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Research Article

# Effects of D-Glucose Exposure on Motor Activity by Swimming Distance During Early Development of Zebrafish (*Danio rerio*)

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**ABSTRACT**

Several studies have been reported behavioral changes in patients with type 1 diabetes mellitus (T1DM) recently. The increasing levels of glucose affects neuronal activity and may inhibit the process of differentiation and regeneration of neurons in the brain. Dopaminergic (DA) neurons produce dopamine as a neurotransmitter which has an important role to regulate motor functions in the brain. This study aimed to determine the effect of exposure to high concentrations of glucose on the activity of dopaminergic neurons on early development of zebrafish (*Danio rerio*). Zebrafish embryos were exposed to glucose (1%, 3% and 5%) as a diabetic animal model at the beginning of development. Co-incubation was performed by incubated both of glucose and L-DOPA. Swimming distance of zebrafish larvae was measured as a parameter to determine the motor function regulated by dopamine activity. Results of statistical analysis in zebrafish embryos exposed to 1% and 3% glucose showed significant differences ($p < 0.05$) reduction on swimming distance. Co-incubation glucose with L-DOPA partially increased the swimming distance. It can be concluded that the administration of excessive exposure to glucose was able to decrease the activity of dopaminergic neurons by decreasing the distance of swim in early zebrafish development.

**Keyword:** glucose, motor activity, dopaminergic neuron, swimming distance.

## INTRODUCTION

Changes in behavior and cognitive lately often associated with diabetes mellitus type 1. One of the consequences of the complications of excess sugar in the blood is disorder of the nervous system in the brain. Many studies showed that the activity of neurons in the brain was influenced by existing glucose levels[1]. Despite the involvement of glucose metabolism in the brain are not directly affected, signaling related to glucose has a strong impact on the activity of neurons[2]. Energy from glucose metabolism in the brain is used for the differentiation of neurons and neurotransmitters biosynthesis[3]. Previous research confirms that there are some changes in mice and humans with diabetes type 1 that increased levels of anxiety, depression, and decreased mental speed and flexibility which these changes are closely related to diabetic complications and decrease the synthesis of dopamine[4].

Impaired glucose balance, including high concentration of glucose in hyperglycemia environment, has been known to also affect different levels of activity in the hippocampus. This region is heavily involved in the process of motor functions controlled by brain neurotransmitter, dopamine[5]. Production of dopamine in Dopaminergic (DA) neuron is regulated by tyrosine hydroxylase (TH). Catalysis tyrosine into L-DOPA by TH is followed by process of decarboxylation by aromatic l-amino acid decarboxylase (AADC) and transform into dopamine.

However, motor activity controlled by dopamine in hyperglycemia condition during early development is still not well known. This study used embryonic zebrafish exposed to high level of glucose as a hyperglycemic model to understand the effects of high concentrations of glucose exposure on swimming activity during early embryonic development. Swimming distance measurement was conducted to observe whether DA neurons function properly in such condition by their motor behavior.

## MATERIALS AND METHODS

*Fish maintenance*

Adult zebrafish (*Danio rorio*) were maintained at 28-30 $^{o}$C in recirculating system (aquatic habitats) and kept in a cycle of 14 hours light; 10 hours dark. The embryos of zebrafish were collected and raised in embryo medium (EM). After 24 hours post-fertilization, the collected embryos were mechanically dechorionated to optimize transdermal drug delivery of glucose exposure.

