Study the Nootropic Potential of Metformin in a Hyoscine-Induced Amnesia Model in Adult Zebrafish

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Abstract

This study investigates the potential of metformin, a widely used diabetes medication, to improve memory function in adult zebrafish. Metformin has long been valued for its ability to lower blood glucose and influence energy balance but recent research has suggested that it may benefit neurological health. Zebrafish, chosen for their suitability in memory and learning research, were divided into several groups: control group, diseased group, standard group, and test group treated with various doses of metformin. The study used T-maze model to test the associative spatial memory. Results demonstrated that metformin-treated zebrafish had a significant reduction in latency to enter the reward zone and an increase in the number of entries, showing improved cognitive performance compared to both control and diseased groups. Dose dependent responses were observed whereas lower dose (0.05 mg/ml) of metformin showed the most promising result compare to standard group in memory-related tasks. These bring to light on metformin as a memory enhancer, though additional research is requisite to elucidate its mechanism of action, particularly its effects on acetylcholine pathways in the zebrafish brain.

Keywords: Metformin, zebrafish, hyoscine, cognition, nootropic

1. Introduction

Metformin, a medication for managing type II diabetes, has roots dating back over a century to the herbal plant *Galega officinalis*, traditionally used in Europe. Scientists identified the blood-sugar-lowering compound guanidine in 1918, eventually leading to the synthesis of metformin. Approved by the FDA in 1995, metformin became a primary treatment for diabetes mellitus type II. It lowers glucose production in the liver, improves insulin sensitivity in muscle and fat, and activates AMPK, influencing energy balance. While primarily for diabetes, metformin has potential benefits for cardiovascular health, polycystic ovary syndrome, weight management, and neurological conditions. Recent research focusing on the application of metformin in diverse neurological ailments such as Parkinson's disease, Alzheimer's disease, and others. In a study carried out by the Alzheimer's Disease Neuroimaging Initiative, when metformin was started early in the course of the disease, patients showed improved cognitive function and decreased brain shrinkage. Another study found that metformin slows down cognitive decline in Alzheimer's patients [1]. Learning and memory is a part of cognitive function which is frequently affected in various neurological diseases. So, improvement of learning and memory can alleviate the symptoms of such diseases [2, 3].

Zebrafish are increasingly used as an animal model due to their similarities with the mammalian brain. They demonstrate cognitive behaviors like spatial learning, object recognition, and memory retention, which are comparable to those in mammals. Zebrafish are valuable for studying drug effects on cognition, as their responses help reveal mechanisms of cognitive enhancement or impairment, aiding in screening treatments for neurodegenerative diseases. Behavioral tests, such as T-maze for spatial memory, are effective and validated against mammalian models. Specific brain regions in zebrafish, like the lateral and medial pallium, resemble the mammalian hippocampus and amygdala in their roles in learning and emotion [4, 5].

Since there isn't much research on metformin as a nootropic for dementia, the aim was to find out its effect on learning and memory in an adult zebrafish model induced by hyoscine. Using the chronic exposure paradigm, we also saw behavioral alterations in zebrafish after they were exposed to different doses of metformin. The final goal of this study was to clarify the possible cognitive advantages of metformin, opening the door for additional investigation into its potential therapeutic uses in maintaining cognitive function and encouraging normal brain activity.

2. Materials

2.1. Animals & Housing

A total of 60 adult short-finned zebrafish, aged between 6 to 10 months and sexually matured, were used in the study. These zebrafish, comprising both males and females, were sourced from a local supplier. During the experiment (n=10 per group), they were kept in a controlled environment with filtered facility water, with the water and room temperatures kept at about 28°C. Illumination was supplied by ceiling and wall-mounted tube lights positioned above the tanks, following a 12-hour- light-12-hour-dark cycle.

The zebrafish was given Tetramin flakes from the Optimum Company (Thailand) two times a day. All fishes were experimentally naive and randomly selected. The zebrafish were divided into several groups: a control group, a disease group, a standard group, and six groups receiving different doses of metformin.

2.2. T-maze Apparatus

The experiment was conducted using a transparent Plexiglas T-maze with three arms. The central stem of the maze had dimensions of 50 x 10 x 10 cm (length x width x height). Dimension of starting box measuring 10 cm by 10 cm by 10 cm, which was separated from the edge of the stem by a transparent sliding door. The three arms of the maze were each 20 cm by 10 cm by 10 cm. At the distal end of each arm, a target compartment measuring 10 cm x 10 cm x 10 cm was located. To restrict access to these target compartments, transparent doors were employed (Figure 1) [6].

