PROCEEDING

The 5th International Conference on Pharmacy and Advanced Pharmaceutical Sciences November 1 – 2, 2017 Yogyakarta, Indonesia

Pharmaceutical Scince & Clinical Pharmacy

Published by:
Faculty of Pharmacy Universitas Gadjah Mada
Sekip Utara, Yogyakarta, 55281,
Indonesia

ISSN : 2614-1779

First Edition, 2017

Printed in Yogyakarta , Indonesia

TOXICOLOGY OF CR(III)- GLUTAMIC ACID AS HYPOGLICEMIC NUTRACEUTICAL ON STREPTOZOTOCIN-NICOTINAMIDE INDUCED DIABETIC RATS

Kun Sri Budiasih, Kartika Ratna Pertiwi, Rizqie Auliana
Universitas Negeri Yogyakarta
kunsb@uny.ac.id

ABSTRACT

Cr-glutamic complex suplementation was conducted in pre-clinical study as the hypoglicemic nutraceutical on nicotinamide-streptozotocin induced diabetic Wistar rats. Three groups were examined on the effect of Cr-glu $[Cr(\mu\text{-}OH)(glu)(OH)_2]_2 \cdot 6H_2O]$ (glu= glutamic) at dose (50, 150 and 300 µg/day). The remain groups are control (+) with chromium picolinate (CrPic), control (-) diabetic group (DM) and non diabetic control (non DM) and control group by glibenclamide. The toxicity of the compound was examined by the liver and kidney function test on SGOT/SGPT, ureum and creatinine determination. The result showed that the SGOT/SGPT was increased until the 7^{th} day, but decrease after treatment in 14 days. There was no significant differences between the start and the end of the treatment ($p>0.05$).The result was also found on the kidney's function by BUN and creatinine measurement. Cr (III)- glutamic acid is potentially developed as nutraceutical product in the management of type-2 diabetes mellitus.
Keywords : Cr(III)-glutamic acid, hypoglicemic nutraceutical, toxicity,

INTRODUCTION

Supplement or nutraceutical is a part of the management of diabetes. Diet, exercise, oral hypoglycemic agents and insulin endogen are other kind of this . Nutraceuticals (often referred to functional foods) are natural bioactive or chemical compounds that have health promoting, disease preventing or medicinal properties[1]**.** This research is a part of application of inorganic nutraceutical, an inorganic compound or metal-containing medicinal product with special purpose as antiperglicemic agent.

Metal complex or organo-metallic compound as trivalent chroium has been used in medicine in the management of diabetes mellitus. Intake of chromium (III) complex showed considerable reduction in the glucose level [2]. Chromium works as a Glucose tolerance factor, by the interaction 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 [3][4]. Chromium increases insulin binding to cells, insulin

receptor number as well as activates insulin receptor kinase leading to increase sensitivity of insulin receptor. Studies in the activity of the Cr(III) based supplement are urgently needed to find the optimum condition in d the prevention and control of diabetes [5].

The amount of Cr(III) intake is about 200μgCr/ day. The well known chromium supplement is chromium picolinate, $Cr(pic)_3$. This compound has a side effect in DNA damage [6], through the catalytic formation of reactive oxygen species. 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.

*S*ome 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 (ochiaim 2008). GTF is an oligopeptide of molecular weight about 1400, and consists of some amino acids : cysteine, glycine, glutamate, and aspartate. It binds the molecule of Cr(III) by 1:4 ratio and acts in the hormonal action of insulin[7].

It is a new chance in developig Cr(III) - amino acid compound as antihiperglicemic supplements. Preparation of Cr-glutamate (Cr-Glu) was reported in previous studies. The structure of the complex is $[Cr(\mu\text{-}OH)(glu)(OH)_2]_2 \cdot 6H_2O$[8].