*Chemical Treatments and Exposure*

Zebrafish embryos were exposed to 1%, 3% and 5% glucose (Wako) in EM. After that, the next experiment was used co-incubation of glucose and 25μM L-DOPA. Embryos were divided into 4 groups: control, glucose

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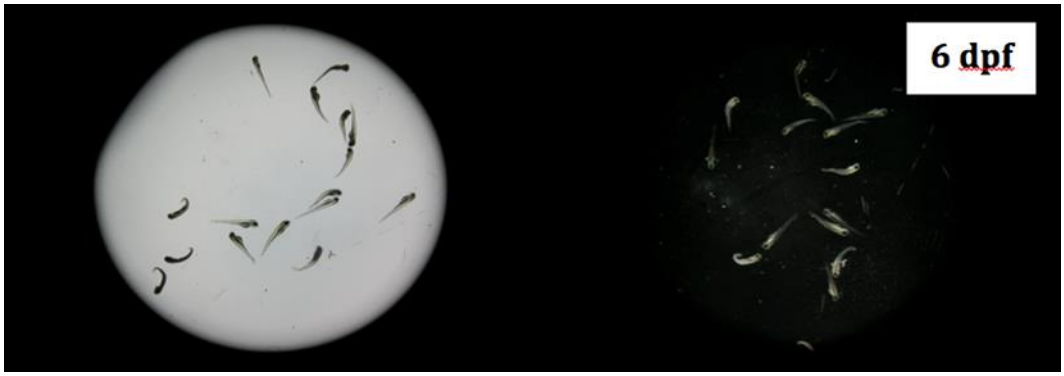


Figure 1: Zebrafish Embryos (6 dpf) exposed to 5% Glucose for 5 days.

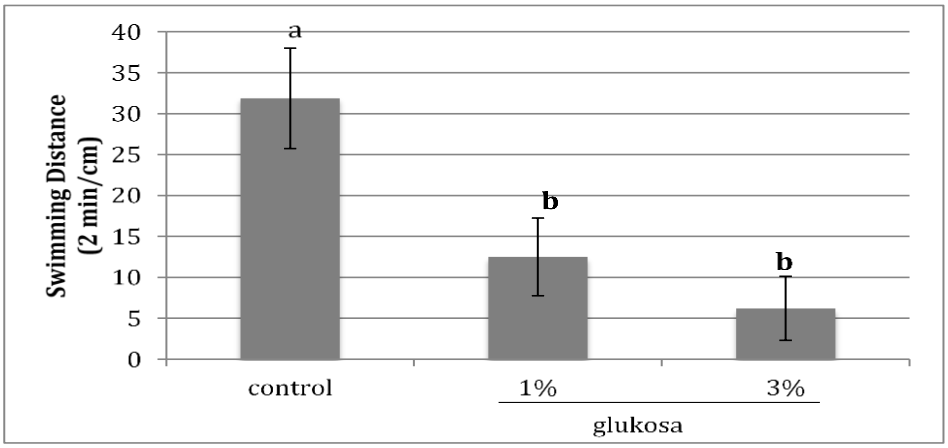


Figure 2: Swimming Distance Results of Glucose Exposure to Zebrafish Embryos for 5 days.

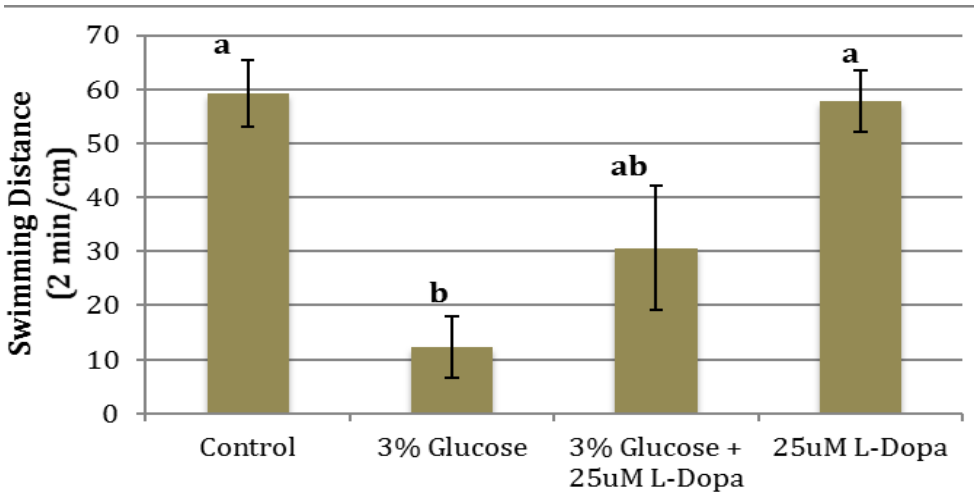


Figure 3: Swimming Distance Results of Co-incubation with L-DOPA for 1 day to Zebrafish Embryos (6 dpf) Exposed to Glucose.