Furthermore, we made cellophane sleeves in yellow and blue to encase the target compartment located at the ends of the arms. We filled the T-maze with three liters of aquarium water and kept the water's temperature constant at 27 ± 1°C. Experiments were carried out between 10:00 AM and 4:00 PM.

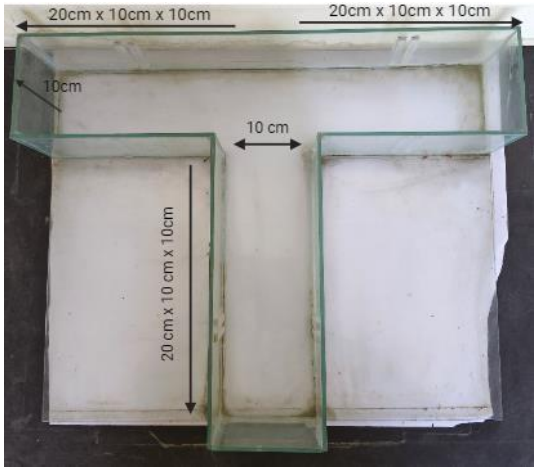

Figure 1 T-maze apparatus

2.3. Drugs

Hyoscine (Sigma Aldrich) was administered to zebrafish by water immersing technique with concentration 100 µM [7], piracetam (Sigma Aldrich) was administered in same fashion with concentration 200 µM [8], and 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mg/ml concentrated solution was used for metformin (Sigma Aldrich) (Table 1). Both the test chamber and the home tank were free of drug exposure. Every fish was drug-naïve and only used it once. The control group was exposed to tank water without any drugs added (Table 2).

Table 1. Different doses of drugs

Drugs	Dose
Hyoscine	100 µM
Piracetam	200 µM
Metformin	0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mg/ml

Table 2. Experimental grouping of animals

Groups	Control	Diseased (Hyoscine)	Standard (Hyoscine + Piracetam)	Test (Metformin)					
				T1 (0.05 mg/ml)	T2 (0.1 mg/ml)	T3 (0.2 mg/ml)	T4 (0.4 mg/ml)	T5 (0.08 mg/ml)	T6 (1.6 mg/ml)
Number of fish	10	10	10	10	10	10	10	10	10

3. Methodology

3.1. T-maze Activity

To minimize stress caused by unfamiliar experimental procedures, all zebra fishes were acclimatized for 2 hours before the start of the experiments. At the beginning of each trial, a zebrafish was placed in the start box with the door closed for 1 minute. Afterward, the transparent door was lifted, and once the zebra fish showed signs of excitement and exited in the start box, the door was closed again. Each zebra fish underwent one trial per day during the training phase. If the fish entered the blue zone, it was gently disturbed or mildly punished using a glass rod, while the yellow zone was designated as a safe area where the fish was rewarded with food. Upon entering either zone, the zebra fish was observed for 5 minutes, and data such as the number of entries into the yellow (safe) and blue (unsafe) zones, along with transfer latency, were recorded. Each zebra fish participated in one trial daily over four consecutive days for training. On the 5th day, memory testing was conducted. All zebra fishes were fasted overnight before experiments. Hyoscine (100 μM) was administered 1 hour prior to the training session, and the fishes were placed in the maze for testing. Piracetam (200 μM) or metformin (0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mg/ml) was administered 30 minutes before the trials (Figure 2).

