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by Fairuz, Nurul Hidayah

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DEVELOPMENT OF INDUCED DIABETIC MODEL USING ZEBRAFISH

Fairuz[a] , Nurul Hidayah[b], Alfi Khatib[b]

[a]Departement of Anatomical Pathology Faculty of Medicine and Health Scince Jambi, Indonesia
Email : Fairuz.quzwain@gmail.com
[b]Department of Pharmaceutical Chemistry, Kulliyyah of Pharmacy, International Islamic University Malaysia, Kuantan 25200, Pahang, Malaysia

***Abstract*—** Diabetes mellitus (DM) is a crucial metabolic disorder that had been increased over the years. The vast majority of diabetic population is suffered from type 2 diabetes mellitus (T2DM), followed by type 1 diabetes mellitus (T1DM). Although many anti-diabetic agents had been established, the incidence of diabetic complication is still rising. Then, further improvement on diabetic therapies is needed to be developed by using various animal model. In recent years, zebrafish is a newly found disease model organism in diabetes research as they have similar glucose metabolism pathways with mammals. Then, in the current study, we aimed to establish a protocol for inducing type 2 diabetes on zebrafish by using alternating immersion sugar solution method. Younger zebrafish (age 4-7 months) were divided into two group: glucose treated group and sucrose treated group with their respective control group. The fish are placed in the sugar solution for 24 hours, followed by free-sugar interval for the next 24 hours. The protocol had been modified by using stepwise increasing concentration of sugar solution for every 2 weeks. The experiment is continued for up to 8 weeks (2 months). The fasting blood glucose level and mortality rate of each treated group and control group are being determined at the end of experiment. The glucose treated group produces an increasing of fasting blood glucose level compared to control group. However, for sucrose treated group, the fasting blood glucose level is quite lower than control group which resulting in no diabetic effect on the fish. Overall, the mortality rate for both glucose and sucrose treated group are higher compared to their respective control group. In summary, the results do not show significant difference between treated group and control group. The method used need to be improvised according to the suitability condition of the zebrafish. As comparison with other research study that used similar protocol, they succeed to obtain desired outcome of hyperglycemic effect of zebrafish model.

Keywords—Diabetic; Model; Zebrafish

I. INTRODUCTION

Based on AstroAwani news on 8th April 2017, it is reported that the number of diabetic patients exceeds ministry's anticipation. However, the total anticipated had occurred five years earlier and in 2017, about 3.492 million people in this country were diagnosed with diabetes. The prevalence of diabetes in adults is 16.9% over the 20.722 million of the total adult population in Malaysia. Then, International Diabetes Federation (IDF) supported that among 425 million people had diabetes in the world, 159 million people are in the Western Pacific (WP) region including Malaysia. This value will rise to 183 million by 2045. Chronic blood glucose elevation is the major parameter of biochemical diagnosis that can be seen in the two major forms of diabetes: Type 1 diabetes or insulin dependent and Type 2 diabetes or non-insulin dependent. Type 1 diabetes mellitus (T1DM) is a disease accompanied by the degradation of pancreatic β-cells with the occurrence of autoimmune disease, causing an absolute insulin deficiency [1]. The T1DM commonly begins at young age and only minority of diabetic population suffers from T1DM. However, for type 2 diabetes mellitus (T2DM) [2]. This condition is known as body's resistance to insulin. T2DM is the most common form of diabetes that affects people of much higher age [3]. Even though there are a lot of treatments exist for diabetic people, the risk of incidence of complications such as diabetic retinopathy, nephropathy and neuropathy is still higher [4]. Therefore, further improvement of the efficiency of diabetic therapies and identification of disease mechanisms need to be established by using various animal model [5]. Then, the most common animal models used for these purposes are rats and mice because they have several important pathophysiological mechanisms in their body. Unfortunately, these models are not reflected perfectly the metabolic background in diabetic people, high labor cost and limit a small groups of animal used due to ethical issues [6]. Hence, in recent

years, a new animal model using zebrafish (Fig.1) has been developed for the study of diabetes and its complication because zebra fish have special features that very attractive research tool. Zebrafish is a good animal model to study diabetes and its related diseases because of the high similarities in organ physiology and metabolism between zebrafish and mammals. Zebrafish share a significant amount of similar genetic identity and organ system with mammals [6,7]. Besides, the body of zebrafish is transparent which can facilitate a non-intrusive visualization of organs and biological processes in vivo. Additionally, this model can reduce both time and cost while carrying the investigation because of the small size of zebrafish leading to relatively inexpensive to maintain, large numbers of offspring produce and rapid development. Thus, in this study, we used zebrafish as a model to induce the type 2 diabetes mellitus by using non-invasive technique. The alternating immersion of zebrafish in an external sugar solution for up to 2 months is used to maintain their hyperglycemia condition.

