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Research Article

Antidiabetic Activity Test of Fe(III) Complex Compound with Arginine Ligand in Male Mice (*Mus Musculus L.*)

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Abstract
Diabetes mellitus is characterized by high blood sugar levels in the body. Treatment for type 1 diabetes mellitus is with insulin injections while treatment for type 2 diabetes mellitus generally uses oral medication. Currently, people are looking for diabetes drugs made from complex compounds using metals. The development of research and utilization of Fe(III) complex compounds is still limited, thus, in this study the synthesis of Fe(III) complex compounds with arginine ligands was carried out. The results of the research obtained a complex compound of Fe(III)-arginine with 96%, in the form of a brown gel with a sample weight of 0.5601 g. Characterization using a UV-Vis spectrophotometer showed absorption at a wavelength of 203 nm which indicated the absorption of the Fe(III)-arginine complex. The results of FTIR analysis showed a typical absorption of Fe–O and Fe–N bonds at a wavelength of 500–600 nm. The results of the calculation of the mice's body weight decreased when induced by alloxan. After 3 and 4 weeks, the mice's body weight returned to stability. The highest decrease in glucose levels was in dose 2, namely 100 µg/kg bw with a decrease in %GL of 66.72%. The results of this study show that the complex compound Fe(III) arginine can reduce blood glucose levels in mice.

Keywords: antidiabetic activity, Fe(III) complex compound, arginine ligand, male mice

1. INTRODUCTION

Indonesia is one of the countries with a fairly high number of diabetes mellitus (DM) cases and continues to increase. According to the 10th edition of the International Diabetes Federation (IDF) Data in 2021, there are 537 million adults in the world who suffer from DM, and it is predicted that it will reach 643 million people in 2030 and 784 million people in 2045 [1]. The increase in the number of DM is directly proportional to the increasing population, the number of obesity sufferers, unhealthy lifestyles and also the lack of physical activity. DM is classified into type 1 DM and type 2 DM. Type 1 DM is a type of diabetes in which the pancreas is unable to produce insulin so that sufferers depend on the administration of insulin from the outside. Type 1 DM occurs due to a viral or autoimmune infection that damages insulin-producing cells, while type 2 DM is a group of metabolic diseases with hyperglycemia characteristics, occurring due to insulin secretion abnormalities or insulin dysfunction. Type 2 DM clinically appears when the body is no longer able to produce enough insulin to compensate for the increase in insulin resistance. Diabetes cases up to 90% are type 2 DM with the characteristics of insulin secretion disorders that attack the sufferer [2]. One of the latest treatments for type 2 DM is by using metallotherapy. Metalotheraphy is a therapeutic treatment using transition metals [3]. A number of inorganic compounds have been previously studied to have a role as antidiabetic agents, namely chromium, manganese, molybdenum, copper, cobalt, zinc, and vanadium.

Research by Ambarwati et al. (2021) stated that the Cr(III)-glycine complex compound has been synthesized and its bioactivity tested as an antidiabetic compound in male rats. The results of the study showed that the best dose of the Cr(III)-glycine complex compound of 200 µg/kg bw was able to reduce blood sugar levels by 44.12%. The results of the study with the Cu(II)-glycine complex compound obtained the best dose of 200 µg/kg bw which was able to reduce blood sugar levels by 41.33% [4][5]. Research on chromium and copper metal complex compounds has been studied computationally and *in vivo* tests have provided significant results, namely that the chromium(III)

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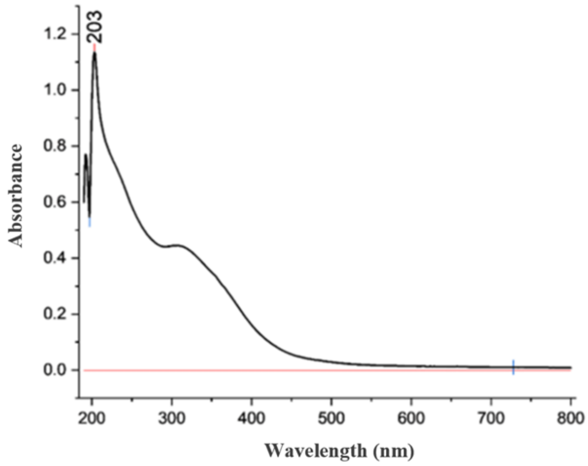

Figure 1. Characterization of [Fe(Arg)$_3$] by UV-Vis.

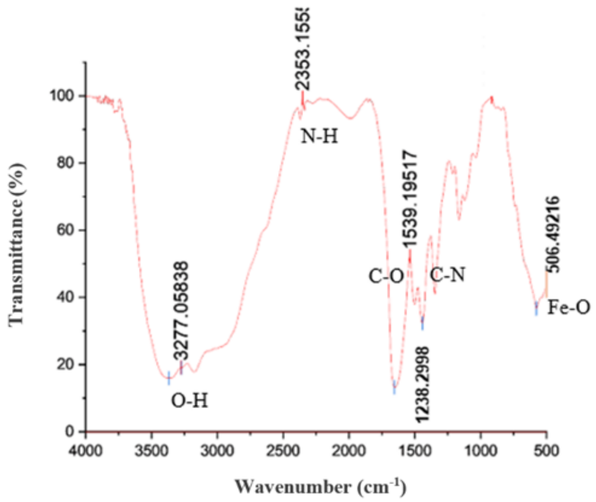

Figure 2. IR spectrum of [Fe(Arg)$_3$].

and copper(II) complex compounds are able to reduce glucose levels [6]-[8]. In addition to the Cr (III) and Cu(II) complexes, the Fe(III) complex is predicted to be a candidate for an antidiabetic drug.

