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ACUTE AND SUBACUTE TOXIC STUDY ON MICE OF GLIPIZIDE SYNTHESIZED IN VIET NAM

Thi Thoi Bui[1, *], Dai Quang Ngo[2], Van Loc Tran[3], Van Chien Tran[3], Thi Nga Nguyen[4], Thi Thao Do[4,5]

[1]*Vietnam Institute of Industrial Chemistry, 22 Pham Ngu Lao, Hoan Kiem, Ha Noi*

[2]*Viet Nam Nationnal Chemical Group,1A Trang Tien, Hoan Kiêm, Ha Noi*

[3]*Institute of Chemistry, VAST, 18 Hoang Quoc Viet, Cau Giay, Ha Noi*

[4]*Institute of Biotechnology, VAST, 18 Hoang Quoc Viet, Cau Giay, Ha Noi*

[5]*Graduate University of Sciences and Technology, VAST, 18 Hoang Quoc Viet, Ha Noi*

[*]Email: *thoibt@gmail.com*

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Abstract. Glipizide is a second generation of sulfonylurea with promising hypoglycemic activity. It acts by stimulating the release of insulin from β-cells of pancreas. Glipizide is absorbed rapidly, uniformly with good mean oral bioavailability. It offers several advantages such as swift and short action, high potency and also does not accumulate in plasma on repeated oral administration. In this paper we report the acute and subacute toxicity of glipizide on BALB/c albino mice. The results showed the safety of our synthesized product.

Keywords: acute, glipizide, hypoglycemia, subacute, sulfonylurea, toxicity.

Classification numbers: 2.3.1; 2.7.1; 2.10.2.

1. INTRODUCTION

Glipizide (Fig. 1) is a second generation of sulfonylurea which lowers the blood glucose levels in patient suffering from non-insulin dependent diabetes mellitus (NIDDM). It acts by stimulating insulin secretion of the pancreatic islets and several other extra pancreatic effects, such as enhancing sensitivity to insulin, decreasing the hepatic glucose production [1-2]. Glipizide is completely absorbed from the gastrointestinal tract and metabolized into five different metabolites in the liver, and has a half-life between 2.5 and 4.7 hours [3]. Glipizide is one of the most effective insulin secretogogue both in the primary phase of insulin secretion and in sustained stimulatory response during long term administration [4]. Consequently, it is one of the most commonly prescribed drugs for treatment of type 2 diabetes mellitus [5]. Its main features are swift and short action with a very high selectivity [6, 7]. It is also regarded as 100 times more effective than tolbutamide in evoking pancreatic secretion of insulin [8].

The acute and subchronic toxicity of glipizide were evaluated. The acute oral toxicity was extremely low in all species tested (LD_{50} greater than 4 g/kg) [9, 10]. Subchronic toxicity was evaluated in rats at oral doses up to 8 mg/kg/day for six months. Findings revealed no drug-related toxicity [10]. It was recommended that glipizide is in the dose range of 2.5 - 20 mg daily. The highest therapeutic dose of glipizide is 40 mg daily. Gradual dosage adjustment usually is required for patients [11].

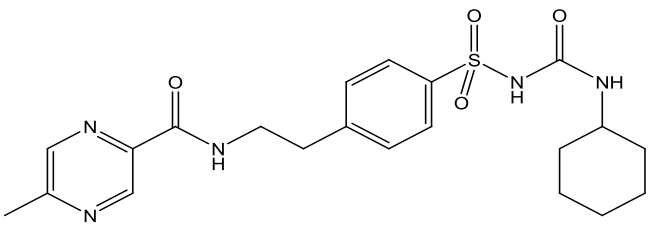

Figure 1. Structure of glipizide.

Glipizide has been synthesized in our laboratory in Vietnam Institute of Industrial Chemistry. Its synthetic procedure had been previously published [12]. The synthesis of glipizide included three steps starting from commercially available 5-methylpyrazine-2-cacboxylic acid, with overall yield of 56 %. The structures of glipizide were determined by IR, MS and NMR spectroscopic methods and its qualities met the USP-40 (The United States Phamarcopeia). In this continuous study, we report the results of acute and subacute toxicity of glipizide, which was produced in our laboratory, on BALB/c albino mice.