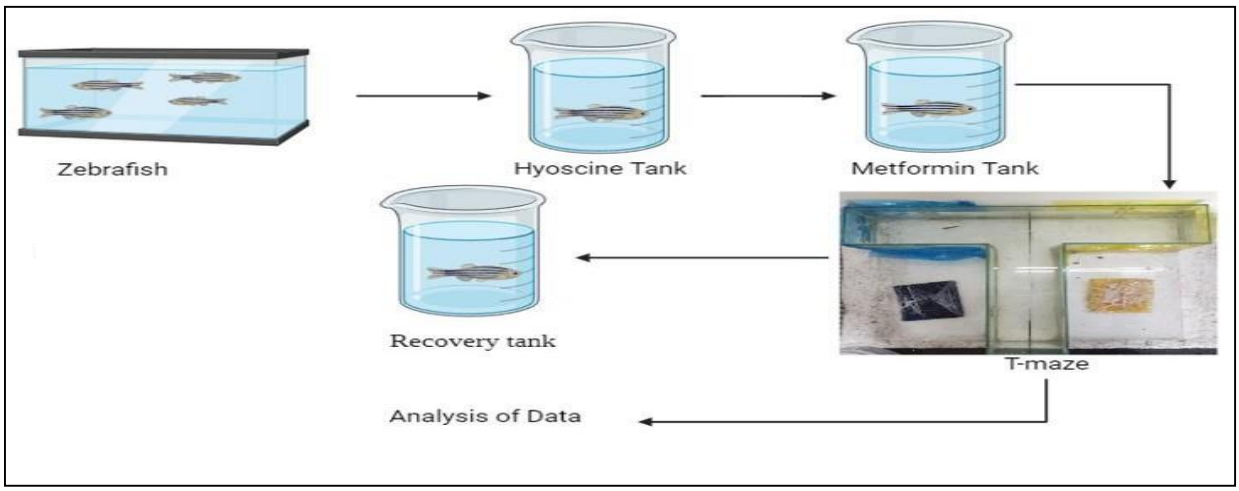

Figure 2 Diagrammatic presentation of the experimental design

3.3. Data Analysis

All data were analyzed statistically in GraphPad Prism software (version 8.0.2) by using a one-way ANOVA (Analysis of Variance). Relevant post-hoc analyses were subsequently applied to identify additional variations of interest.

4. Results

4.1. Assessment of latency time

The latency time in the reward arm (favorable arm) was measured for all groups on day 5. Zebrafish treated with various doses of metformin (0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mg/ml; n=10) shown a notable reduction in latency time contrast to the control group ($p<0.05$) and the disease group ($p<0.001$). As shown in figure 3, different doses of metformin exhibited significant differences compared to the standard drug, except for the 1.6 mg/ml dose ($p<0.05$). The group treated with 0.05 mg/ml of metformin appeared a remarkable difference when compared to the 0.8 and 1.6 mg/ml metformin-treated groups ($p<0.05$).

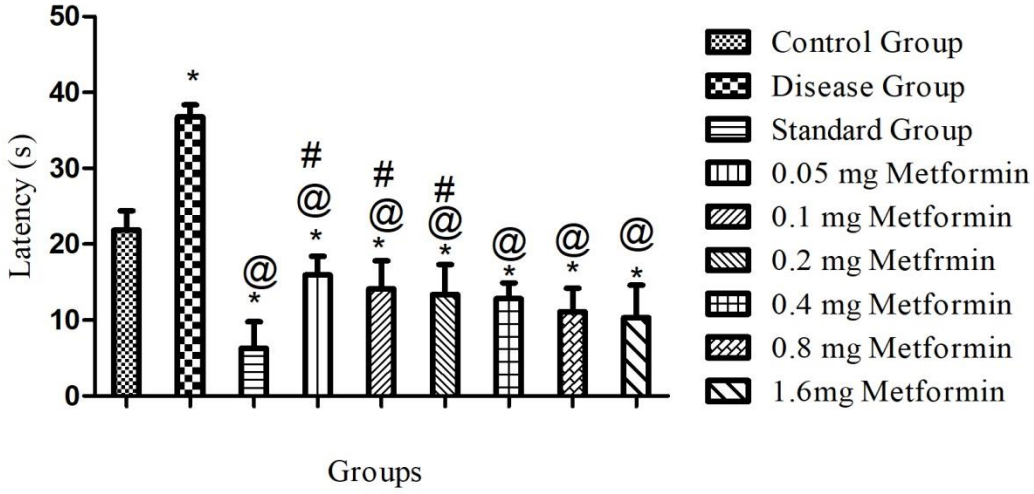

Figure 3 Outcome of metformin treatments in T-maze representing transfer latency to reward armin different groups. [The data are presented as Mean ± S.D.*$p < 0.05$ vs Control, $^{@}p < 0.001$ vs Disease, $^{\#}p < 0.05$ vs standard]

4.2. Assessment of number of entries in reward arm

Number of entries in reward arm for all groups was assessed at day 5. Different doses of metformin 0.05, 0.1, 0.2, 0.4, 0.8, 1.6 mg/ml (n=10) treated groups showed increased number of entries in reward arm in contrast to control ($p<0.05$) and disease group ($p<0.001$). 0.05 and 0.1 mg/ml treated groups of metformin was shown significance difference when compared with standard drug ($p<0.01$). 0.8 and 1.6 mg/ ml dose of metformin treated groups showed significant difference when compared with 0.05 mg/ml dose of metformin ($p<0.05$) (Figure 4), indicating formation of associative spatial memory.