The Fe(III) metal is one of the compounds that acts as a cofactor that helps the activity of primary antioxidants, namely the superoxide dismutase enzyme in defense against free radicals, thereby preventing the emergence of oxidative stress and vascular complications related to diabetes [9]. Transition metal complexes, including Fe(III), have the potential for antidiabetic activity. This study can be a reference for understanding the mechanism of action of Fe(III) in complex compounds and its potential as an antidiabetic agent [10]. Fe complexes with various ligands, including amino acids such as arginine, are explored for their potential in regulating blood glucose levels [11]. The role of arginine in insulin secretion and vascular function provides a basis for how arginine as a ligand in the Fe(III) complex can have antidiabetic activity [12]. The use of Fe(III) metal complexes with several amino acids such as arginine, aspartic acid and glycine as antidiabetic compounds has not been widely reported to date, therefore research was conducted on Fe(III) complex compounds with amino acids arginine, aspartic acid and glycine and characterized using UV-Vis, and IR spectrophotometer instruments.

2. MATERIALS AND METHODS

The procedure for the synthesis, characterization, and antidiabetic activity test of complex compounds used in this study, is based on the procedure that has been carried out in the research of Ambarwati et al. (2021) [5].

2.1. Fe(III)-arginine Synthesis

The manufacture of Fe(III) complex compounds with arginine is synthesized in a ratio of 1:3 (metal:ligand). $FeCl_3 \cdot 6H_2O$ was dissolved in 25 mL of distilled water and arginine was dissolved in 25 mL of distilled water. Then both solutions were mixed, the pH was checked, and refluxed at 60 °C with time variations (1, 2, 3, 4, and 5 h), then freeze -dried for 48 h. The synthesized complex compound was weighed until a constant weight was obtained to obtain the optimum time.

2.2. Characterization of Fe(III) Complex Compounds

The results of the synthesis of complex compounds that have been formed is characterized by UV-Vis, Infrared (IR) spectrophotometer to determine the functional groups contained in Fe(III) complex compounds at wavenumbers of 4000–400 cm^{-1}.

2.3. Antidiabetic Test In Vivo (Oral Method)

The method of testing antidiabetic activity in mice follows previous studies using the *in vivo* method [13]. Mice in the test group (D, E, and F) who had blood glucose levels ≥ 200 mg/dL, were given with complex compounds orally on day 13 with a reference dose of 18.2, 36.4, and 72.8 mg/kg BW was administered daily for 14 days [14].

2.4. ANOVA Test

ANOVA is a test used to analyze the difference in the average population to find out the significant difference of two or more data groups. The principle of the ANOVA test is to analyze the variability of the data into two sources of variation, namely variations within groups (within) and variations between groups (between). The variation between groups in question is the relationship between variables in the study.

3. RESULTS AND DISCUSSIONS

3.1. Synthesis of Fe(III)-Arginine Complex Compounds

The synthesis results that have been obtained from the reflux process with time variations of 1, 2, 3 and 4 h were then carried out UV-Vis test with the aim of determining the optimum time in the synthesis process in this study. The complex compound $[Fe(Arg)_3]$ that has been freeze-dried is in the form of a brown gel with a yield of 96.9%, weighing 0.5061 g. The $[Fe(Arg)_3]$ complex is brown because the complex compound Fe(III)

Table 1. Absorption peaks of Fe(III) complex compound.

No.	Function Clusters	Wavenumber (cm^{-1})	
		Arginine [18]	Fe(III)-Arg
1	O–H	3426	3277
2	N–H	3280	3230
3	C=O	1614	1539
4	C–N	1174	1238
5	Fe–O	-	506

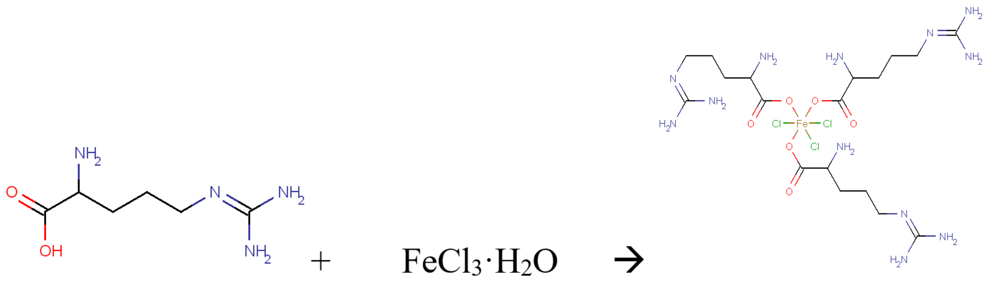

Figure 3. Fe(III)-arginine formation reaction.

Table 2. Average percentage of blood sugar levels in male mice**.**

Treatment	Percentage of Blood Sugar Levels (%)
Fe-Arg-D1 (50 µg/kg bw)	64.65
Fe-Arg-D2 (100 µg/kg bw)	65.66
Fe-Arg-D3 (200 µg/kg bw)	63.44
Fe-D1 (50 µg/kg bw)	58.43
Fe-D2 (200 µg/kg bw)	59.17
Arg-D1 (50 µg/kg bw)	60.07
Arg-D2 (200 µg/kg bw)	61.00
K(+)	70.02
K(-)	1.51
K(n)	4.45

