

# **S.T. Yau High School Science Award**

## **Research Report**

### **The Team**

Name of team member: Jasper Shi  
School: Dougherty Valley High School  
City, Country: San Ramon, CA, USA

Name of supervising teacher: Karen Dennis  
Job Title: Science Teacher  
School/Institution: Dougherty Valley High School  
City, Country: San Ramon, CA, USA

### **Title of Research Report**

**ZebraX**: An Innovative Artificial Intelligence Approach to Analyzing Zebrafish Behavioral Indicators for Herbal Diabetes Treatment

### **Date**

08/16/2025

# **ZebraX: An Innovative Artificial Intelligence Approach to Analyzing Zebrafish Behavioral Indicators for Herbal Diabetes Treatment**

## **Abstract**

*Danio rerio*, commonly known as zebrafish, have been used as model organisms for studying various diseases, including diabetes, because of its genetic similarity to humans, its fast reproductive capacity, and low-cost maintenance. However, current studies using zebrafish are mainly focused on non-metabolic diseases, and most importantly, these studies largely depend on manual data collection techniques that are prone to errors, time-consuming, and decrease the sample size of fish maintained. To aid the development and analysis of treatments for diabetes, this study presents a novel Artificial Intelligence (AI) based approach called ZebraX, designed to bridge these gaps by analyzing diabetes related behavioral Indicators in zebrafish and evaluating the therapeutic potential of herbal remedies. This study successfully induced type 2 diabetes in zebrafish through dietary and environmental adjustments, from a baseline of 2.71 mmol/L to 12.73 mmol/L ( $p < 0.001$ ). The ZebraX system was used to detect the behavioral patterns of zebrafish by integrating automated tracking and predictive algorithms. Initial results indicated that zebrafish movement patterns, average speed, swimming episode duration, and episode frequency were all negatively affected by increasing the severity of diabetes. The maximum speed during swimming episodes of zebrafish with diabetes was reduced by 50% and irregular movements increased when compared to healthy control group fish. In addition, natural treatments consisting of mulberry leaves, aloe vera, and bitter melon extracts were evaluated for therapeutic effects. The most significant effect was in lowering blood glucose, which decreased blood glucose by about 52% to 6.08 mmol/L ( $p < 0.001$ ). As the first study of its kind, this novel approach not only establishes zebrafish as an expandable model for diabetes-related research but also opens a significant possibility of integrating AI technologies with herbal remedies to improve the

management of diabetes. It offers a promising direction for future interdisciplinary research bridging artificial intelligence and life science.

*Keywords: ZebraX, AI, Type 2 Diabetes, Behavioral Indicators, Herbal Treatments, Mulberry Leaves, Aloe Vera, Bitter Melon, Blood Glucose Test.*

## **Acknowledgement**

I would like to extend my sincere gratitude to my mentor, Ms. Karen Dennis at Dougherty Valley High School, for her guidance, encouragement, and support throughout this research.

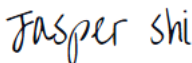
## Commitments on Academic Honesty and Integrity

We hereby declare that we

1. are fully committed to the principle of honesty, integrity and fair play throughout the competition.
2. actually perform the research work ourselves and thus truly understand the content of the work.
3. observe the common standard of academic integrity adopted by most journals and degree theses.
4. have declared all the assistance and contribution we have received from any personnel, agency, institution, etc. for the research work.
5. undertake to avoid getting in touch with assessment panel members in a way that may lead to direct or indirect conflict of interest.
6. undertake to avoid any interaction with assessment panel members that would undermine the neutrality of the panel member and fairness of the assessment process.
7. observe the safety regulations of the laboratory(ies) where we conduct the experiment(s), if applicable.
8. observe all rules and regulations of the competition.
9. agree that the decision of YHSA is final in all matters related to the competition.