The in vivo experiment was studied on Streptozotocin (Stz) – nicotinamide induced diabetic albino rats. 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 [9-10]. The induction models were applied on some studies of natural product as antidiabetic agents[11-12]. Some previous study also reported the application of Cr(III) based drug and supplement on in vivo investigation[13-14].The histophatological studies on the effect of alloxan [15], and nicotinamide-stz induction were reported [16].

Materials And Methods

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) (E-Merck), Streptozotocin / Stz (Sigma Aldrich). The control were prepared Na CMC. The subjects of the study were 10 male Wistar albino rats (±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 induction of diabetes was done by intraperitonial injection of 120mg/kg nicotinamide then followed 15 minutes later by 60 mg/kg

streptozotocin [13]. 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.

The Supplement in this work were synthesized in previous study[8]. The formula is Cr-glutamate ($[Cr(\mu\text{-}OH)(glu)(OH)_2]_2 \cdot 6H_2O$]). The supplement was adiministered in 50,150 and 300 µg Cr/ day respectively, marked by Cr-AA1 (A), CrAA2 (B) and CrAA3 (C). The blood glucose level od diabetic subject is 126 mg/dL or more. Blood samples were taken and measured at H_0 (start), H_8 (a week after induction) and H28 (4^{th} week). The blood was collected from the eye vein.

RESULT AND DISSCUSSION

The rats were inducted intraperitonially by nicotinamide and streptozotocin. In this case, nicotinamide prevent the occurence of type 1 diabetes, by acting as a cytoprotectant against streptozotocin induced diabetic damage in wistar rats brain. Collaboration of two induction agent in this work give the most similar condition with type 2 diabetic subject.

The blood glucose level (in mg/dL) was measured during the supplementation. At the day 8^{th} (7 days after induction), all treatment samples classified as diabetes with the blood glucose level minimum 126 mg/dL. Generally, the blood glucose level was decreased after treatment in 35 days in all groups except the control groups. Treatment in 5 weeks showed that the glucose level were in the normal level[17].

The acute and sub chronical toxicity and the function of hepar

The diabetic rats were administered with oral suppementation of Cr-AA, by 300 µg/day. CMC-Na was given to the control group. The treatment of these subject were carried out in 5 weeks. The acute and sub chronical toxicity and the the function of the hepar were measured by the value of SGOT/SGPT, ureum, and creatinine.

The 1 st pre clinical test showed the potent of Cr(III)-glu as hypoglicemic agent to the stz- nicotinamide diabetic rats(17). The effect of supplementation showed that Cr-Glu give a better histophatological performance of proximal tubules compared to standard oral therapy with *glibenclamide*. The Cr-Glu groups also have the less degeneration and necrosis, although still have a small part of congestion and blooding. The dose of Cr-Glu 150 by µg/day was not give a significant effect in the regeneration of diabetic subject in this research. It is necessary to pay attention to the dose 200-300 µg/day, for futher research. It is also

important to to investigate the acyte toxicity of the highest dose, 300 µg/day. According to this result, the evaluation of the toxicity was carried out at the highest dose. 300 µg/day[18].

Acute toxicity was investigated in 1-14 days. This aimed to khow the general performance of he subject after administered by the supplement. The specific organ chosen in the evaluation of the toxicity is the hepar, based on the role of hepar as the center of the metabolism of human body. It changes the lipophilic materials to hydrophilic so that can excreted by the urine. The acute toxicity of the hepar is a response of the supplementation of Cr(III)-glu in this research was determined by the investigation of the value of SGOT- SGPT which is indicated the integration of the cells. The dose applied in the test is 300 µg/ day, based on the previuous experiment of the 1st pre-clinical test on the histopatology of the hepar.

The activity of Alanin Transaminase enzime / SGPT and Aspartat Transaminase (AST) enzme / SGOT increaser if there are change in the permeability of the cell wall. It indicated the disturbance in the cell integrity (hepatocellular). SGOT (Serum Glutamic Oxaloacetic Transaminase) is a normal enzime that exist in the hepar cell and other organ. SGOT was excreted when the hepar was disturbed. The level of the SGOT of the blood then related to the abnormality of the hepar cell.