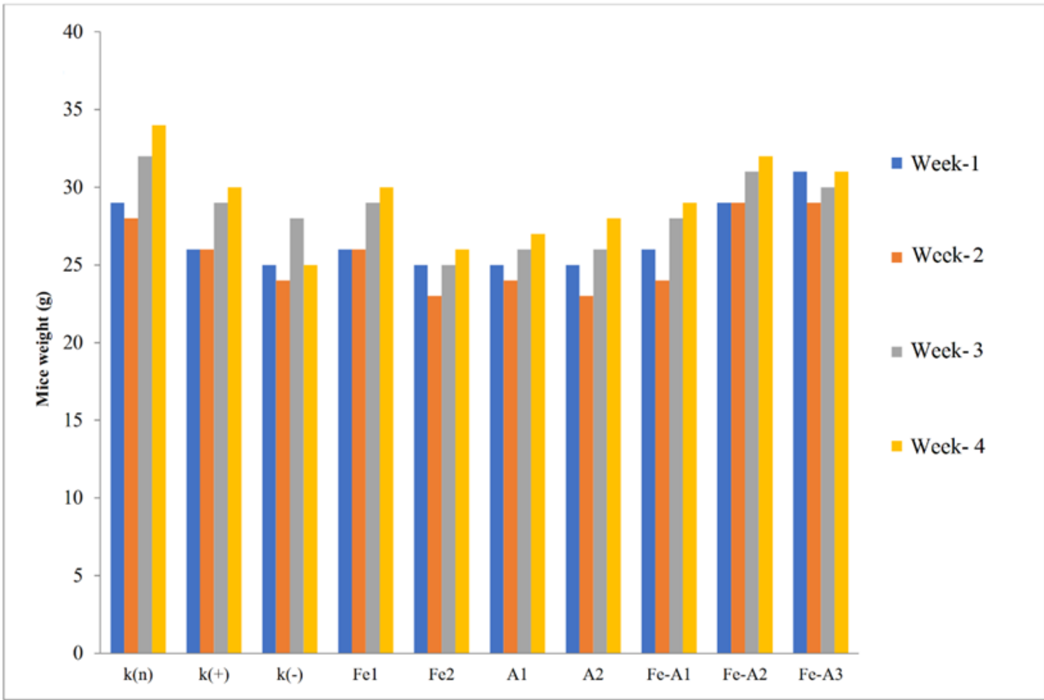

Figure 4. Average body weight of male mice.

arginine has a brown sample color. This is due to several factors related to the chemical properties of the Fe^{3+} metal ion transition and its complex coordination structure. Fe^{3+} transition metal ions have chromophoric properties that are capable of absorbing light in the visible spectrum. These ions have orbitals that are not fully filled, so electrons in that orbital can transition. This electron transition results in the absorption of light in the red-brown spectrum [15].

The mechanism of formation of complex compounds [Fe(Arg)$_3$] begins with the replacement of the Cl group in the $FeCl_3 \cdot 6H_2O$ compound by the OH group of arginine. The replacement of the group can occur due to the difference in ligand strength between the OH group as a stronger electron donor ligand than the Cl group which is a weak ligand and a good leaving group. The Cl is replaced by NH_3 and OH groups of leucine. The NH_3 and OH groups in arginine act as nucleophiles. Nucleophiles are species (ions or molecules) that are strongly attracted to a region that is positively charged to something else [16].

3.2. Results UV-Vis and IR Characterization of Fe (III)-Arginine Complex Compounds

The UV-Vis spectrophotometer is used to see the shift in the maximum wavelength of Fe(III) after it is reacted with an arginine ligand. The results of the spectrophotometer measurements of the compound are explained in the following Figure 1. Based on Figure 1, there is absorption of the Fe(III)-arginine complex compound at 203 nm. This indicates a hypochromic shift or a shift towards lower wavelength absorption when compared to $FeCl_3{\cdot}6H_2O$ wavelength absorption Hypochromic shift is an absorbent shift to a shorter wavelength region due to substitution or solvent effects, thus causing a hypochromic effect or a decrease in absorbant intensity.

The characterization of complex compounds is then continued by using an IR spectrophotometer to see the absorption of functional groups between the central atomic metal and the ligand (Figure 2).

The results of the IR spectrophotometer measurement of the $[Fe(Arg)_3]$ complex in Figure 3, show that a typical absorption has formed a Fe(III)-arginine complex with an absorption peak at 506 cm $^{-1}$ which is a typical absorption of Fe–O and Fe–N, the O and N atoms of arginine have been bound to the Fe(III) metal [17]. Complete IR absorption data in Table 1, shows the absorption peaks that appear, including the NH_2 peak in the 3,277 cm^{-1} area, absorption at 1,539 cm^{-1} is an asymmetric C=O, which is a typical absorption of arginine which has amine and carboxylate functional groups. The Fe–O and Fe–N bonds appear bands in the range of 400–600 cm^{-1} indicating the presence of metal bonds with oxygen or nitrogen donors.

The absorption of the Fe–O functional group in the complex compound $[Fe(Arg)_3]$ that has been characterized and appears at 506 cm^{-1}. The synthesized compound shows a bond between the

Table 3. Results of ANOVA and BNT 5% of blood sugar levels of mice.

Treatment	Average Total Blood Glucose Levels of Mice (Mean ± Std. Deviation)
Fe-Arg-D1 (50 µg/kg bw)	(112.33 ± 5.507) [a]
Fe-Arg-D2 (100 µg/kg bw)	(112.66 ± 8.504) [a]
Fe-Arg-D3 (200 µg/kg bw)	(116.66 ± 3.511) [a]
Fe-D1 (50 µg/kg bw)	(122.00 ± 5.567) [a]
Fe-D2 (200 µg/kg bw)	(116.33 ± 4.163) [a]
Arg-D1 (50 µg/kg bw)	(120.00 ± 2,000) [a]
Arg-D2 2100 µg/kg bw)	(117.33 ± 5.507) [a]
K(+)	(97.00 ± 12.529) [a]
K(-)	(342.33 ± 5.131) [b]
K(n)	(105.00 ± 6.245) [a]

Table 4. Results of determining pharmacokinetic tests using *the Lipinski Rule of Five* compound Glibenclamide.