2. MATERRIAL AND METHODS

2.1. Sample preparation

Glipizide was obtained from the laboratory of Vietnam Institute of Industrial Chemistry according to method as published in the paper [12]. Purity: 98.5 %, mp: 205 ^{o}C, insoluble in water, partly soluble in $CHCl_3$ and EtOH. White powder product was packed in sealed PE bags.

2.2. Animal

Healthy BALB/c mice in range of 19-22 gram were used in acute and subacute toxicity experiment. Mice were grown in light and temperature standard condition at Institute of Biotechnology. They were obtained food and water *ad bilitum.*

2.3 Experiments

2.3.1. Acute toxicity

Mice were weighed and divided to 5 groups (10 mice/group) and fasted overnight before treatment. Group 1 served as physiological control in which mice were administered 0.3 ml distilled water. Group 2, 3, 4 and 5 were treated with glipizide at a single oral dose of 2000, 3000, 4000 and 5000 mg/kg b.w, respectively. All the animals were objected for the clinical signs and weighed for the first 2 hours, 1 day, 4 day and 7 day after treatment. The mortality

was also recorded for 3 days. The LD_{50} (Lethal dose at 50 percent) of sample was calculated by using Karber Behrens formular as following [13, 14]:

$$LD_{50} = LD_{100} - \Sigma a \times b/N$$

in which: LD_{50} is the amount of the sample required to kill 50 % of the tested animal population. LD_{100} is the lowest dose of sample inducing 100 % of animal death, **N** is the number of animals in each group; **a** is the dose different while **b** is the mean of mortality.

2.3.2. Subacute toxicity

Based on the result of acute toxicity test, 24 mice were divided into 4 groups. Mice in group 1 were administered only distilled water and served as control group. The experimented groups 2, 3, 4 were treated orally glipizide at doses of 125, 250 and 500 mg/kg b.w, respectively, for 4 weeks at specific time daily. All experiment animals were observed daily for the mortality and any clinical signs and symptoms, the behavior alteration, food and water intake. In addition, the body weight of tested mice was also recorded once a week.

At the end of experiment, the blood samples were collected from orbital sinus for haematological and biochemical parameters. The haematological indexes were studied including red blood cell count, white blood cell count, platelet count, hemoglobin, mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration by using a hematology analyzer. For biochemical analysis, the serum was separated from the blood sample and alanine aminotransferase (ALT), aspartate aminotransferase (AST), and creatinine were estimated by using a semi-automated Biochemical Analyzer.

After blood collection, mice were sacrificed and the organs (liver, kidney and spleen) were dissected, washed in saline solution and weighed.

2.3.3. Data analysis

The data were expressed as Mean ± SE. The results were analyzed statistically by Student t' Test, one-way Analysis of Variance (ANOVA). The $P < 0.05$ was considered to be statistically significant in comparison with control.

3. RESULTS AND DISCUSSIONS

3.1. Acute cytotoxic

There was no toxicity and mortality observation in mice treated with glipizide. None of mice showed the toxic clinical signs in behavior, skin, fur, eyes or diarrhea. The sign of tremors, convulsion, lethargy, sleep and coma did not reported in testing mice. During 7 days of observation, the food consumption and water intake at all dose treatment were also similar to control group.

Beside clinical observation, the body weight of mice was also determined. As showed in Table 1, the body weight of mice was totally similar between control group and test groups. After glipizide administration, the body weight gradually increased in various time points. However, the body weight of all tested groups was not significantly different from control group ($P > 0.05$). From these results, glipizide showed the LD_{50} to be much higher than 5000 mg/kg b.w.

According to the World Health Organization and Organization for Economic Cooperation and Development – OECD [15, 16], of which object possesses LD_{50} greater than 5000 mg/kg b.w. could be assigned as Class 5 and considered as non toxic or at the lowest toxic potent.