The SGOT and SGPT were investigated as the response of injury of the hepatocite which excreted the *Aspartat Transaminase (AST)*/SGOT and SGPT/ *Alanin Transaminase* ALT. The effect of acute supplementation of Cr(III)-glu was expressed by the averafe value of the SGOT and SGPT of the blood at the start (before induction, H_0), after induction and the firts day of *supplementation* (H_8) and two weeks after (H_{14}) as showed in Table 1.

Table 2. The SGOT and SGPT value in acute supplementation of Cr-Glu (in U/l)*

Parameter	Start (H_0)	Induction (H_7)	Acute suppl (H_{14})	P**
SGOT/AST	162.42±2.87	276.2±0.0	164.3±0.0	0.142
SGPT/ALT	60.00±2.69	88.5±0.0	43.1±0.0	0.163

*average ± SD ***Anova single factor* ($p<0.05$)

The increasing of SGOT and SGPT indicated the damage in the cells of the hepar. The normal SGOT and SGPT value in wistar albino rat are 30,2-45,7 U/l and 2,1-23,8 U/l, respectivelly. As showed in Table 2, the average of SGOT and SGPT of the treatment group by Cr(III)-glu (300 µg/ day) was increased until the 7th day. Supplementation in 7 days later decreased the SGOT level to the value of the normal condition (starting time). The SGPT

decreased until lower level than the begining (H_0). Statisctical analysis by *Anova single factor* showed that there is no difference between the SGOT level between non induced subject and after supplementation. In the other word, the are a significant effect of the supplementation of Cr(III) in acute interval (14 days) ($p>0.05$).

Kidney is also a target organ in the investigation of the acute toxicity of Cr(III)-glu supplementation.The pre-clinical test of acute toxicity in kidney was determined in the *Blood Urea Nitrogen / BUN*) and creatinine. Ureum is is the residue of the protein metabolism which is toxic in the body. It must be excreted by the activity of the kidney in urine. The incresing of the ureum indicated the disturbance of the kidney's function. Creatinine is a product of the decomposition of the creatine, which is syntesyzed in the hepar as (*creatin phosphate, CP*), as an energy storege. In the usage of teh energy, the process produced creatinine, which is filtered by the glomerulus in the kidney and excreted as urine. The amount of ureum and creatinine were used in the assesment of the kidney function. The measurement of BUN-creatinine of the treatment subject in this research was listed in table 2.

Table 2 . Blood Urea Nirogen (BUN) and creatinine by acute Cr-Glu (mg/dl)*

Parameter	(H0)	(H8)	(H14)	P**
BUN	35.00±5.80	37.2±0.00	33.60±0.00	0.109
creatinine	0.20±0.05	0.29±0.00	0.34±0.00	0.604

*average ± SD ***Anova single factor* ($p<0.05$)

The normal value of BUN and creatinine of albino rats are 13.9 – 28.3 mg/dl and 0,30-1.00 mg/dl respectively. The result of BUN and creatinine in Table 2 showed the difference in trend. The highest BUN is in the 7^{th} day, but the highest creatinine is at 7th day. The BUN level then decreased until the lower value than the day before induction. Statistical analysis showed that there was no significant difference of both the BUN and creatinine between before and after diabetic induction. It means, the diabetic condition increased the BUN and creatinine level according to the disturbance of kidney activity, but after supplementation of Cr-III)-glu, they came back to the normal level. The increasing of BUN and creatinine of the diabetic subject in this research was not sufficient high. Overall, the kidney was normaly work during the supplementation of Cr-glu (300 µg/day).

Sub-chronical toxicity aimed to know the effect of the supplement to the subject. The interval is about 10% of the lifetime (about 1-3 month). The investigation of sub chronical toxicity was carried out during supplementantion of Cr-glu in 4 weeks (28 days). The result was showed in Fig 1.