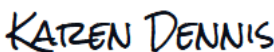
**We understand and agree that failure to honour the above commitments may lead to disqualification from the competition and/or removal of reward, if applicable; that any unethical deeds, if found, will be disclosed to the school principal of team member(s) and relevant parties if deemed necessary; and that the decision of YHSA is final and no appeal will be accepted.**

*(Signatures of full team below)*

Jasper Shi   
Name of team member: \_\_\_\_\_

\_\_\_\_\_  
Name of team member:

\_\_\_\_\_  
Name of team member:

Ms. Karen Dennis   
Name of supervising teacher: \_\_\_\_\_

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# ZebraX: An Innovative Artificial Intelligence Approach to Analyzing Zebrafish Behavioral Indicators for Herbal Diabetes Treatment

**Abstract**—*Danio rerio*, commonly known as zebrafish, have been used as model organisms for studying various diseases, including diabetes, because of its genetic similarity to humans, its fast reproductive capacity, and low-cost maintenance. However, current studies using zebrafish are mainly focused on non-metabolic diseases, and most importantly, these studies largely depend on manual data collection techniques that are prone to errors, time-consuming, and decrease the sample size of fish maintained. To aid the development and analysis of treatments for diabetes, this study presents a novel Artificial Intelligence (AI) based approach called ZebraX, designed to bridge these gaps by analyzing diabetes related behavioral Indicators in zebrafish and evaluating the therapeutic potential of herbal remedies. A schematic overview of the ZebraX framework is shown in Figure 1. This study successfully induced type 2 diabetes in zebrafish through dietary and environmental adjustments, from a baseline of 2.71 mmol/L to 12.73 mmol/L ( $p < 0.001$ ). The ZebraX system was used to detect the behavioral patterns of zebrafish by integrating automated tracking and predictive algorithms. Initial results indicated that zebrafish movement patterns, average speed, swimming episode duration, and episode frequency were all negatively affected by increasing the severity of diabetes. The maximum speed during swimming episodes of zebrafish with diabetes was reduced by 50% and irregular movements increased when compared to healthy

control group fish. In addition, natural treatments consisting of mulberry leaves, aloe vera, and bitter melon extracts were evaluated for therapeutic effects. The most significant effect was in lowering blood glucose, which decreased blood glucose by about 52% to 6.08 mmol/L ( $p < 0.001$ ). As the first study of its kind, this novel approach not only establishes zebrafish as an expandable model for diabetes-related research but also opens a significant possibility of integrating AI technologies with herbal remedies to improve the management of diabetes. It offers a promising direction for future interdisciplinary research bridging artificial intelligence and life science.

**Keywords**—*ZebraX, AI, Type 2 Diabetes, Behavioral Indicators, Herbal Treatments, Mulberry Leaves, Aloe Vera, Bitter Melon, Blood Glucose Test.*

## 1. Introduction

Diabetes is a complex disease that has emerged as one of the leading causes of mortality and morbidity across the globe. More than 463 million people are affected by this disease worldwide, and it is expected that this will increase to more than 700 million by the year 2045 [1]. In the United States, around 37 million have been diagnosed with diabetes, now it is prevalent in about 11% of the population [2]. One of the great concerns is the fact that diabetes is now being found at increasingly younger ages—evidence of changes occurring over generations in lifestyle and eating

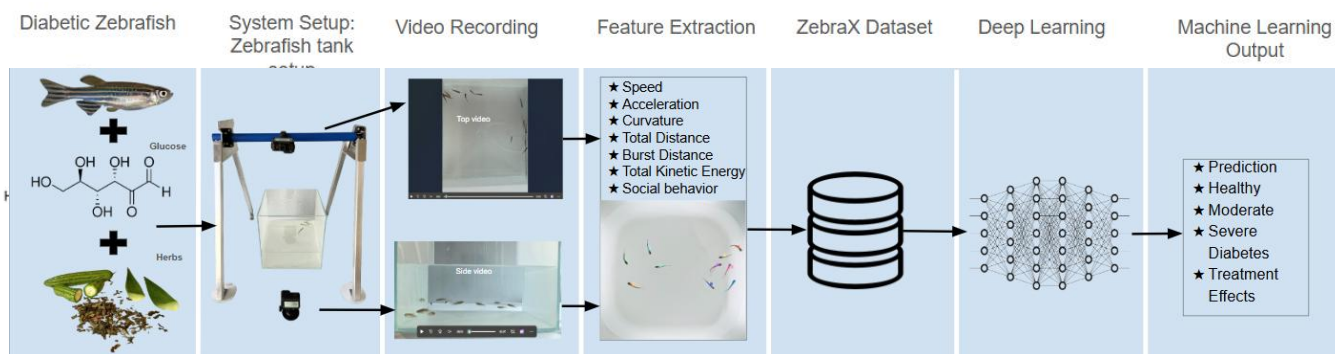


Figure 1: Diagram of the proposed ZebraX AI-Based System for monitoring zebrafish behavior and measuring blood glucose levels.