	Molecular Weight	Donor Association H	H-Acceptor Bond	LogP	RM
Condition	<500	<5	<10	<5	40–130
Glibenclamide	493	3	8	3.9	123

Table 5. Pre-ADME test results.

Compound	Absorption		Distribution
	HIAs (%)	Caco-2 cell (nm sec)	Plasma Binding Protein (%)
Glibenclamide	95.90	5.99	100

Table 6. Toxicity test results for the Fe(III)-arginine complex compound.

Target	Shorthand	Prediction	Probability
Hepatotoxicity	dili	inactive	0.63
Carcinogenicity	carcino	inactive	0.76
Immunotoxicity	immuno	inactive	0.73
Mutagenicity	mutagen	inactive	0.80
Cytotoxicity	cyto	inactive	0.77

central atom Fe^{3+} with the amine and carboxyl group ligands [18][19]. The reaction between $FeCl_3 \cdot 3H_2O$ metal and arginine to form a complex compound $[Fe(Arg)_3]$ is shown in Figure 3.

3.3. Antidiabetic Test

The bioactivity test of the antidiabetic compound $[Fe(Arg)_3]$ has been carried out with test parameters, namely body weight and blood glucose levels. The synthetic complex compounds obtained at the optimum time of 4 h were tested for antidiabetic bioactivity *in vivo*, by inducing male mice using alloxan at a dose of 150 mg/kgBB by injection under the skin of the nape of the neck. The blood sugar levels of diabetic male mice were obtained on day 14.

Male mice positive for diabetes were given oral treatment using the $[Fe(Arg)_3]$ complex at doses of 50, 100, and 200 µg/kg bw, as well as $FeCl_3 \cdot 3H_2O$ solution, and arginine, at doses of 50 and 200 µg/kg bw. The positive control group was given glibenclamide at a dose of 5 mg/kg bw, the negative control group was only induced with alloxan, and the normal control group was not given any additional treatment only given feed and drink. Observations were carried out for 4 weeks and sugar levels and body weight were measured every week. The description of the results of the bioactivity test of the compound $[Fe(Arg)_3]$ is explained as follows.

3.4. Body Weight of Male Mice

The body weight of male mice was weighed to determine the dose of alloxan, glibenclamide, and the complex compound used. Male mice were weighed once a week according to the test stages described above. The results of measuring the body weight of male mice can be seen in Figure 4.

Figure 4 shows the results of the average body weight of male mice during 4 weeks of treatment. In the first week, male mice were acclimated, showing a stable body weight of 25–31 g, then continued with alloxan induction in the second week. Giving alloxan in the second week caused weight loss, namely to 23–29 g. This is because alloxan competes with glucose for recognition by glucose receptor cells, because alloxan is structurally similar to glucose [20]. A reduction in glucose entering these cells leads to a decrease in fat and glycogen synthesis, resulting in a tendency for weight loss [21]. Male mice with diabetes also experience dehydration, so they drink more and urinate more often. The normal group tended not to experience weight loss because this group was not given any treatment except eating and drinking.

In the third and fourth weeks, male mice from the positive control group were treated orally with the drug glibenclamide and showed an increase in body weight (28–34 g). Glibenclamide is a sulfonylurea drug to lower blood sugar levels in people with type 2 DM, which is widely sold on the market. Glibenclamide works by stimulating pancreatic β cells to release the hormone insulin, thereby increasing insulin levels in the blood. Insulin has an anabolic effect where it can stimulate the absorption and metabolism of glucose by body tissues, especially muscle and fat. Glucose will be used for the synthesis of fatty acids, glycogen and protein. This causes an increase in body weight due to increased muscle and fat mass. Studies in diabetic mice treated with glibenclamide showed a significant increase in body weight after 4 weeks of treatment compared to controls. This is because glibenclamide can reduce blood sugar levels to normal conditions, thereby triggering the synthesis of body tissues such as muscle and fat [22].

The treatment group for complex compounds was not treated with glibenclamide but instead used the $[Fe(Arg)_3]$ complex. Just like the drug glibenclamide, the $[Fe(Arg)_3]$ complex also provides an anti-hyperglycemic effect on male mice that have been induced by alloxan, thereby increasing the body weight of male mice in the third and fourth weeks to 28–34 g. This can be seen from its ability to reduce blood glucose levels in male diabetic mice through the mechanism of stimulating insulin release and increasing glucose absorption by body cells [23]. In addition to glibenclamide, the commonly used antidiabetic drug metformin has been studied for its effects on COVID-19 due to its anti-inflammatory properties and ability to reduce hyperglycemia in people with diabetes [24].

Insulin plays a role in glucose metabolism and protein synthesis. Increased insulin levels due to administration of zinc complex can stimulate the assimilation and synthesis of glucose into glycogen, triglycerides and protein in muscle and fat tissue, causing an increase in cell size and number of muscle cells and fat tissue. Overall, it has an impact on increasing the body weight of mice and this process occurs in the $[Fe(Arg)_3]$ complex [25]. Based on measurements of the body weight of male mice, the complex compound $[Fe(Arg)_3]$ showed the ability to restore the condition of diabetes in male mice induced by alloxan, with an increase in body weight that occurred so that it was close to the body weight before treatment. This shows that diabetes in male mice actually reduces body weight, in contrast to what happens in humans which causes obesity. The test was carried out by examining the blood sugar levels of male mice.

3.5. Blood Sugar Levels on Mus musculus L

Examination of blood sugar levels in male mice was carried out 4 times using the results of the synthesis of a complex compound $[Fe(Arg)_3]$ with varying doses, namely 50, 100, and 200 µg/kg bw with each dose repeated 3 times. Male mice were induced with alloxan in the second week with doses adjusted for body weight group. This treatment caused male mice to selectively experience damage to pancreatic *β* cells and induce complications of

Table 7. *Lipinski Rule of Five* test results $[Fe(Arg)_3]$.