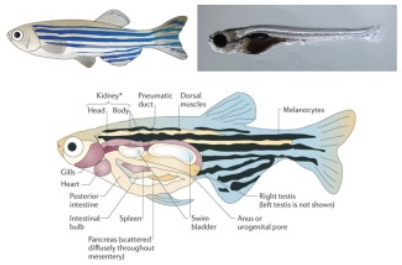


Fig.1 The wild type of Zebrafish (Daniorerio)

II. MATERIAL AND METHODE

Materials are obtained from the following sources: D(+)-Glucose from Merck KGaA, Germany and caster sugar from Central Sugar Refinery SDN. BHD., Selangor, Malaysia; reverse osmosis (RO) water from laboratory of Kuliyyah of Pharmacy, International Islamic University Malaysia (IIUM); needle for blood sampling from BD Ultra-Fine II insulin syringe, Becton, Dickinson and Company, USA; blood glucose meter and blood glucose test strip from EasyTouch®, Bioptik Technology, Inc., Taiwan. The type of zebrafish (Daniorerio) used in this experiment are wild-type of both sexes. The fish used are randomly chosen younger fish approximately age within four to seven months. The fish are maintained in the laboratory of Kuliyyah of Pharmacy IIUM in rack system under constant environmental conditions. Fish are fed two times daily with Lovely Guppy flakes from Daya Aquatics SDN. BHD. The animals are submitted to hypothermia by exposure to ice water at the end of experiment. To determine the effect and maintenance of hyperglycemia on zebrafish, the fish were immersed in sugar solution for longer time period. There were two groups of zebrafish which were exposed to glucose solution and sucrose solution with their respective control. 15 zebrafish were used as glucose-treated group and 8 zebrafish were used for its control group. Meanwhile, for sucrose-treated group, the zebrafish used were 10 fishes for both sucrose group and its control group. The experiment used alternating immersion protocol for 8 weeks (2 months). The treated groups of zebrafish were placed in 3-L aquarium containing glucose solution or sucrose solution, which were diluted in reverse osmosis water for 24 hours. Then, the protocol was followed by free-sugar interval by exposing to control solution for the next 24 hours before repeating the previous step. For control group, the fish were maintained in 3-L aquarium containing reverse osmosis water for the same period and conditions as the treated groups. During the experiment, fish were maintained at 27.0°C and a pH ≈7. The fish were fed every day (i.e before transfer into sugar solution) with Lovely Guppy flakes.

As a modification of the alternating immersion protocol, the fish were exposed to a stepwise increasing sugar exposure protocol. There was difference in the initial sugar percentage of both glucose and sucrose treated group. For glucose treated group, fish were initially exposed to an alternating 1% external glucose solution for 2 weeks. After this time, the glucose concentration was increased to 2% for the next 2 weeks (until experimental week 4). Then, the next 2 weeks, glucose concentration increased to 2.5% until experimental week 6. The last glucose concentration used for an additional 2 weeks (until experimental week 8) was 3% external glucose. Next, for sucrose treated group, fish were initially exposed to an alternating 0.5% external sucrose solution for 2 weeks. After this time, the sucrose concentration was increased to 1% for the next 2 weeks (until experimental week 4). Then, the sucrose concentration increased to 2% for the next 2 weeks until experimental week 6. Lastly, 3% external sucrose was used for the next last 2 weeks of experiment until week 8. All treatments involved alternating immersion in the external glucose solution for 24 hours and then in control solution for 24 hours. Blood sampling of fish were collected at the end of experiment (last day of week 8). The fish were transferred to other 3-L aquariums containing control solution with river stone at the bottom for more than 24 hours before taking blood sample. The fish were

anesthetized in ice water gradually from 15°C to 5°C for approximately 2-3 minutes. Zebrafish were considered to be anesthetized when all movement in the water had stopped. The fish were removed from water, patted dry with Kimwipe tissue. The fish were injected using needle as described above behind the gills, where the heart is located for taking about 10µL of blood sample. The blood was collected on the glucose test strip and measured fasting blood sugar using EasyTouch® blood glucose meter, as described above.

