment for DM has been done using various therapies, such as insulin injections used for the treatment of type 1 diabetes.

The treatment for DM has been done using various therapies, such as insulin injections used for the treatment of type 1 diabetes. Whereas the treatment for type 2 diabetes generally uses oral medications, for example glibenclamide [2]. The latest type 2 DM treatment has been widely studied, such as using metalotherapy. Metallotherapy is a new therapy for the treatment of type 2 DM with metal complexes [3]. The metals that have been studied as having effective benefits as antidiabetic supplements are chromium(III) and copper(II). The results of Sharma et al., (2011) showed Cr (III) and Cu (II) can activate the hormone insulin by increasing systemic insulin sensitivity and glycemic control in diabetics [4]. El-Megharbel (2015) studied a complex of Cr(III) with metformin, the in vivo results in mice showed a percentage decrease of up to 60% in blood sugar levels compared to the group of diabetic rats who were not given additional Cr(III) metformin [5]. Vanco et al., (2008) have also conducted antidiabetic research studies of complex compounds of Cu(II) with salicylaldehyde ligands, the results showed that Cu(II)-salicylaldehyde complex compounds can reduce blood sugar levels in mice by 34-62% [6].

The research data shows the success of antidiabetic properties of Cr(III) and Cu(II), to get new complex compounds from the metal Cr(III) and Cu(II) in this study used amino acid alanine as a ligand. This research has synthesized Cr(III) and Cu(II) complex compounds with the amino acid alanine. The results of the synthesis obtained were then tested for antidiabetic activity in alloxan-induced mice.

METHOD

The materials used in this study include aquades, aquabides, $CrCl_3 \cdot 6H_2O$, $CuCl_2 \cdot 2H_2O$, amino acid alanine, NaOH, Aloxan, NaCl 9%, pH indicator, ice cube, straw (rice seed coat) and white mice (*Mus musculus L*.). The equipment used include glassware, a set of reflux devices, hot plate stirrers, desiccators, test tubes, a set of bioactivity test kits (tubs for mice beds, drinking and eating mice, glucometers, injections, oral devices (sonde), glucose kit), and instrumentation: Uv-Vis spectrophotometer, FT-IR spectrophotometer, EDX analysis.

Synthesis and Characterization

The method used in the synthesis of chromium(III) and copper(II) complex compounds with the amino acid alanine is carried out based on procedures in the synthesis of chromium phenylalanine complex compounds [7].

The procedure is complex 1, $CrCl_3 \cdot 6H_2O$ (0.26 g) with amino acid alanine (0.27 g), and complex 2, $CuCl_2 \cdot 2H_2O$ (0.17 g) with amino acid alanine (0.18 g) prepared with ratio of 1: 3 and 1: 2, all compounds in the form of solution, then mixed and then added 0.1 M NaOH to reach pH 4-4.5. The complex compound mixture solution is then refluxed and stirred using a magnetic stirrer for 4 hours at a temperature of 80°C. The reflux solution is then frozen in the refrigerator and freeze-dried. Synthesis was carried out with several variations of pH namely (2,3; 4; 5; 6 and 7) and also time variations (1, 2, 3 and 4).

Antidiabetic Test

Diabetes induction is done by injecting alloxan subcutaneously at the nape of the neck 3 times for 6 days. The dose of alloxan used was 150 mg/KgBW. The alloxan was then dissolved with 9% NaCl, the weight and volume used in the injection were adjusted to the average weight of the mice. The group test of mice were given daily treatment orally (po) with the formula $[Cr(ala)_3]$ and $[Cu(ala)_2]$ at a dose of 50, 100 and 200 µg/KgBW for 15 days. This dose was used based on the research of Selcuk et al., [8] and Dogukan et al., [9] who used a dose of 100 µg/KgBW. Control group 1 was given glibenklamide solution, control group 2 was not given medication and control group 3 was not given medication. The overall assessment of antidiabetic activity in vivo is expressed as a decrease in glucose (%GL) ie glucose levels before treatment minus glucose levels after treatment divided by glucose before treatment multiplied by 100%.

RESULTS AND DISCUSSION

THE SYNTHESIS RESULTS

The results of chromium(III) synthesis with alanine obtained optimum time and pH variations, namely at 4 hours and pH 4 with a temperature of 80°C, the results obtained were thick purple colored clumps and sticky samples with a weight of 0.27 grams and a yield of 85.4%. While the synthesis of copper(II) with alanine obtained the same time, pH and temperature variations as the synthesis of $[Cr(ala)_3]$, the synthesized sample in the form of a blue solid with a weight of 0.2 grams and a yield of 84.03%. The complex compounds formed were characterized using a Uv-Vis spectrophotometer, FT-IR spectrophotometer and EDX.