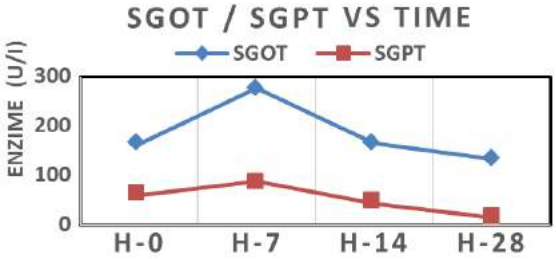

Fig 1. SGOT and SGPT in supplementation of Cr-glu supplementation by 300 µg/day in 28 days

Fig. 1 indicated that Cr –glu tends to decerase the SGOT and SGPT after increasing of both them according to the induction of diabetic by Stz-nicotinamide. Sub-chronikal toxicity in kidney was observed by determiantion of BUN) and creatinine until 28th day. The result showed in Fig. 2 and Fig. 3.

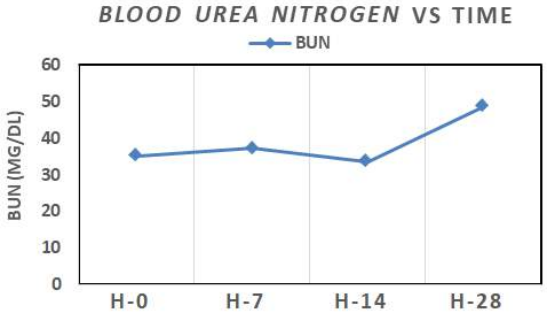

Fig 2. BUN value in supplementation of Cr-glu (300 µg/day) in 28 days

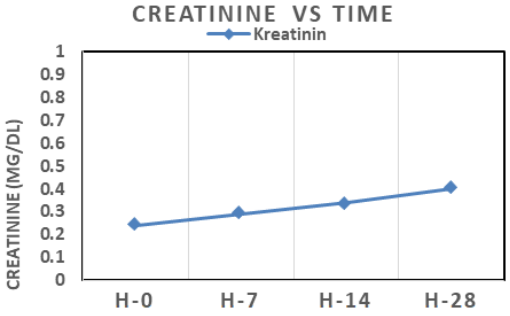

Fig 3. Creatinine value in supplementation of Cr-glu (300 µg/day) in 28 days

The increasing of BUN and creatinine in 28 days supplementation were also affected by the hypovolemic (dehidration) or the over protein

intake. It means that the increasing of the value was not the one and only indicator of the damage in the kidney. As found in the previous research on the histopatological performace if the kidney, the supplementation of Cr- glu (300 μg/day) was not affected to the hepatotoxic and neufrotoxic and the activity of the hepar dan kidney[18]. In the other word, the supplementation of Cr(III)-glu is safely recomended.

CONCLUSSION

Cr (glu) complex is potentially as *nutraceutical product to type 2* diabetes mellitus. Toxicity of this product has been evaluated to acute and sub chronical interval. The supplementation of Cr(III)-glu is safely recommended until 300 μg/day in 28 days. Chronical toxicity was needed to investigate the further effect in toxicity.

ACKNOWLEDGMENT :

The research was funded by The Ministry of Research and Higher Education, by "Hibah Bersaing" research scheme.

REFFERENCES

[1] Pandey, M., Vijayakumar., 2011, Nutraceutical Supplementation for Diabetes : a Review, *Int. J. Pharm. Pharmaceutical Sci.*, 3 (4), 33-40.

[2] Z Krejpcio, Essentiality of Chromium for Human Nutrition and Health, *Pol.J, Environ.* Studies, 2001, 10 (6) 399-404.

[3] JB Vincent, (ed), The Nutritional Biochemistry of Chromium(III), Elsevier,New York, 2007 : 139-151.