	Molecular Weight	Donor Association H	H-Acceptor Bond	LogP	RM
Condition	<500	<5	<10	<5	40–130
$[Fe(Arg)_3]$	235	4	6	-3.15	58.25

Table 8. Toxicity test results for complex compounds $[Fe(Arg)_3]$.

Target	Shorthand	Prediction	Probability
Hepatotoxicity	dili	inactive	0.78
Carcinogenicity	carcino	inactive	0.51
Immunotoxicity	immuno	active	0.84
Mutagenicity	mutagen	inactive	0.55
Cytotoxicity	cyto	inactive	0.66

Table 9. Pre-ADME test results.

Compound	Absorption		Distribution
	HIAs (%)	Caco-2 cell (nm sec)	Plasma Binding Protein (%)
$[Fe(Arg)_3]$	52.32	2.44	73.69

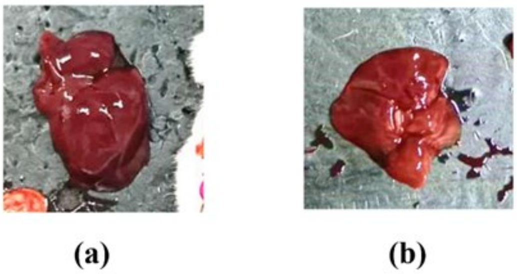

Figure 5. Liver organs of male mice (a) Fe(III)-Arginine, (b) normal control.

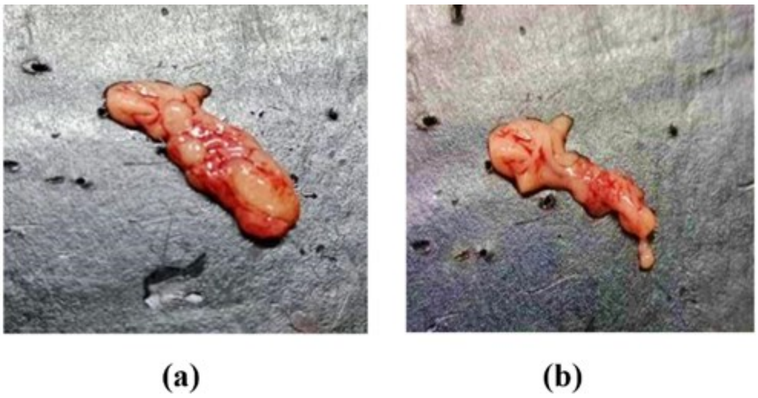

Figure 6. Pancreatic organs of male mice (a) Fe(III)-Arginine, (b) normal control.

type 1 DM. Damage to pancreatic cells, either due to inflammation, insulin resistance, or other metabolic factors, can reduce insulin secretion and lead to increased blood sugar levels [26]. The effect of administering metals, amino acids and complexes on reducing blood sugar levels was carried out by measuring blood sugar levels and calculating the average percent glucose lowring (% GL). The mean percent glucose lowring (%GL) of male mice after administration of the $[Fe(Arg)_3]$ complex can be seen in Table 2.

The information obtained in Table 2 shows the results of the average percentage reduction in blood glucose levels in mice after being treated with $FeCl_3$ metal, arginine ligand, and the complex compound $[Fe(Arg)_3]$. The group treated with the amino acid arginine was also stated to be able to reduce blood sugar levels in mice with a %GL value of 60.07% for dose 1 (50 µg/kg bw) and 61.00% for dose 2 (100 µg/kg bw). The highest %GL was at the lowest dose, namely dose 1. This was influenced by the mice's body's absorption capacity for the drug given. A dose that is better absorbed by the mice's body will be more effective in lowering blood sugar levels. This is because at this dose, the amount of active compound circulating and reaching the target organs (eg pancreas/liver) is greater. The better the absorption capacity, the less dose is needed to achieve a hypoglycemic effect, so it can be concluded that arginine has better absorption than arginine in the body of mice.

In the $[Fe(Arg)_3]$ complex, the dose that had the best percentage of glucose levels was dose 2 (100 µg/kg bw), which was 65.66%. The results of this dose are almost close to the comparison percentage for the control group (+) glibenclamide as a drug, namely 70.02%. Apart from the average percentage reduction in blood glucose levels, data on blood glucose levels of mice using the complex compound $[Fe(Arg)_3]$ was also followed by statistical data analysis using the ANOVA test and the BNT test at the 5% level as shown in Table 3.

The information obtained in Table 3 shows the complex compound [Fe(Arg)$_3$] which was tested on male mice and the mean blood glucose levels were measured using the one-way ANOVA test. The results showed that all treatment groups showed a significant reduction in blood glucose levels ($P<0.05$). The complex compound test [Fe(Arg)$_3$] was continued with BNT at 5% level. There was a real difference between the negative control group and all treatment groups, then there was no real difference between the normal control group and the positive control, complex [Fe(Arg)$_3$] dose 1, and [Fe(Arg)$_3$] dose 2, complex [Fe(Arg)$_3$] dose 3, metal $FeCl_3$ dose 1, metal $FeCl_3$ dose 2, arginine dose 1, and arginine dose 2. Based on data on the percentage of glucose lowring (%GL) and the One-way ANOVA test, it can be stated that a dose of 100 µg/kg bw has a mean value and percentage of blood sugar levels that are close to K(+). This shows that [Fe(Arg)$_3$] has activity in reducing blood glucose levels.