III. RESULT AND DISCUSSION

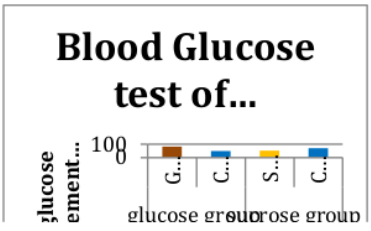
3.1 Blood glucose reading

Table 1. The fasting blood glucose level of glucose treated group and control group

	Glucose treated group	Control group
Glucose reading (mg/dL)	89	26
	68	*212*
	98	36
	87	78
	80	57
	88	-
	69	-
	79	-
Average reading (mg/dL)	**82.25**	**49.25**

Table 2. The fasting blood glucose level of sucrose treated group and control group

	Sucrose treated group	Control group
Glucose reading (mg/dL)	30	76
	56	*150*
	65	42
	75	102
	35	92
	-	38
Average reading (mg/dL)	**52.20**	**70.00**



Graph 1. The average fasting blood glucose level of both glucose and sucrose group

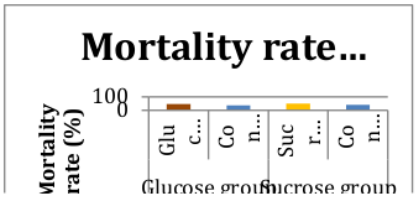
3.2 Mortality

Table 3. The mortality rate of glucose treated group and control group

	Glucose treated group	Control group
Number of fish (N)	15	8
Fish alive	8	5
Fish died	7	3
Mortality rate (%)	**46.67**	**37.50**

Table 4. The mortality rate of sucrose treated group and control group

	Sucrose treated group	Control group
Number of fish (N)	10	10
Fish alive	5	6
Fish died	5	4
Mortality rate (%)	**50.00**	**40.00**



Graph 2. The mortality rate of both glucose and sucrose group

In the present study, the development of type 2 diabetes model induced on zebrafish is exposed to an alternating external glucose and sucrose solution for up to 2 months by stepwise increasing the concentration for every 2 weeks. Then, zebrafish are anesthetized by using ice water instead of tricaine solution because hypothermia state yield more consistent glucose reading than tricaine solution. Besides, tricaine solution might increase blood glucose in zebrafish as it is known as ion channel blocker agent that may interfere the β-cells function [8]. For blood sampling, cardiac puncture on live fish is used instead of decapitation because repeated measurement of same individual fish can be done. However, inserting needle into heart chamber needs precise anatomical knowledge and significant surgical skills [8]. Otherwise, the blood sampling failed.

For the result analysis, generally, the fasting blood glucose level for treated group are not significantly different with the control group. The average of fasting blood glucose level for glucose treated group is 82.25mg/dL meanwhile for the control group is 49.25mg/dL. The glucose treated group produces hyperglycemic effect to the fish by approximately 50% elevation compared to control group. However,

for the sucrose treated group, the fasting blood glucose reading is contradicted with the glucose treated group. The average of fasting blood glucose level for sucrose treated group is 52.20mg/dL meanwhile for the control group is 70.00mg/dL. The blood glucose level of sucrose treated group is much lower than control group by approximately 20mg/dL reduction. This proves that sucrose treated group did not produce hyperglycemic effect to the zebrafish. Besides, the initial concentration of sucrose solution is much lower than the initial concentration of glucose solution. Thus, this might produce a delay effect of hyperglycemia to the sucrose treated group compared to glucose treated group.

In terms of mortality rate, all treated group and control group have high mortality rate for about 45% risk of death. There is no significant difference of mortality rate between treated group and control group for both glucose and sucrose exposure. The mortality rate of glucose treated group is 46.67% meanwhile the control group has mortality rate of 37.50%. Next, the mortality rate of sucrose treated group is 50% meanwhile the control group is 40%. This shows that glucose exposure and sucrose exposure produce low survival rate of zebrafishcompared to control group throughout the 8 weeks of experiment. The low survival rate of zebrafish in treated solution might be due to the reduction of oxygenation and osmotic stress of the solution [9]. However, when comparing between the treated group of sucrose and glucose, the percentage value is not reliable due to the different number of fish used at the beginning. However, when observing throughout the experiment, glucose treated group causes high mortality rate compared to sucrose treated group especially during the first few days of changing solution concentration for every week. This might be due to the glucose molecule is much smaller compared to sucrose molecule, causing rapid absorption of glucose molecule in the body. Thus, this may lead to the accumulation of glucose inside body that can cause toxicity to the fish.