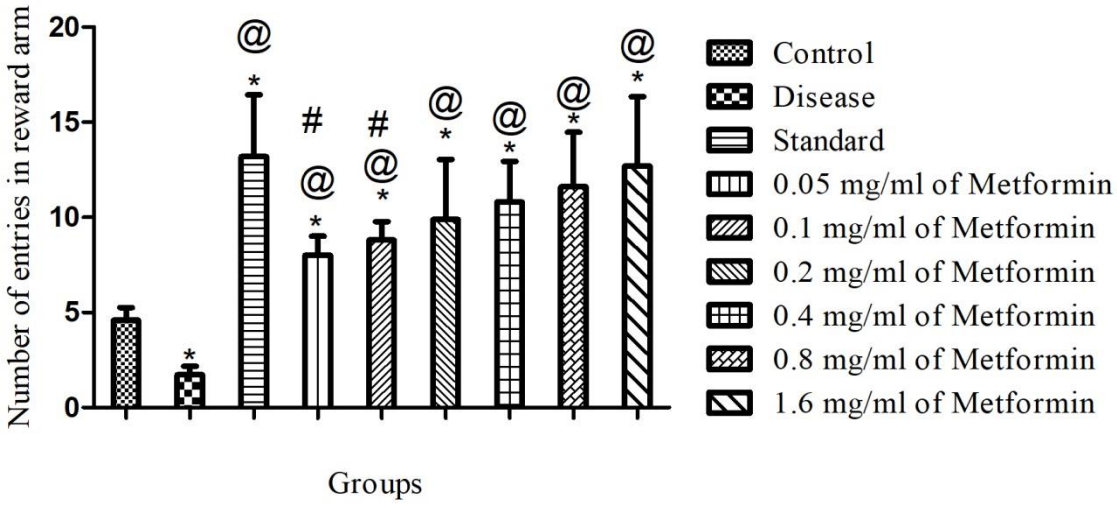

Figure 4 Effect of metformin treatments in T-maze representing the number of entries in reward arm of different groups. [Data are presented as Mean ± S.D.*$p < 0.05$ vs Control,$^{@}p < 0.001$ vs Disease, $^{\#}p < 0.01$ vs standard]

4. Discussion

The colour-cued food rewards has emerged as an effective technique for assessing the memory function of zebrafish, as demonstrated in Kim et al. in 2017 [9]. Using the T-maze apparatus, we used hyoscine, a muscarinic receptor antagonist, to create memory impairments in zebrafish. This resulted in fewer entries into the reward arm (yellow) and a longer latency period during colour discrimination tests. Our evaluation of memory encompassed colour discrimination and associative spatial memory tests within the T-maze, a well-established tool for assessing memory in both rodents and zebrafish models [10]. The inherent colour discrimination ability of zebrafish played a pivotal role in these learning and memory assessments. Interestingly, our study showed that metformin significantly improved zebrafish memory in hyoscine-induced amnesia. As shown in Figure 4, the metformin-treated groups exhibited a noticeable increase in the number of entries into the reward arm (yellow). Moreover, as evidenced by the data presented in figure 3, zebrafish treated with metformin consistently distinguished between two distinct colours (yellow and blue) compared to the group with the induced memory impairment. Our results also revealed that different doses of metformin (0.05, 0.1, 0.2, 0.4, 0.8, and 1.6 mg/ml) enhanced learning and memory performance. This was demonstrated by a higher number of entries into the reward arm (yellow) and shorter latency times compared to both the diseased and control groups. It's important to highlight that the effectiveness of different metformin doses showed variability when compared to the standard treatment group. Among them, 1.6 mg/ml of metformin had an effect on latency that was equivalent to the standard group and 0.05 and 0.1 mg/ml of metformin had an effect that was more pronounced than the standard group. It has been observed that low dose (0.05 mg/ ml) of metformin showed better response compared to higher dose of metformin (0.8 and 1.6 mg/ml). The dose-dependent effects of metformin remained somewhat inconclusive, though the lower dose (0.05 mg/ml) appeared promising as a potential nootropic agent. Previous research had already suggested metformin's cognitive enhancement properties in rat model, and our study provided further evidence of its nootropic effects, particularly in the context of associative spatial memory in adult zebrafish. Given that the pallium is structurally akin to the mammalian hippocampus, which plays a pivotal role in spatial learning, it is conceivable that metformin may modulate the acetylcholine pathway in the pallium and sub-pallium regions.

5. Conclusion

In conclusion, metformin ameliorated hyoscine induced amnesia in the adult zebrafish model. The exact mechanism of metformin's action against amnesia remains elusive. Our data suggest that it counteract the damage caused by cholinergic neurons in zebrafish brain. Nevertheless, a more comprehensive investigation needed to delve into the involvement of specific pathways and neurotransmitter systems.

Ethical Approval

All the ethical approvals were obtained from the Committee for Control and Supervision of Experiments (CCSEA) of Sanaka Educational Trust's Group of Institutions.

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Conflict of interest
There is no conflict of interest both personally and financially.

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Data availability
Data will be available on request.

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