habits. Diabetes has also become the seventh leading cause of death within the United States due to its major impact on public health. The economic and social burdens related to this disease are enormous, involving health care expenditure, lost productivity, and reduction in the general quality of life. Traditional biological models, particularly mouse models and its derivatives, have provided significant contributions towards a better understanding of diabetes. However, these biological models are often hampered by high costs, long experimental times, and obstacles related to genetic manipulations. By contrast, zebrafish larvae, which are transparent, share 87% genetic similarity to humans [3]. Their strong proliferation, low costs, and ease of maintenance make them well-suited for high-throughput drug screening and disease modeling. Their accessibility to gene-editing technologies further increases their usefulness in biomedical research.

## 2. Related Work

Recent developments have highlighted the effectiveness of AI in zebrafish research. In a 2021 study, Barreiros et al. developed an AI-based system for real-time monitoring and assessment of zebrafish behavior. They studied zebrafish behavior and noticed that there was a difference in learning from the red led light and stimuli of vibracall [4]. This system helped

zebrafish research by allowing careful study of their behavior when they face outside signals. Zhao et al., in 2024, offered a more dynamic, real-time approach to disease monitoring and prediction. The convergence of data integration techniques with AI enhances the accuracy of predictions and facilitates a proactive response to potential outbreaks [5]. Through their study, significant contributions toward improving early disease detection with data-driven predictions have been made. In a 2021 study, Choi et al. discovered that with the aid of CRISPR-based-knockout technology and big data from next-generation DNA sequencing, functional validation of GWAS candidates in zebrafish is greatly enhancing the ability and accuracy of identifying causative genes and molecular mechanisms underlying the pathogenesis of human genetic diseases [6]. This study improved the testing of genetic factors that cause disease. In a 2021 study, Konadu et al. demonstrated that zebrafish is an excellent system to model fetal hyperglycemia by focusing on direct glucose exposure of the embryo without altering maternal glucose levels [7]. Their study provided a foundation for understanding metabolic disorders during development. In a 2019 study, Sawaki et al. succeeded in utilizing AutoML, a web-based machine learning program, to identify the abnormalities in the phenotypes of fluorescent zebrafish using their images

[8]. This development increased the efficiency of discovering phenotypic abnormalities in zebrafish models. Lubin et al., in 2021, developed an easy-to-use, customizable automated screening procedure suitable for high-throughput phenotype-based screens of live zebrafish [9]. Their study has made large-scale studies simpler to understand while increasing the efficiency of drug screening and phenotype assessment. As summarized above, significant progress has been made in the following areas: real-time monitoring, disease prediction, functional validation of genes, fetal hyperglycemia modeling, and more [10]. However, there is still a notable lack of studies using AI to investigate diabetes-related issues in zebrafish logical model. Moreover, more research is needed to apply the findings of the zebrafish model into practical applications for the prevention, diagnosis, and treatment of human diabetes to verify their clinical relevance.

This study is the first to utilize AI to systematically monitor zebrafish behavior, identify diabetes-specific Indicators, and evaluate the efficacy of natural therapeutic interventions. This study focuses on three key objectives: developing the AI-based ZebraX system to monitor and analyze changes in zebrafish behavior and identify potential behavior Indicators for diabetes, establishing a reliable method for inducing type 2 diabetes in zebrafish, and evaluating the effectiveness of natural therapies in treating diabetes for potential translation to human treatment.