[4] RA Anderson., Chromium and the Prevention and Control of Diabetes, *Diabetes & Metabolism*, 2007,vol.26, p. 22-27.

[5] D Boghchi, SJ Stohs, BW Downs, Cytotoxicity and Oxidative Mechanism of Different Forms of Chromium *Toxicol*, 2002, No. 180 (1), 5-22.

[6] Tawkir, M., & Iqbal S.A., Synthesis, Characterization and Medical Efficacy of Cr(III) Complexes of Sulphonil ureas as Oral Antidiabetics, 2012, *Asian J, Pharm and Clin. Res*,5(4).

[7] E.Ochiai, *Bioinorganic Chemistry*, John Willey & Sons, New York. 2008, p.235.

[8] K.S. Budiasih, C.Anwar, S.J.Santosa, H.Ismail, 2013, Synthesis and Characterization of Chromium (III) Complexes with L-Glutamic Acid, Glycine and L-Cysteine, *Waset Journal*, 78, pp 1095-1909.

[9] EU Etuk, 2010, Animals Models for Studying Diabetes Mellitus, *Agric. Bio. J. of. N. Am.*, 2010:1 (2), 130-134.

[10] SS Ibrahim, SF Rizk, Nicotinamid: A cytoprotectant against streptozotocin-induced diabetic damage in wistar rat brains, *Afr.J. Biochem.Res*, 2008, 2 (8), pp.174-180.

[11] A Shirwaikar, K Rajendran, CD Kumar., R Bodla, Antidiabetic Actiivity of Aqueous Leaf Extract of Anona Squamosa in Streptozotocin-nicotinamide type 2 Diabetic Rats, *J. Of. Etnopharmacology* : 2004, 91 : 171-175.

[12] M Sharma, M. Siddique, M.W.., Shamim, A.M., Gyanesh, ., and K.K. Pillai, Evaluation of Antidiabetic and Antioxidant Effects of Seabuckthorn (Hippophaerhamnoides L.) in Streptozotocin-Nicotinamide Induced Diabetic Rats *, The Open Conference Proceedings Journal*, 2011, 2, 53-58.

[13] Selcuk MY.,, B Aygen, A Dogukan, Z Tuzcu, F Akdemir, J Komorowski, M Atalay, K Sahin, Chromium Picolinate and Chromium Histidinate Protects Against Renal Dysfinction by Modulation of NF-B pathway in high-fat diet fed and Streptozotocin-induced Diabetic Rats *, Nut. & Met.*, 2012, 9:30.

[14] Ragavan,B., Krishnakumari .S., Effect of T Arjuna Stem Bark Extract on Histopathology of Liver, Kidney And Pancreas Of Alloxan-Induced Diebetic Rats, 2006, *African J Biomed.Res*, 9 : 189-197.

[15] Kante, K., Reddy, CS., Anti Diabetic activity of Dolichos Lablab (seeds) in Sterptozotocin-nicotinamide induced diabetic rats, 2013, *Hygeia J.Diabetes.Med*., 5 (1), 32-40.

[16] Madhavi, M., Kiran.S., Ramesh.D., Evaluation of antidiabetic, Antihyperlipidemic and Antioxidant activities of Acacia leucophloea in Sterptozotocin-Nicotinamide induced type II Diabetic in rats, 2014, G*lobal J. Pharm.* 8 (1): 64-72.

[17] Budiasih ,KS, Pertiwi, KR.,Pre Clinical Study of Cr(III)-based Hypoglicemic Supplement in –Type 2 Diabetic Rats, ICB-Pharma International Conference, Universitas Muhammadiyah Surakarta, 31 Oktober 2015.

[18] Budiasih, KS. Pertiwi, KR., *A Study on the Histopathology of Cr(III)-glutamic acids Supplementation on Streptozotocin-Nicotinamide induced Diabetic Wistar Rats, 6 Th Euchem Chemistry Congress, Seville, Spain , 13-15 September 2016.*