Nevertheless, in comparison with other research study of the similar sugar immersion protocol, there are four to five times increment in blood glucose level for all different concentration of glucose solution than control group. Even though the glucose had been withdrawn for 7 days, the blood glucose level is still higher about 2 times than the control group [9]. Furthermore, another study of Capiotti et al. (2016), the immersion of adult zebrafish in 111mM glucose solution showed 150% increase of blood glucose level in relation to the control group [10]. In addition, Shin, Hong and Kang (2012) reported that the exposure of zebrafish in 3% water glucose solution increased significantly to 198mg/dL even in acute period of 30 minutes. They also proved the elevation of blood glucose level of zebrafish is in dose-dependent and time-dependent manner [11].

Besides the immersion glucose protocol, there is also other method to induce the type 2 diabetes on the zebrafish by using overfeeding method. The fish were constantly fed with high fat diet for about 3 months. One of those study reported that the body weight high diet group increased significantly with the significant elevation of fasting blood glucose level compared to normal diet group [6]. Another study also supported with the similar outcome even though overfeeding for up to 2 months [12,13]. The increase of blood glucose level associated with obesity and overfeeding is due to the defective downstream signaling of insulin and eventually causing impaired glucose tolerance [13,6]. Therefore, the use of zebrafish as animal model to study the diabetes and its complication have been extensively established in this recent years. Besides, the zebrafish model can be considered more reliable in producing hyperglycemic effect compared to other animal model. This is because the system regulating glucose homeostasis in zebrafish are similar to those in humans in terms of its morphology, development and function [14]. For example, the pathway of insulin secretion in pancreas and insulin resistance in liver of both zebrafish and mammals are quite similar and had been illustrated in figure 4 and 5. Besides, similar to humans, the homeostasis of glucose in zebrafishalso involves brain, skeletal muscle, liver and pancreatic endocrine cells due to the presence of glucose transporter to respective organs above. To be more specific on insulin regulating organ, the pancreas of zebrafish is consists of exocrine and endocrine compartments connected by a ductal system to the digestive tract, as equal to humans (Fig. 2). The pancreatic islets of zebrafish have a central core of insulin producing β-cells surrounded by glucagon-producing α-cells, δ-cells and ε-cells. These cells can be identified as early as 1 day of post fertilization and their development is regulated by pathways similar to humans [15].

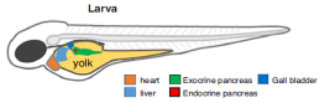


Fig 3. Overview of functional organ of zebrafish model [16].

Furthermore, zebrafish shows a β-cell compensatory response which is similar to rodents and humans. The number of β-cells in T2DM

zebrafish increases when in the over-nutrition state, indicating insulin resistance in these zebrafish [6]. Additionally, zebrafish provide unique advantage for studying β-cells development and regeneration. This because zebrafish strains in which the pancreatic β-cells can be visualized using fluorescent proteins placed under the control of the insulin promoter (Fig.3).

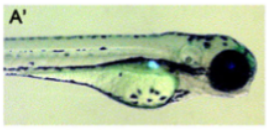


Fig 3. Fluorescent image of islet of pancreas in zebrafish model [7]

IV. CONCLUSION

As the conclusion, we created a zebrafish model of type 2 diabetes by using a modification method of alternating immersion in sugar solution of stepwise increasing concentration for every 2 weeks. The hyperglycemic effect of zebrafish can only be seen in glucose treated group but not sucrose treated group as compared with control group. However, the elevation of fasting blood glucose level is not significantly different between treated group and control group. The survival rate of both glucose treated and sucrose treated group is low. Thus, this study needs to be improvised in the future by study the correlation of blood glucose level with body weight, pancreatic morphology and histology. Moreover, the mortality rate of zebrafish also needs to be identified by determining the possible toxicity effects or any unsuitable conditions to the fish that lead to death throughout the experiment.