Table 1. The effect of glipizide on body weight of mice in acute toxicity test.

Groups		Mean of body weight (gr)			
		Day 0	***Day 1st***	***Day 4th***	***Day 7th***
Control group	Mean ±SE	20.58 ± 0.44	20.77 ± 0.41	21.27 ± 0.40	21.83 ± 0.34
	P value	-	-	-	-
Group 2	Mean ± SE	20.83 ± 0.54	21.00 ± 0.57	21.47 ± 0.58	22.05 ± 0.56
	P value	> 0.05	> 0.05	> 0.05	> 0.05
Group 3	Mean ± SE	20.42 ± 0.30	20.47 ± 0.38	20.78 ± 0.48	21.86 ± 0.30
	P value	> 0.05	> 0.05	> 0.05	> 0.05
Group 4	Mean ± SE	20.42 ± 0.24	20.52 ± 0.26	20.82 ± 0.33	21.85 ± 0.61
	P value	> 0.05	> 0.05	> 0.05	> 0.05
Group 5	Mean ± SE	20.47 ± 0.31	20.10 ± 0.32	20.73 ± 0.34	21.90 ± 0.33
	P value	> 0.05	> 0.05	> 0.05	> 0.05

(LD_{50} > 4000 mg/kg) (up to 8 mg/kg/day for six months).

3.2. Subacute toxicity test

3.2.1. Observation

Table 2. The change of body weight of glipizide treated mice in sub-acute toxicity test.

Groups		Mean of body weight (gr)				
		Day 0	***Day 7th***	***Day 14th***	***Day 21st***	***Day 28th***
Control group	Mean ± SEM	21.20 ± 0.52	22.87 ± 0.22	26.23 ± 0.81	27.49 ± 0.77	28.33 ± 0.81
	P value	-	-	-	-	-
Group 2	Mean ± SEM	21.2 ± 1.12	22.23 ± 1.36	24.87 ± 1.67	26.9 ± 2.15	28.1 ± 1.16
	P value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Group 3	Mean ± SEM	21.3 ± 0.46	22.32 ± 0.52	24.25 ± 0.44	26.2 ± 0.44	27.6 ± 0.57
	P value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05
Group 4	Mean ± SEM	21.8 ± 0.55	22.30 ± 0.83	24.28 ± 0.70	26.1 ± 0.80	27.1 ± 0.91
	P value	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05

In order to determine subacute toxic effects of glipizide, mice were daily treated with different doses of glipizide based on the result of acute toxicity test. During the 28 – day period of glipizide administration, there were no signs of abnormal change in skin, fur paten, eyes and behavior of glipizide treated mice. All of animals also were alive in experiment period of time.

The body weights of mice were recorded on initial day, 7th, 14th, 21st and 28th day. Glipizide did not show strong effects on the growth of mice (Table 2). The body weight of glipizide treated mice continuously increased in the experiment duration. Although the body weight of mice treated with high dose (500 mg/kg) and medium dose (250 mg/kg) of glipizide was slightly lower than that of mice in control group by end of the experiment, there was no significantly different ($P > 0.05$).

3.2.2. Hematological and biological parameters

Table 3. The affect of glipizide on hemalogical and biological parameter.