The results of Uv-Vis characterization of chromium(III)-alanin there are two peaks at wavelengths of 426 and 617 nm and the copper(II)-alanine one peak at a wavelength of 811 nm. The obtained

wavelengths indicate a shift of hypsochromic or shift towards wavelengths that are lower than the metal wavelengths of 436 and 629 nm ($CrCl_3 \cdot 6H_2O$) and 825 nm ($CuCl_2 \cdot 2H_2O$).

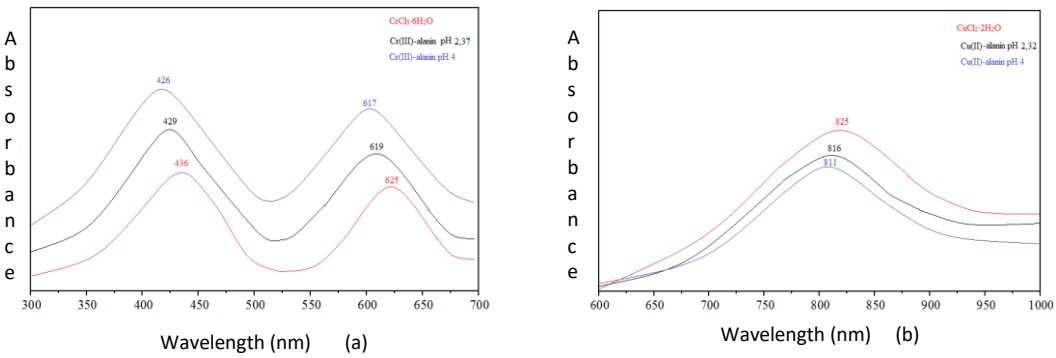

Figure 1. The Results of Uv-Vis characterization of complex compounds (a) $[Cr(ala)_3]$ and (b) $[Cu(ala)_2]$

In the two images above, there is a shift in maximum wavelength uptake towards smaller wavelengths, this indicates the reaction between the metal Cr(III) and Cu(II) with alanine has formed complex.

The results of the FT-IR characterization on chromium(III)-alanin there was an absorption of Cr-O and Cr-N in the area of 579.19 cm^{-1} and 489.63 cm^{-1}. The uptake of Cr-O and Cr-N also occurs in copper(II)-alanine the other found in the regions of 569.23 cm^{-1} and 439.31 cm^{-1}. This result shows that the amino acid alanine as ligand has bind to the central atom, Cr^{3+} and Cu^{2+}.

Table 1. IR analysis of complex compounds $[Cr(ala)_3]$ and $[Cu(ala)_2]$

Functional Group	Wavelength (cm^{-1})			
	Reference	Alanin	$[Cr(ala)_3]$	$[Cu(ala)_2]$
O-H	3500-3300	3450.77	-	-
N-H	3400-3250	3257.29	3379.39	3375.13
C-H	3100-2700	2987.19	2909.53	2994.63
C=O	1760-1600	1679.54	1619.89	1617.90
C-O	1350-1000	1315.67	1260.23	1245.31
C-N	1280-1050	1220.19	1197.48	1198.94
Cr-O	600-500	-	579.19	569.23
Cr-N	500-400	-	489.63	439.31

The alanine absorption peaks and complex compounds $[Cr(ala)_3]$ and $[Cu(ala)_2]$ in Table 1, show that the complex compounds formed have two Cr-O and Cr-N uptakes that indicate ligand replacement in the metal and complex the desired has been formed.

The EDX results of complex compounds $[Cr(ala)_3]$ in Figure 1. shows the peaks of the constituent elements of $[Cr(ala)_3]$ compounds namely Cr, O, C and N.

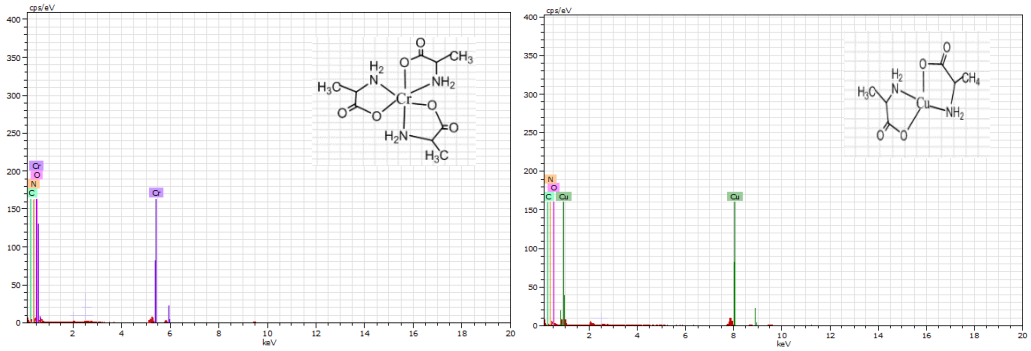

Figure 2. The Results of EDX characterization of complex compounds $[Cr(ala)_3]$ and $[Cu(ala)_2]$