Fe(III) is known to have antioxidant activity that can help reduce oxidative stress. Oxidative stress contributes to insulin resistance and pancreatic beta cell dysfunction. By reducing free radicals and inhibiting excessive oxidation reactions, the Fe(III) complex can protect pancreatic beta cells, increase insulin secretion, and improve insulin sensitivity in peripheral tissues such as the liver, muscle, and adipose tissue. In addition, Fe(III) in the complex likely interacts with the body's antioxidant defense system such as the superoxide dismutase, strengthening the body's capacity to neutralize free radicals. It can be concluded that the Fe(III)-arginine complex can help improve beta cell function through protection against oxidative damage to pancreatic beta cells, thereby increasing insulin secretion, and increasing insulin sensitivity in peripheral tissues (liver, muscle, adipose tissue), thereby helping to normalize blood sugar levels.

3.6. Pharmacokinetic Test of Complex Compounds

Pharmacokinetic determination was carried out using *the Lipinski Rule of Five*, Pre-ADME and Protox websites. This pharmacokinetic determination aims to see whether the synthesized compound is suitable as a drug candidate or not. In this pharmacokinetic test, the compound to be tested is [Fe(Arg)$_3$] with the compound glibenclamide as a comparison. The following is an

(a) (b)

Figure 7. Kidney organs of male mice (a) Fe(III)-Arginine, (b) normal control.

(a) (b)

Figure 8. Heart organs of male mice (a) Fe(III)-Arginine, (b) normal control.

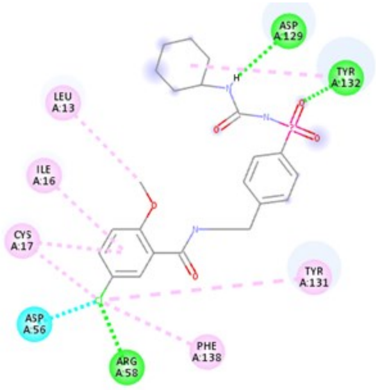

Figure 9. 2D visualization of Glibenclamide *docking*.

explanation of the glibenclamide compound.

3.7. Glibenclamide

Glibenclamide is an antidiabetic drug used in the treatment of type 2 diabetes mellitus. The mechanism of action of glibenclamide is by inhibiting potassium channels that are sensitive to adenosis triosphate (ATP) in pancreatic beta cells. Widespread use of glibenclamide causes side effects, namely liver damage and thrombocytopenia and long-term use requires monitoring. Computational calculations using glibenclamide as a normal control for comparison of the $[Fe(Arg)_3]$ complex as a candidate antidiabetic drug [27]. Analysis of drug-like properties is carried out based on the *Lipinsk Rule of Five* which states that a compound has properties similar to a drug if the molecular weight (BM) of the compound is less than 500 Daltons, the value of the partition coefficient log P is less than 5, the number of hydrogen bond donors is less than 5, and the number of hydrogen bond acceptors is less than 10 [28]. *The Lipinski Rule of Five* pharmacokinetic results of the Glibenkalmid compound in Table 4.

Determining the pharmacokinetics using *the Lipinski Rule of Five* on glibenclamide obtained a molecular weight of 493 g/mol, has 3 hydrogen donor bonds, has 8 hydrogen acceptor bonds, a LogP value of 3.9 and a molar refractivity of 123. So based on these results the glibenclamide compound is declared to be used as a drug candidate because it meets the *Lipinski Rule of Five*.

Further pharmacokinetic determination is done using Pre-ADME (Absorption, Distribution, Metabolism and Excretion) which provides information regarding the value of pharmacokinetic properties such as absorption *(gastrointestinal absorption, human skin permeability coefficient),* distribution *(blood-brain barrier* (BBB), and metabolism (CYP2D6). and CYP3A4 *inhibitors*). HIA is the sum of bioavailability and absorption which is evaluated from the ratio of excretion through urine, bile, and feces. Distribution parameters are predicted based on attachment to plasma proteins and interacts with pharmacological targets so that plasma protein binding plays an important role in drug efficacy. Plasma protein binding (PPB) is a drug fraction that is available in free form for distribution to various tissues. Caco-2 cells are an *in vitro model* to determine drug transport through intestinal epithelium derived from human colon adenocarcinoma which has dual transport pathways. A compound is categorized as being well absorbed if the % HIA value is in the range of 70–100%, adequate in the range of 20–70%, and poor in the range of 0–20%. A PPB value of > 90% indicates that the drug is strongly bound.

The results of the determination using Pre-ADME of the glibenclamide compound can be seen in Table 5. For the glibenclamide compound, the HIA *value* was 95.90%, Caco-2 cell 5.99 and PPB was 100%. Based on the results of these 3 parameters, the glibenclamide compound has a good condition in terms of its ability to absorb and distribute in the blood so that the glibenclamide compound can be used as a drug candidate. Apart from using *the Lipinskie Rule of Five* and PRE-ADME for determining pharmacokinetics, the ProTox *server is also used*. The ProTox-II server provides comprehensive information on toxicity parameters including prediction of oral rat acute toxicity (LD_{50}), toxicity class, hepatotoxicity, carcinogenicity, immunotoxicity, mutagenicity, and cytotoxicity. The pharmacokinetic results of the proTox compound glibenclamide can be presented in Table 6.

Based on Table 6, the results of pharmacokinetic determination with glibenclamide proTox have 5 inactive classes, thus it can be stated that the glibenclamide compound can be used as a drug candidate because it meets the requirements of the proTox-II server and is not toxic.

3.8. Complex Compound [Fe(Arg)$_3$]

Based on Table 7, the Fe(III)-arginine complex compound meets the requirements of *the Lipinskie Rule of Five* because the molecular weight is 235, the donor bond is 4, the acceptor bond is 6, the LogP value is -3.15, and the molar refractivity is 58.28.

