

Pre Clinical Study of Cr(III)-based Hypoglycemic Supplementin–Type 2 Diabetic Rats

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Abstract—The chromium (III)-amino acid complex based hypoglycemic agent was investigated on pre-clinical study by the response of the blood glucose level. This study was conducted on nicotinamide-streptozotocin induced diabetic Wistar rats. The rats were divided into 7 groups each consist of 4 animals. Three groups are control (+) with chromium picolinate (CrPic), control (-) diabetic group (DM) and control non diabetic (non DM). Furthermore, three groups were examined on the effect of Cr-AA [Cr(μ -OH)(glu)(OH)₂]₂·6H₂O (glu= glutamic) at dose (50, 150 and 300 μ g/day). In addition, the last group was studied on the effect of control group by glibenclamide. The blood glucose levels were measured before and after treatment. The results show that supplementation of Cr(III)-complex in 8 weeks decreased the blood glucose level in range 46.446 - 79.593 %.

Keywords—*hypoglycemic; chromium (III); amino acid; complexes; nicotinamide-streptozotocin; diabetic rats*

I. INTRODUCTION

The World Health Organization (WHO) defined that diabetes is a metabolic disease which characterized by hyperglycemia and glycosuria resulting from the defect of the secretion or the action of insulin, or both of them. The prediction of this WHO explained that the amount of diabetic patients in Indonesia will increase from 8.4 million at 2000 to 21.3 million in 2030. *International Diabetes Federation* (IDF) also predicted the similar condition. Prevalence of diabetes mellitus in Indonesia is 5.7% at 2010 [1,2].

The particular management of diabetes were

diet, exercise, supplement or nutraceutical, oral hypoglycemic agents and insulin endogen. Nutraceuticals (often referred to functional foods) are natural bioactive or chemical compounds that have health promoting, disease preventing or medicinal properties [3]. Some natural product from plants were used as antidiabetic or hypoglycemic agent, for example *Raphanus Sativus L. Leaves* [4], and *Allium Cepa, L.* [5].

Most nutraceutical usually come from organic compounds. This research is a part of application of inorganic nutraceutical. Inorganic compound or metal-containing medicinal product may contain one of these probabilities: (a) chemical elements essential to life forms, for example, iron for anemia (b) non essential/toxic elements that carry out specific medicinal purposes, for example platinum as antitumor agents [6].

Some metal complexes or organo-metallic compounds have been used in medicine for centuries. Metal complexes play essential role in pharmaceutical industry and in agriculture. The metallo-elements present in trace quantities play vital roles at the molecular level in living system. In diabetes, intake of chromium metal complex shown considerable reduction in the glucose level [7].

Supplement contains trivalent Chromium is needed for a person with type 2 diabetes mellitus, according to it's important role in glucose metabolism. Cr (III) interact with the insulin and its receptors on the first step in the metabolism of glucose entry into the cell, and facilitates the interaction of insulin with its receptor on the cell surface [8][9]. Chromium increases insulin binding to cells, insulin receptor number as well as activates insulin receptor

kinase leading to increase sensitivity of insulin receptor. Additional studies are urgently needed to elucidate the mechanism of the action of chromium and its role in the prevention and control of diabetes [10].

The amount of Cr(III) intake is about 200 μ gCr/day. The well known chromium supplement is chromium picolinate, Cr(pic)₃. The disadvantage of Cr(pic)₃ is the effect of this compound in DNA damage [11]. The damage presumably through the catalytic formation of reactive oxygen species. As this supplement enters cells intact and it potentially capable of generating oxidative damage.

Administration of the Cr(pic)₃ to rats has demonstrated for the first time that it can give rise to oxidative DNA damage in whole animals [12]. The research for compounds with ability to maintain the blood glucose level or called 'antihyperglycemic agent' is still in progress.

Chromium ascorbate complex also proposed for Cr supplement. However, these ascorbate complex also have a potential damage to DNA [13]. The next potential compound is the complex of Cr(III) with amino acid. For example, the Cr(III) complex with D-phenylalanine, Cr(pa)₃. Unlike chromium picolinate, Cr(pa)₃ does not cleave DNA under physiological conditions [14].