The results of the percentage produced by EDX characterization of compound $[Cr(ala)_3]$ having the empirical formula $C_9H_{15}N_3O_6Cr$, and copper(II)-alain has the empirical formula $C_6H_{10}N_2O_4Cu$ the data are according to the theory.

Antidiabetic Test

The effect of giving chromium (III) -alanin complex compounds to alloxan-induced mice can be seen in Figure 3.

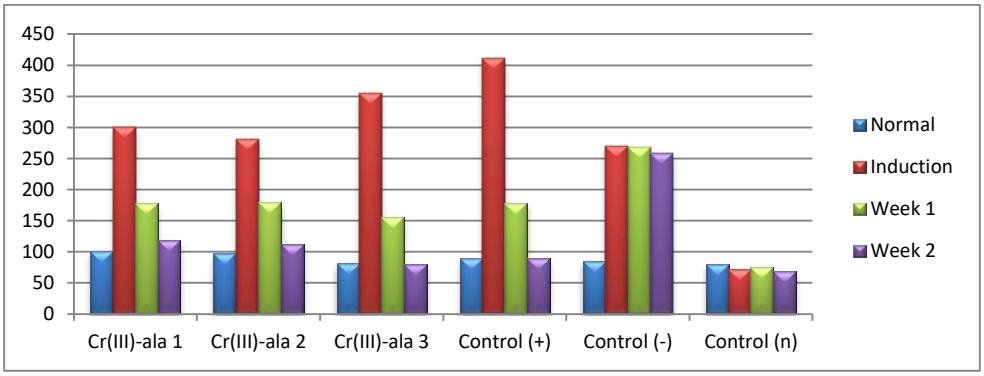

Figure 3. The graph of blood sugar levels of mice giving of $[Cr(ala)_3]$

In Figure 3. the blood glucose levels of the early mice on the first day before all chromium(III)-alanine and also the control groups were in normal condition which is around 80-100 mg/dL, this shows that the mice were in good health. On the 7th day after alloxan-chromium(III)-alanin was induced using alloxan, positive and negative control, the increase in blood sugar level was more than 126 mg/dL, which was about 270-411 mg/dL so that it could be stated that the mice were already in state of diabetes [10].

In the normal control group there was no increase in blood sugar levels, because the normal control group was not induced by alloxan, so it was seen that in the second week the blood sugar level of the control group was stable under normal conditions ie 68.7 mg/dL. To prove the effectiveness in reducing blood sugar levels of all chromium(III)-alanin treatment groups, the percentage of reduction in blood sugar (% GL) was calculated [4].

Tabel 2. The test results of the antidiabetic activity of the treatment group [Cr(ala)$_3$] in % glucose lowering (%GL)

Group	Dose	%GL
[Cr(ala)$_3$]	1	47.67
	2	57.64
	3	76.98
Control	K(+)	78.35
	K(-)	0.86
	K(n)	4.81

Table 2 shows that the percentage decrease in blood sugar levels in the treatment group [Cr(ala)$_3$] dose 3 (%GL=76.98) was the highest compared to doses 1 and 2. All treatment groups [Cr(ala)$_3$] showed a decrease blood sugar levels in% GL are quite high. Qualitatively the treatment group [Cr(ala)$_3$] at dose 3 was the most effective because it approached the %GL value of the positive control group. The compared with the negative control group and the normal control group, the decrease in blood sugar levels of all treatment groups [Cr(ala)$_3$] was not influenced by environmental factors, food and drink from mice, this was evidenced from the %GL of the negative control group and the normal control group which is very low, respectively 0.86% and 4.81%.