Based on Table 7, the Fe(III)-arginine complex compound meets the requirements of *the Lipinskie Rule of Five* because the molecular weight is 235, the donor bond is 4, the acceptor bond is 6, the log P value is -3.15, and the molar refractivity is 58.28. This states that the Fe(III)-arginine complex compound has the same properties as a drug. The pharmacokinetic test was then continued with a toxicity test with web proTox-II. Based on the results of the toxicity test of the complex compound [Fe(Arg)$_3$], it is stated that this complex compound is not toxic because it enters class 5 in the toxicity parameters even though in *the prediction target* there is an active part in the carcinogenicity target with a probability of 0.51 (Table 8).

The pharmacokinetic test is carried out by testing the absorbance or absorption of complex compounds on the Pre-ADME website by looking at the HIA, Caco-2 cell and PBP values on the Pre-ADME website. Following are the results of the pharmacokinetic test with Pre-ADME explained in Table 9.

Based on the results of these 3 parameters, the compound [Fe(Arg)$_3$] has a fairly good condition for drug absorption in the body because the HIA value of the complex compound [Fe(Arg)$_3$] is 53.32%, which means it is in the range 0–20%, including in quite good parameters for drug absorption.

3.9. Testing the Vital Organs of Male Mice

Male mice with diabetes will experience damage to vital organs in the body, because hyperglycemia (high blood sugar levels) that occurs during diabetes causes excessive oxidative stress in the body's cells. The free radicals formed can damage cell membranes and cause tissue damage. Several

Table 10. Glibenclamide docking results.

Amino acid	Bond Length (Å)	Types of Bond Interactions
ARG58	3.28	Hydrogen
TYR132	3.04	Hydrogen
ASP129	2.01	Hydrogen
ASP56	3.43	Halogen
LEU13	4.29	Alkyl
CYS17	4.15	Alkyl
TYR131	5.19	Pi-Alkyl
TYR132	4.89	Pi-Alkyl
PHE138	4.71	Pi-Alkyl
ILE16	5.25	Pi-Alkyl
CYS17	4.22	Pi-Alkyl

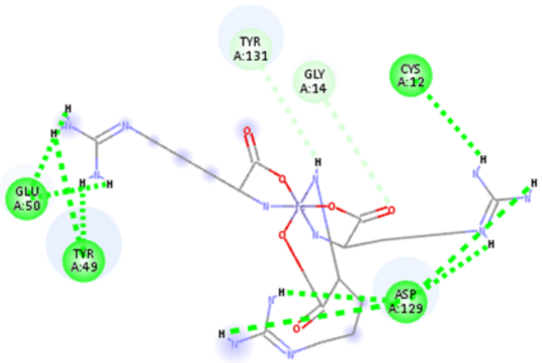

Figure 10. 2D docking visualization [Fe(Arg)$_3$].

vital organs in male mice were damaged.

3.9.1. Liver

The first vital organ in male mice that experiences problems when they have diabetes is the liver. When diabetes has high blood glucose levels, the liver has to work harder to lower and stabilize blood glucose levels. This can be burdensome and damage the liver if it occurs for a long time (Figure 5).

An excessive increase in glucose intake causes the liver to produce more fatty acids. This condition can develop into hepatitis (inflammation of the liver) and liver cirrhosis (permanent liver tissue damage) in mice that suffer from diabetes for a long time. Diabetes is often accompanied by overweight or obesity. This can accumulate in the liver and trigger inflammation. This condition causes the liver to become larger (hypertrophy).

3.9.2. Pancreas

The pancreas is responsible for producing insulin. Insulin is an important hormone that regulates blood sugar levels. If there is an abnormality in the pancreatic β cells (cells that produce insulin), it will cause reduced or no insulin production. This causes blood sugar levels to become uncontrolled and causes diabetes (Figure 6).

Impaired function of beta cells and excessive workload of the pancreas can cause gradual damage, ranging from inflammation to total failure of pancreatic function. The pancreas in male mice with diabetes experiences changes in size and tends to increase in size due to the proliferation of pancreatic cells in response to high blood glucose levels.

3.9.3. Kidney

The kidneys have many small blood vessels that are susceptible to damage due to high blood glucose levels in diabetes. High blood glucose levels can damage the kidney blood vessels, thereby inhibiting filtration and the kidneys' work to remove waste (Figure 7).

Diabetes is also associated with high blood pressure, which is a risk factor for kidney damage. High blood pressure can worsen damage to the kidney blood vessels. The kidneys experience fibrosis and an increase in the number of renal tubules that experience lesions (damage or injury). Nerve damage due to diabetes (diabetic neuropathy) can disrupt the work of kidney smooth muscle, thereby reducing filtration function.

3.9.4. Heart

The heart organs of male mice affected by diabetes will experience changes such as enlargement of the heart size (cardiomegaly). This is caused by hypertrophy of the heart muscle due to the increased workload of the heart. Thinning of the heart wall. Heart muscle cells become thinner due to the damage that occurs. Changes in the color of heart tissue. Healthy heart tissue is brownish red, whereas in diabetes it can become paler (Figure 8).

This is because diabetes increases the risk of atherosclerotic plaque formation on the artery walls. This happens because prolonged hyperglycemia can damage the blood vessel endothelium and trigger inflammation. Atherosclerotic plaque can narrow or harden, causing disruption of blood flow to the heart and damage to heart tissue. From the image of the vital organs above, it can be concluded that there is a slight disturbance in the function of the male mice's vital organs, such as swelling of the liver and pancreas, as well as a paler color change in the kidneys and heart. However, there were no significant physical changes in the vital organs of male mice after alloxan induction, when compared to the normal control group and the Fe(III)-Arginine complex group. Thus, when you look at it at a glance it looks the same. This means that the two complex compounds given did not affect the vital organs of male mice.