### 3. Methodology

#### 3.1 Zebrafish Cultivation

Zebrafish were purchased from Carolina Biological Supply and were housed in 15-gallon aquariums (30 ×

30 × 60 cm). The aquariums were maintained under controlled environmental parameters, such as temperature—about 75°F, pH ranging between 7.0 and 8.0—and a light cycle of 14 hours of lighting followed by 10 hours of darkness to simulate the natural environmental rhythm. Feeding was carried out twice a day at an interval of 12 hours. The fish tank was cleaned once a week to maintain a hygienic and stable environment for the fish. The conventional diet comprised diminutive shrimp flakes. For optimal health, zebrafish necessitate a typical blood glucose index (i.e., blood glucose concentration) ranging from 2.5 mmol/L to 6.1 mmol/L.

#### 3.2 Video Setup for Behavior Monitoring



Figure 2: Zebrafish movement tracking system setup

To monitor the behavior of zebrafish, a dual-camera system was set up with one top-mounted camera for an overhead view and a side-mounted camera for lateral view. The top camera was fixed using a steel frame to ensure the stability as shown in Figure 2. The side camera mounted at an optimal angle, recorded lateral movements and side activities of zebrafish. This dual-camera setup was chosen to track the movements of zebrafish more precisely, in two dimensions: up-

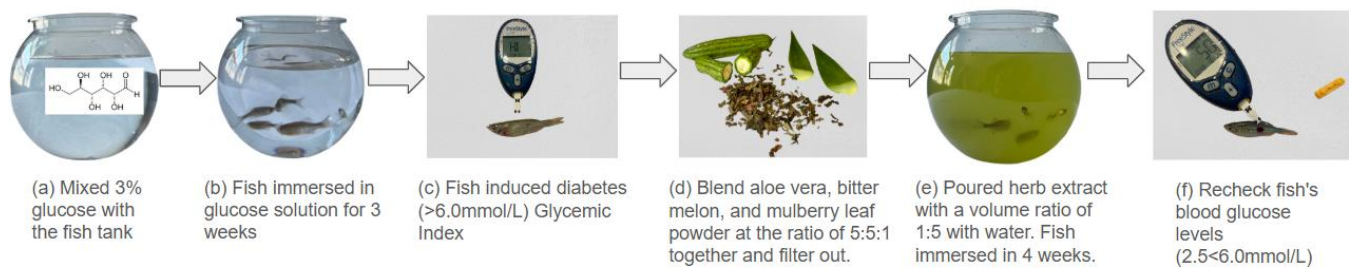


Figure 3: Diagram of the whole process of zebrafish-induced diabetes and cure

and-down and side-to-side. These clear images provide a powerful dataset for analyzing crucial details about the zebrafish's swimming speed, acceleration, and movement path. By utilizing two distinct viewpoints, the system reduces blind spots and the phenomenon of fish overlap, thereby improving the accuracy of behavioral analysis. This combined data is crucial for observing the link between zebrafish behavior and diabetes severity. A visual overview of the zebrafish diabetes induction and recovery protocol is provided in Figure 3.

### 3.3 Zebrafish Diabetes Modeling

To investigate the progression of diabetes and the efficacy of herbal treatments, the zebrafish were divided into four distinct experimental groups. The Control Group was fed a standard diet of shrimp flakes only and served as the healthy baseline to establish normal physiological and behavioral parameters. To ensure a valid starting point for the experiment, baseline measurements, referred to as the Experimental Group (Normal/Initial Data), were taken from all fish prior to any dietary changes to confirm they started from a comparable healthy state. Subsequently, the Diabetic Group was fed a high-glucose diet (3% glucose mixed with shrimp flakes) to induce a diabetic state[11]. This group's condition was monitored over several weeks, allowing for the

observation of a progression from Moderate Diabetes, characterized by intermediate hyperglycemia after approximately one week, to Severe Diabetes, which presented with significant and sustained hyperglycemia after two or more weeks. The final group was the Cured Group, which consisted of fish that were first made diabetic and then administered a therapeutic treatment of mulberry leaf, aloe vera, and bitter melon extracts to evaluate the restorative effects of the herbal remedies. The experimental process lasted 21 days. Each group contained 20 zebrafish, and the diabetic group was fed at 7:00 am & 7:00pm each day, 0.5 grams each time. The feeding time on Saturday was 7:00am, and 0.25 grams each time in the morning. The diabetic zebrafish received glucose solution and overfeeding for 21 days. Fresh water was changed every five days to make sure consistent water quality and minimize environmental stress. The high-sugar environment simulated the high blood glucose and diet-induced stress in human diabetic patients. All cholesterol was maintained under the same conditions without supplementation. The allowed researchers to see changes in behavior and body functions directly related to high glucose levels. All cholesterol was maintained under the same conditions without supplementation.