The most effective dose of all [Cr(ala)$_3$] groups (K1, K2 and K3) was seen from the average blood sugar level with mean ± SD (oneway ANOVA analysis) which was the closest to the positive control group (K+) 89.00 ± 4,000 is the group [Cr(ala)$_3$] (K3) with a mean ± SD 89.85 ± 4.155. The results show that the group [Cr(ala)$_3$] with a dose of 200 µg/KgBW is the most effective dose in reducing blood sugar levels in alloxan-induced mice [11]. Among all treatment groups, the best dose to reduce blood glucose levels in mice was dose 3, the% GL value of 76.98% approached the %GL control group that was 78.35%.

The effect of giving copper(II)-alanine complex compound can be seen in Figure 4.

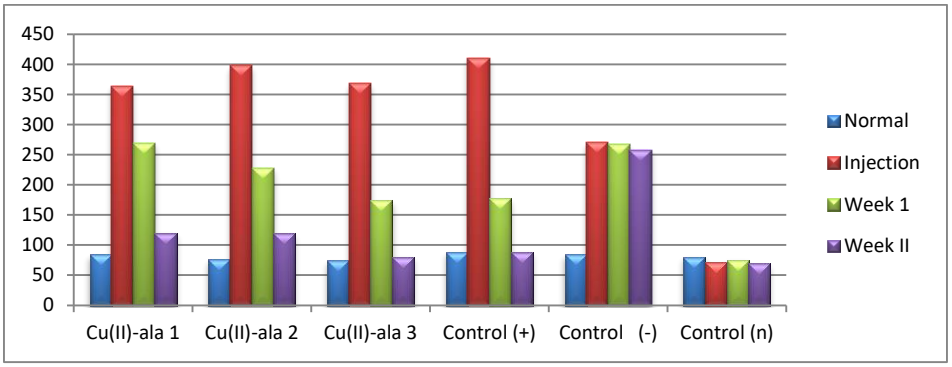

Figure 4. The graph of blood sugar levels of mice giving of [Cu(ala)$_2$]

In Figure 4, the blood glucose levels of the early mice on the first day before all copper(II)-alanin and also the control groups were induced under normal conditions, which was around 74-85.7 mg/dL, this showed that the mice were in good health. On the 7th day after being induced using alloxan all copper(II)-alanin treatment groups, positive and negative controls so that an increase in blood sugar levels of more than 126 mg/dL is around 270-411 mg/dL, so it can be stated that the mice have already in a state of diabetes [10]. In the normal control group there was no increase in

blood sugar levels, because the normal control group was not induced so that it was seen in the second week the blood sugar level of the control group was stable under normal conditions ie 68.7 mg/dL.

Tabel 3. The test results of the antidiabetic activity of the treatment group [Cu(ala)$_2$] in % glucose lowering (%GL)

Group	Dose	%GL
[Cu(ala)$_2$]	1	52.27
	2	60.73
	3	72.51
Control	K(+)	78.35
	K(-)	0.86
	K(n)	4.81

Table 3. shows that the percentage decrease in blood sugar levels in the copper(II)-alanin dose group 3 (%GL=72.51) is the highest compared to doses 1 and 2. All copper(II)-alanin treatment groups showed a significant decrease in blood sugar levels in %GL. Qualitatively it can be concluded that the copper(II)-alanin treatment group at dose 3 is the most effective in reducing blood sugar levels. The compared with the negative control group and the normal control group, the decrease in blood sugar levels of all copper(II)-alanin treatment groups was not influenced by environmental factors, food and drink from mice, this was evidenced from the %GL of the negative control group and the normal control group very low ie 0.86% and 4.81%, respectively.

Based on Table 3, the average blood sugar levels of all copper(II)-alanine (T1, T2 and T3) treatment groups were tested with oneway ANOVA, the mean±SD values closest to the positive control group (K +) 89.00 ± 4.000 were copper(II)-alanine 3 (T3) group. The results showed that the copper(II)-alanin 3 (T3) group with a dose of 200 µg/KgBW was the most effective dose in reducing blood sugar levels in alloxan-induced mice according to the decrease in blood sugar levels using %GL where the group copper(II)-alanin 3 (T3) had the highes t%GL among all treatment groups namely 72.51% approaching the %GL control group that was 78.35%. All doses copper(II)-alanine given to mice can reduce glucose levels.

Conclusions

The results of antidiabetic bioactivity tests show that the chromium(III)-alanin and copper(II)-alanin complex compounds can reduce blood sugar levels significantly. The complex compound [Cr(ala)$_3$] is more effective than complex compound [Cu(ala)$_2$] with a decrease in blood glucose level %GL respectively respectively 76.98% and 72.51%, the most effective dose in reducing blood sugar levels is the third dose of 200 µg/KgBW.

Conflicts of interest

"There are no conflicts to declare."

Acknowledgments

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