Parameter		Control	Group 2	Group 3	Group 4
WBC	Mean ± SE	8.23 ± 0.13	8.34± 0.21	8.36±0.14	8.34±0.14
($\times 10^9$/L)	*P* value	-	> 0.05	> 0.05	> 0.05
RBC	Mean ± SE	8.68 ± 0.21	8.72±0.45	8.91±0.18	8.87±0.20
($\times 10^{12}$/L)	*P* value	-	> 0.05	> 0.05	> 0.05
Hemoglobin	Mean ± SE	132.83±2.77	133.23 ± 2.08	133.17 ± 2.54	134.33 ± 1.69
(g/L)	*P* value	-	> 0.05	> 0.05	> 0.05
HCT (fl)	Mean ± SE	0.44 ± 0.01	0.44 ± 0.02	0.43 ± 0.01	0.44 ± 0.01
	P value	-	> 0.05	> 0.05	> 0.05
MCV (g/L)	Mean ± SE	49.27 ± 0.76	49.25 ± 0.41	49.20 ± 0.62	49.08 ± 0.82
	P value	-	> 0.05	> 0.05	> 0.05
MCH (pg)	Mean ± SE	14.95 ± 0.27	14.82 ± 0.32	14.97 ± 0.14	14.77 ± 0.28
	P value	-	> 0.05	> 0.05	> 0.05
MCHC (g/L)	Mean ± SEM	292.17 ± 5.38	296.17 ± 3.18	300.17 ± 4.47	300.50 ± 3.68
	P value	-	> 0.05	> 0.05	> 0.05
PLT ($\times 10^9$/L)	Mean ± SE	804.00 ± 10.95	791.58 ± 12.26	782.33 ± 13.58	789.33 ± 10.47
	P value	-	> 0.05	> 0.05	> 0.05
AST UI/L)	Mean ± SE	88.93 ± 2.15	92.69 ± 1.32	94.37 ± 1.30	98.78* ± 1.69
	P value	-	> 0.05	> 0.05	**< 0.05**
ALT (UI/L)	Mean ± SE	42.40 ± 1.05	42.33 ± 0.64	42.10 ± 0.73	41.83 ± 0.87
	P value	-	> 0.05	> 0.05	> 0.05
Creatinin	Mean ± SE	26.83 ± 0.63	27.06 ± 0.47	27.95 ± 0.53	30.12 ± 0.61
(UI/L)	*P* value	-	> 0.05	> 0.05	> 0.05

*Note: *P<0.05 in comparision with that of control group.*

The effect of glipizide on haematological index was presented in Table 3. The indices including red blood cells, total white blood cells, platelet counts, hemoglobin, hematocrit, the mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular hemoglobin (MCH) in blood of all tested mice were similar to those of control group.

Liver and kidney are toxic cleavage organs in the body. Therefore, the effect of glipizide on liver and kidney function was accessed by determining alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatin phosphokinase (CPK) levels in serum. The results from Table 3 showed that glipizide had no effect on level of CPK, a kidney function enzyme or ALT, a biomarker of hepatic damage. However, the AST level was slightly increased at 500 mg/kg b.w glipizide treated mice ($P < 0.05$). The AST level increases in blood when the liver

was affected and damaged in some manner. This enzyme could be used for the safety evaluation of developmental compounds in the pharmaceutical industry [17]. The higher AST level in 500 mg/kg b.w. glipizide treated mice indicated that high dose of glipizide for long time may affect the liver function.

3.2.3. The change of organs' weight

The assessment of organs' weight in toxicity test is an essential process in evaluating pharmaceuticals. The toxicity of drug may relate to the change of organs' weight. The changes are often accompanied by corresponding histopathological findings [18].

Liver and kidney are the major organs which have detoxification and excretion functions. Spleen also plays an important role in the immunological activities. As shown in the Table 4, glipizide did not effect on the relative organ weights of liver, spleen or kidney at different doses when compared with control ($P > 0.05$). On the other hand, neither lesion nor necrosis was observed in liver, spleen or kidney. There was also no water retention in kidney. Therefore, the samples were not collected for histopathological assessment.

Table 4. Relative organ weights of mice treated glipizide (gram/10 gram b.w).

Groups		***Relative organs weight (gram/10 gram b.w)***		
		liver	**kidney**	**spleen**
Control	Mean	0.385 ± 0.018	0.095 ± 0.015	0.034 ± 0.014
	P value	-	-	-
Group 2	Mean	0.384 ± 0.022	0.095 ± 0.021	0.035 ± 0.012
	P value	> 0.05	> 0.05	> 0.05
Group 3	Mean ±	0.383 ± 0.021	0.094 ± 0.019	0.035 ± 0.022
	P value	> 0.05	> 0.05	> 0.05
Group 4	Mean ±	0.387 ± 0.016	0.096 ± 0.024	0.035 ± 0.017
	P value	> 0.05	> 0.05	> 0.05

4. CONCLUSIONS

All obtained results from our study report that the synthesized glipizide at all different tested dosages is safe with no signs of acute toxicity. The LD_{50} was much greater than the highest usage dose which was 5000 mg/kg b.w. since no death of animal was recorded at this dose. Also, in the subacute toxic test, the glipizide treated mice showed no change in body weights, in haematological and biochemical parameters as well as in relative organ weights. However, the AST level in the 500 mg/kg b.w. glipizide treated group exhibited higher number meaning of liver partially damaged.