3.10. In Silico Glibenclamide Test Results

The glibenclamide compound is *docked* with *a gridbox* (x = 40, y = 40, z = 40), *gridbox center* (x = -1.436, y = 0.91, z = 2.031), *spacing* = 0.375 Å, and *number of runs* (20) to obtain a bond energy value of -9.11 kcal/mol. Below we can present the interactions that occur between the glibenclamide compound and the receptor shown in Figure 9 and Table 10.

Based on the validation information from *the docking results* shown in Table 10, the bonds that occur in the glibenclamide compound are found in the amino acid residues CYS17, ILE16, LEU13, TYR131 and ASP129 with a bond length of 2.10–5.25 Å. The results of the docking interaction of glibenclamide as a diabetes drug with the 5KQG protein, which is a cyclin-dependent kinase 8 inhibitor protein found in humans (*Homo sapiens*) showed good results with an interaction energy of -9.11 kcal/mol. The 5KQG protein regulates several transcription factors that are important for glucose metabolism and insulin response. Dysfunction in this transcriptional regulation can affect the glucose regulatory pathway, which plays a role in diabetes. The insulin signaling pathway often involves various protein kinases, and the activity of kinases involved in transcriptional regulation (such as 5KQG) can affect the expression of genes involved in insulin resistance or insulin secretion. The docking results showed that there were 4 hydrogen bonds interacting with glibenclamide, this strengthens glibenclamide as an antidiabetic drug.

3.11. In Silico Test Results [Fe(Arg)$_3$]

Compound [Fe(Arg)$_3$] docked *with a gridbox* (x = 40, y = 40, z = 40), *gridbox center* (x = -1.436, y = 0.91, z = 2.031), *spacing* = 0.375 Å, and *number of runs* (20) to obtain a value bond energy of -10.38 kcal/mol.

Based on the validation information from *the docking results* shown in Figure 10 and Table 11, hydrogen bonds occur in the compound [Fe(Arg)$_3$] found in the amino acid residues GLY14, TYR131 and ASP129 with a bond length of 1.70–3.10 Å. Hydrogen bonds are very important because in general the molecular interactions that occur in the

Table 11. Docking results [Fe(Arg)$_3$].

Amino acid	Bond Length (Å)	Types of Bond Interactions
ASP129	2.11	Hydrogen
ASP129	1.70	Hydrogen
ASP129	2.09	Hydrogen
ASP129	2.08	Hydrogen
TYR49	2.78	Hydrogen
TYR49	1.92	Hydrogen
GLU50	2.11	Hydrogen
GLU50	1.73	Hydrogen
CYS12	3.01	Hydrogen
GLY14	3.10	Hydrogen
TYR131	2.74	Hydrogen

body are non-covalent interactions, namely interactions between atoms that are not covalently bound to each other. The main amino acids seen in the active site of this interaction are GLY14, TYR131 and ASP129. This is because these amino acids are included in the validation of native ligand *redocking*. As in the interaction of glibenclamide with the 5KQG protein, the interaction of the Fe $(Arg)_3$ complex shows a more negative interaction energy value, namely -10.38 kcal/mol. This more negative number indicates that the $Fe(Arg)_3$ complex can be a candidate for antidiabetic drugs such as glibenclamide. There are 11 hydrogen bond interactions between metal complex compounds and amino acid residues in the target protein increasing the stability of the complex. The more hydrogen bonds formed, the more stable the interaction of the metal complex with the protein. These interactions often help maintain the position of the metal and its ligands in the active site or binding area of the protein. The hydrogen bond interactions formed in metal complexes are better than the interactions with glibenclamide. Computational predictions are not only for metal complex compounds, but can also be used to predict organic compounds as drug candidates, such as predicting compounds contained in pineapple and ginger as anticovid-19 [28].

The docking results showed that the $[Fe(Arg)_3]$ complex has a high affinity energy for the 5KQG insulin receptor, this may mean that the complex is able to enhance insulin signaling or reduce insulin resistance which is important in controlling blood sugar levels. In addition, the $[Fe(Arg)_3]$ complex shows a high affinity for glucokinase, this can increase glucose phosphorylation, improve glucose metabolism, and improve hyperglycemia conditions. Pharmacokinetic testing includes the journey of the $[Fe(Arg)_3]$ complex in the body, including ADME. This assessment is important to ensure that the complex has optimal bioavailability, duration of action, and safety. The results of Lipinski, ADME and toxicity calculations show that the $[Fe(Arg)_3]$ complex can be a candidate because it can be distributed well in the body and is not toxic.

4. CONCLUSIONS

The complex compound $[Fe(Arg)_3]$ was successfully synthesized using the reflux method. The UV-Vis results showed absorption at 203 nm, which is the absorption of $\pi \rightarrow \pi^*$ transition of the Fe^{3+} ion with the arginine ligand. The IR spectrum shows typical absorption (Fe–O) at 506 cm^{-1} of the complex structure. The complex compound [Fe $(Arg)_3]$ has the potential as an antidiabetic agent in alloxan-induced mice, administration of this complex reduces blood sugar levels with a dose of 100 µg/kg bw capable of reducing blood glucose in mice by 65.66%. The results of the ANOVA calculation showed no significant difference between the group averages with the $[Fe(Arg)_3]$ complex, and the 5% BNT test on the $[Fe(Arg)_3]$ complexs showed that the control (-) group had a significant difference, but the Fe(III) metal group, arginine ligand and control (+), (n) were not significantly different. The results of the computational calculation of the complex compound $[Fe(Arg)_3]$ have a better interaction energy of -10.38 kcal/mol compared to Glibenclamide (-9.11 kcal/mol). Pharmacokinetic test results that meet the Lipinski Rule of Five, Pre-ADME, and Protox parameters as a candidate antidiabetic drug.

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Conflicts of Interest

The authors declare no conflict of interest.

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