Ochiai [15] reported that some amino acids with Cr(III) act as a part of GTF (*Glucose Tolerance Factor*). GTF is a low molecular weight Chromium (LMWCr), which is, involved in the action of insulin in processing glucose into energy. It is an oligopeptide of molecular weight about 1400, and consists of glycine, cysteine, aspartate and glutamate with the acidic amino acid. This compound binds the molecule of Cr(III) very tightly by ratio 1:4 and involved in the hormonal action of insulin. Natural GTF is a fraction isolated from brewer's yeast which has a biological activity in glucose metabolism. A solution which contains chromium (III), glycine, glutamic acid and cysteine mimics the biological activity of the naturally occurring GTF [16]. Another study reported the relationship between chromium (III)-amino acids complexes with GTF activity using a yeast assay [17].

Development of new Cr(III) - amino acid complexes as antihyperglycemic supplement is still in progress. In previous study, preparation of Cr-glutamate (Cr-Glu) was carried out in aqueous solution, varying three variables: reflux duration, pH, and temperature. Ratio of Cr(III):L-glutamic acid which is used in this research was 1:3. The structure of the resulting complex was [Cr(μ -OH)(glu)(OH)₂]₂·6H₂O [18].

The in vivo experiment was studied on Streptozotocin (Stz)-nicotinamide induced diabetic albino rats [21][22]. Collaboration of the two induction agent according to the cytotoxicity of Streptozotocin (Stz) when given alone. Nicotinamide-Stz induction is a new experimental diabetic model that mimic some features of type 2 diabetes [23].

II. MATERIALS AND METHODS

A. Materials

The Cr(III) complex was produced from the previous study [19]. All supplement samples were diluted in 0.2% sodium carboxy methyl cellulose (CMC-Na). Nicotinamide (Sigma Aldrich) was diluted in 0.9% NaCl (E-Merck), whereas Streptozotocin / Stz (Sigma Aldrich) was diluted in citrate buffer solution (pH=4.5). The control were prepared by commercial product of Cr-Pic (Diabetasol) and oral hypoglycemic agent (glibenclamide).

B. Animals

C. The subjects of the study were 28 male Wistar albino rats (\pm 8 weeks old, 200-290 grams). The animals were kept and maintained with standard laboratory condition. Feeding was done with standard laboratory diet and drinking was allowed with water *ad libitum*. The amount of animals were determined by Freederer formula, $(n-1)(k-1) \geq 15$, where n is the amount of animals per groups, and k is the amount of groups [24]. In this case, the amount of animals in each group were 4 ($n \geq 3$).

C. Induction of Diabetes

The induction of diabetes was done by intraperitoneal injection of 120mg/kg nicotinamide then followed 15 minutes later by 60 mg/kg streptozotocin [20]. Streptozotocin was dissolved in citrate buffer (pH 4.5) and nicotinamide was dissolved in NaCl 0.9%. Hyperglycemia was confirmed by the elevated blood glucose levels at day 7 after injection. Blood sample was collected from the eye vein. Measurement of blood glucose level was conducted by using spectroscopic methods.

D. Experimental Design

The experimental design was modified from several previous work [24-29]. The rats were divided into 7 groups consist of 4 animals. The supplements administered orally 1 ml per day for 8 weeks. Treatment of these groups was described in Table 1.