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References

[1] Harris, M., & Zimmet, P. (1997). Classification of diabetes mellitus and other categories of glucose intolerance, In: Alberti, K., Zimmet, P., Defronzo, R. (Eds.) (Second edi). John Wiley and Sons Ltd., Chichester: International Textbook of Diabetes Mellitus

[2] Kahn, S. E. (2003). The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of Type 2 diabetes. *Diabetologia*, *46*(3–19)

[3] Shi, Y., & Hu, F. B. (2014). The global implications of diabetes and cancer. *Lancet*, *383*, 1947–1948

[4] Li, L., Jick, S., Breitenstein, S., & Michel, A. (2016). Prevalence of diabetes and diabetic nephropathy in a large U.S. commercially insured pediatric population, 2002-2013. *Diabetes Care*, *39*, 278–284.

[5] Heckler, K., & Kroll, J. (2017). Zebrafish as a Model for the Study of Microvascular Complications of Diabetes and Their Mechanisms. *International Journal of Molecular Sciences*, *18*(2002), 1–9. https://doi.org/10.3390/ijms18092002

[6] Zang, L., Shimada, Y., & Nishimura, N. (2017). Development of a Novel Zebrafish Model for Type 2 Diabetes Mellitus. *Scientific Reports*, *7*, 1461. https://doi.org/10.1038/s41598-017-01432-w

[7] Seth, A., Stemple, D. L., & Barroso, I. (2013). The emerging use of zebrafish to model metabolic disease. *Disease Models & Mechanisms*, *6*, 1080–1088. https://doi.org/10.1242/dmm.011346

[8] Philipson, L. H., Prince, V. E., & Kinkel, M. D. (2010). Blood Sugar Measurement in Zebrafish Reveals Dynamics of Glucose Homeostasis. *Zebrafish*, *7*(2), 205–213. https://doi.org/10.1089/zeb.2009.0640

[9] Capiotti, K. M., Junior, R. A., Kist, L. W., Bogo, M. R., Bonan, C. D., & Silva, R. S. Da. (2014). Persistent impaired glucose metabolism in a zebra fish hyperglycemia model. *Comparative Biochemistry and Physiology, Part B*, *171*, 58–65. https://doi.org/10.1016/j.cbpb.2014.03.005

[10] Capiotti, K. M., Siebel, A. M., Kist, L. W., Bogo, M. R., Bonan, C. D., & Silva, R. S. Da. (2016). Hyperglycemia alters E-NTPDases, exto-5'-nucleotidase and ectosolic and cytosolic adenosine deaminase activities and expression from encephala of adult zebrafish (Danio rerio). *Purinergic Signalling*, *12*, 211–220. https://doi.org/10.1007/s11302-015-9494-z

[11] Shin, E., Hong, B. N., & Kang, T. H. (2012). An optimal establishment of an acute hyperglycemia zebrafish model. *African Journal of Pharmacy and Pharmacology*, *6*(42), 2922–2928. https://doi.org/10.5897/AJPP12.327

[12] Landgraf, K., Schuster, S., Meusel, A., Garten, A., Riemer, T., Schleinitz, D., … Korner, A. (2017). Short-term overfeeding of zebrafish with normal or high-fat diet as a model for the development of metabolically healthy versus unhealthy obesity. *BMC Physiology*, *17*(4), 1–10. https://doi.org/10.1186/s12899-017-0031-x

[13] Meng, X., Chen, B., & Zhang, J. (2017). Intracellular Insulin and Impaired Autophagy in a Zebrafish model and a Cell Model of Type 2 diabetes. *International Journal of Biological Sciences*, *13*(8), 985–995. https://doi.org/10.7150/ijbs.19249

[14] Maddison, L. A., & Chen, W. (2017). Modeling Pancreatic endocrine Cell Adaptation and Diabetes in the Zebrafish. *Front. Endocrinol*, *8*(9). https://doi.org/10.3389/fendo.2017.00009

[15] Field, H. A., Dong, P. D., Beis, D., & Stainier, D. Y. (2003). Formation of the digestive system in zebrafish. II Pancreas morphogenesis. *Develop- Mental Biology*, *261*, 197–208.

[16] Kamel, M., & Ninov, N. (2017). Catching new targets in metabolic disease with a zebrafish. *Current Opinion in Pharmacology*, *37*, 41–50. https://doi.org/10.1016/j.coph.2017.08.007

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