group, co-incubation group (3% glucose and 25 µM L-DOPA), and L-DOPA group. Incubation was performed to embryos at 24 hours post-fetilization (hpf) for 5 days. The co-incubation was conducted at the last 24 hours before the measurement.

*Swimming Distance Assay*

Swimming activity test performed to determine if incubation with certain drugs in early embryonic development affects zebrafish neurobehavioral function. This test was performed at 6 dpf by placing ten fish were placed in the well, the well of the fish, with 5 mL of embryo medium and incubated for 5 minutes in a 37 ° C incubator. After that, the fish was habituated out for 1 minute and recorded for 2 minutes with a ruler placed beside the well. Recordings obtained were analyzed the distance of swim of the embryos using the ImageJ 1.47v software.

*Statistical Analysis*

Data are expressed as mean ± the standard error of the mean (SEM). For quantitative analysis of multiple

comparisons using Kruskal-Wallis test followed by Mann-Whitney test due to the abnormal and inhomogeneous data. A *p*-value less than 0.05 considered statistically significant. Statistical analysis was performed using SPSS version 16 software.

## RESULTS AND DISCUSSION

Previous research has shown that high glucose exposure affects the production of TH expression in dopaminergic neurons, thereby directly disrupting the dopamine production. Dopamine is often associated and has an important role on locomotor activity by acting as neurotransmitter[6]. Measurement of swimming distance is one of the common ways to determine the function of dopaminergic neurons in the fish through their behavior. Three dose of glucose concentrations were administrated to embryos by incubation, which were 1%, 3%, 5% glucose. However, on day 5 of incubation (embryos aged 6-day-old), the dose of 5% glucose caused of death to the embryo as shown in Figure 1. Hence, it was not possible to do the measurement of swimming distance and only did to the rest two doses (1% and 3% glucose).

Results of statistical analysis using the non-parametric test showed that both doses of incubation in 1% and 3% glucose caused decreasing of swimming distance at 6 dpf zebrafish embryos significantly ($p<0.05$) compared to control (Figure 2). These data showed that incubation to zebrafish embryos (24 hpf) for 5 days in 1% glucose was able to cause hyperglycemia followed by decreasing the activity of swimming. These results in accordance with previous studies, where the environment hyperglycemia caused down-regulation of insulin then started disturbance in motor activity as a result of changes in the expression of DA neurons[7].

Furthermore, the other results showed that embryos were co-incubated in 3% glucose and 25 μM L-DOPA, which has a similar action with dopamine, was able to help a significant effect of glucose exposure partially. Based on Figure 3, data showed that there was significant ($p<0.05$) difference between the groups, the swimming distance embryos incubated with glucose dropped significantly compared to the control embryos and the embryos were only exposed to L-DOPA. It states that the incubation of 25 μM L-DOPA alone on a normal embryo does not cause adverse effects to the swimming distance. Meanwhile, co-incubation with 25 μM L-DOPA to the embryo that had been exposed to glucose caused an increase in distance of swim. These results indicate that the treatment of co-incubation on embryonic hyperglycemia, will cause a repair effect on the emergence of motor activity embryos. Although it was improve the swimming distance partially, through the administration of L-DOPA on embryos exposed to glucose, compared to the control embryos. These data demonstrate that the effects of high glucose exposure decreased swimming activity of embryonic zebrafish may through dopaminergic neurons, since there was declining on motor function.

## CONCLUSION

Motor activity may down in hyperglycemic condition caused by D-glucose exposure since it decreased swimming distance of embryos through dopaminergic neuron during early development of zebrafish (*Danio rerio*).

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