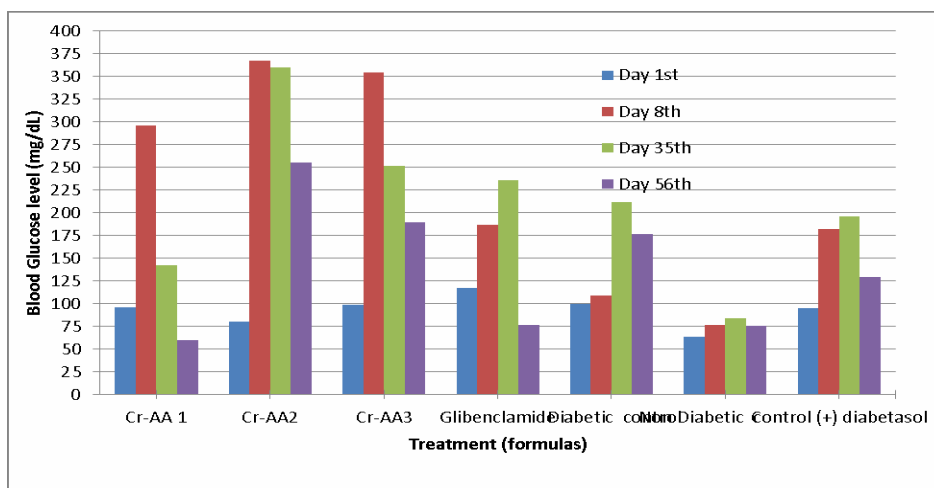


Figure 1. Profile of the blood glucose level (mg/dL) during the supplementation

Table 1. Experiment design of [Cr(III)]supplementation

Group	Treatment	Intake
1	Control (+)	Cr-Pic (Diabetasol [®])
2	Formula A(Cr-glu) doseI	50µg/day
3	Formula A(Cr-glu) doseII	150µg/day
4	Formula A(Cr-glu) doseIII	250µg/day
5	Glibenclamide	
6	Diabetic Control (-)	CMC-Na(0.2%)
7	NormalControl	CMC-Na(0.2%)

E. Data Collection and Analysis

Blood samples were taken and measured at D₀ (starting time, Week 0), D₈ (a week after induction, 1st week) and D₅₆ (9th week). The blood was collected from the eye vein. The result were expressed as mean±SD. Overall assessment of in vivo antihyperglycemic activity was stated as % glucose lowering (%GL):

$$\%GL = \frac{(glucose)_{before\ treatment} - (glucose)_{after\ treatment}}{(glucose)_{before\ treatment}} \times 100\% \quad (1)$$

III. RESULT AND DISCUSSION

Induction of diabetes was conducted by nicotinamide and streptozotocin. Generally, induction agents work as the destructive agent of beta cells of the Langerhans, so that cause the type 1 diabetes. Streptozotocin induced diabetes by free radical generation, which causes a massive reduces of insulin secreting beta cells of the islets of langerhans, resulting in decrease in endogenous insulin release. Damage and destruction of beta cells may occur via oxidative stress. Increasing of reactive oxigen species in beta cells may result in oxidative damage of DNA, means the DNA strand breaking [29]. In this case, nicotinamide prevent the occurrence of type 1 diabetes, by acting as a cytoprotectant against streptozotocin induced diabetic damage in

wistar rats brain [32]. Collaboration of two induction agent in this work give the most similar condition with tipe diabetic subject.

The materials applied in this work were synthesized in previous study [18]. The formula of supplements were A, Cr-glutamate ([Cr(µ-OH)(glu)(OH)₂]₂·6H₂O]). The applied dose are 50,150 and 250 µg Cr/ day respectively, marked by Cr-AA1, CrAA2 and CrAA3. Effects of these formulas to the blood glucose level lowering were shown in Figure 1.

Figure 1 showed the profile of the blood glucose level (mg/dL) during the supplementation. At the day 8th (7 days after induction), all treatment samples surpassed the value to be classified as diabetes with the blood glucose level minimum 126 mg/dl. The blood glucose levels was decreased after treatment in 35 days in all groups except the control groups. Further treatment until 9 weeks keep the glucose level in the normal level.

The hypoglycemic activity was calculated by equation (1) and stated as percent glucose lowering (%GL). The result of %GL were 30.564; 46.446; and 79.593% respectively. The diabetasol group also indicated the blood glucose lowering by 28.64%. There was no blood glucose level lowering in DM control group. It was lead to the conclusion that the formula showed significant lowering of the blood glucose levels.

IV. CONCLUSION

Study in 9 weeks of the CrAA complex, ([Cr(µ-OH)(glu)(OH)₂]₂·6H₂O]) on Nicotinamide-Sreptozotocin induced diabetic Wistar rats, give significant effect in lowering glucose level (p 0.05) compared to diabetic rats control group. The highest glucose lowering of this study was 79.53%. This is a high opportunity to use this compound as an hypoglycemic agent.

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