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REFERENCES

1. Thombre A. G., Denoto A. R., Gibbes D. C. - Delivery of glipizide from asymmetric membrane capsules using encapsulated excepients, J. Control release **60** (1999) 333-341.
2. Rendell M. - The role of sulphonylureas in the management of type 2 diabetes mellitus. Drugs **64** (2004) 1339-1358.
3. Balkate R., Baokar S., Joshi H., and Patil R. - The complete review on analytical and formulation techniques of glipizide, IJPSR **9** (2018) 1000-1008.
4. Verma R. K., Garg S. - Development and evaluation of osmotically controlled oral delivery system of glipizide, Eur J. Pharm Biopharm **57** (2004) 513-525.
5. Ammar H. O., Salama H. A., Ghorab M., El-Nahhas S. A., and Elmotasem H. – A transdermal delivery system for glipizide. Curr. Drug Deliv **3** (2006): 333.
6. Colin Dollery, Therapeutic Drugs, 2nd Ed., Churchill Livingstone **1** (1999) p. G56.
7. Prabhakara P., Nayari M. H., Gulzar A. M., Yadav B., and Narayana Charyulu R. - Formulation and In Vitro evaluation of Gastric Oral floating Tablets of GlipizideIndian. Indian J. Pharm. Edu. Res **42** (2) (2008)174.
8. Semalty M., Semalty A., and Kumar G. - Formulation and characterization of mucoadhesive buccal films of glipizide, Indian J. Pharm Sci. **70** (2008) 43.
9. http://www.pfizer.com/files/products/material_safety_data/288.pdf , 2007-Jan-2.
10. https://pubchem.ncbi.nlm.nih.gov/compound/glipizide#section=FDA-Orange-Book-Patents. 2019-Jan-19.
11. Lebovitz H. E. - Glipizide: a second-generation sulfonylurea hypoglycemic agent. Pharmacology, pharmacokinetics and clinical use. Pharmacother **5** (1985) 63.
12. Thi Thoi Bui, Dai Quang Ngo, Van Loc Tran, Van Chien Tran, Thi Phuong Thao Tran - Synthesis of glipizide for the treatment of type 2 diabetes, Vietnam Journal of Chemistry **54** (6e2) (2016) 233-236.
13. Yeo D., Rita Bouagnon, Bernard Nazaire Djyh, Chonta Tuo and Jean David N'guessan - Acute and subacute toxic study of aqueous leaf extract of combretum molle. Tropical Journal of Pharmaceutical Research April **11** (2) (2012) 217-223.
14. Akhila J. S., Deepa S., Alwar M. C. - Acute toxicity studies and determination of median lethal dose. Curr Sci **93** (2007) 917-920.
15. Organization for Economic Co-operation and Development (OECD). Guidance Document on Acute Oral Toxicity Testing 420; Organization for Economic Co-operation and Development: Paris, France, (2008).
16. http://www.ilo.org/legacy/english/protection/safework/ghs/ghsfinal/ghsc05.pdf. 2001-July - 5
17. Ozer J., Ratner M., Shaw M., Bailey W., Schomaker S. - The current state of serum biomarkers of hepatotoxicity. Toxicology **245** (3) (2008) 194-205.
18. Bello I., Bakkouri A. S., Tabana Y. M., Al-Hindi B., Al-Mansoub M. A., Mahmud R., Asmawi M. Z. - Acute and sub-acute toxicity evaluation of the methanolic extract of Alstonia scholaris stem bark. Medical Sciences **4** (1) (2016) 4.