### 3.4 Blood Collection and Measurement Method

Blood collection from zebrafish is very challenging due to their small size and fast blood coagulation rate [12]. They are relatively weak, and their heart rate is fast. Capillary blood can coagulate within 20-30 seconds, making blood collection even more difficult. The blood measurement method this study presents first takes blood from the heart artery to prevent the leakage of effective tissue fluid and cause low blood glucose. Then, a free-style micro-measurement blood glucose meter is used, which only requires 0.3 microliters or more of micro-blood to ensure the accuracy of blood glucose measurement.

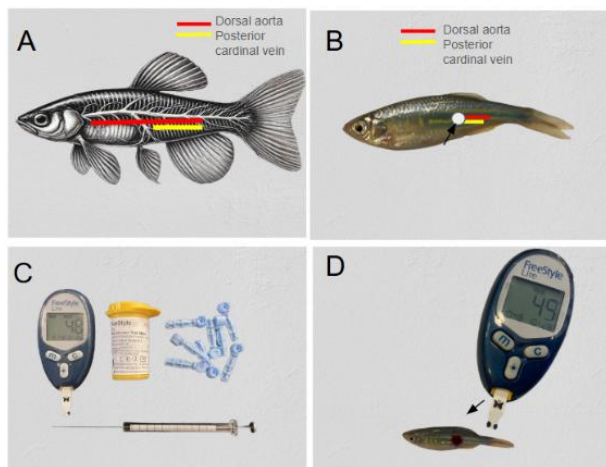


Figure 4: Zebrafish's lab procedures for measuring blood glucose levels. (A) Dorsal aorta of zebrafish; (B) The point where the needle is stabbed; (C) Materials; (D) Blood glucose levels.

As shown in Figure 4, first, zebrafish were picked up and put in an anesthetic solution of 0.008% Syncaïne. After more than 30 seconds, the zebrafish will lose consciousness and be unable to move. This method did not pose any harm to the zebrafish. Following anesthesia, excess water was carefully removed with absorbent paper, then zebrafish were placed on a glass slide. A 40x microscope was used to insert a fine needle at a 40-degree angle into the dorsal aorta of the fish. The needle was aimed at the point where the vertical and horizontal midlines of the fish's body meet. Blood was collected quickly to stop it from clotting and to

keep the sample good. A small blood glucose meter, needing only 0.3 microliters of blood, measured glucose levels very accurately. This method reduced stress on the fish and helped get reliable data.

### 3.5 Herbal Selection Rationale

Type 2 diabetes involves several processes at the same time: fast rises in blood glucose after meals, reduced insulin action, and stress or inflammation in tissues. To address more than one process, this study selected three botanicals that act in different ways and are commonly reported in the literature: mulberry leaf, aloe vera, and bitter melon. Each extract was tested individually and also as a three-herb combination because their actions are complementary to each other. In the manuscript, the combination produced the largest improvement in blood glucose among the tested conditions.

#### 3.5.1 Mulberry Leaf Extract

Mulberry leaves contain 1-deoxynojirimycin (DNJ) and flavonoids. DNJ inhibits  $\alpha$ -glucosidase in the intestine, which slows the breakdown of carbohydrates and helps reduce post-meal glucose spikes. Prior reports in animals and humans show better post-prandial glucose after Mulberry Leaf Extract intake.

#### 3.5.2 Aloe Vera Extract

Aloe vera provides polysaccharides (e.g., acemannan) and other small molecules. These compounds have been reported to support  $\beta$  cell function, to help glucose transport into cells, and to reduce inflammation/oxidative stress, which are linked to insulin resistance.

#### 3.5.3 Bitter Melon Extract

Bitter melon contains charantin, polypeptide-p (an insulin-like peptide), and triterpenoids. Studies suggest insulin-mimetic effects, better peripheral glucose

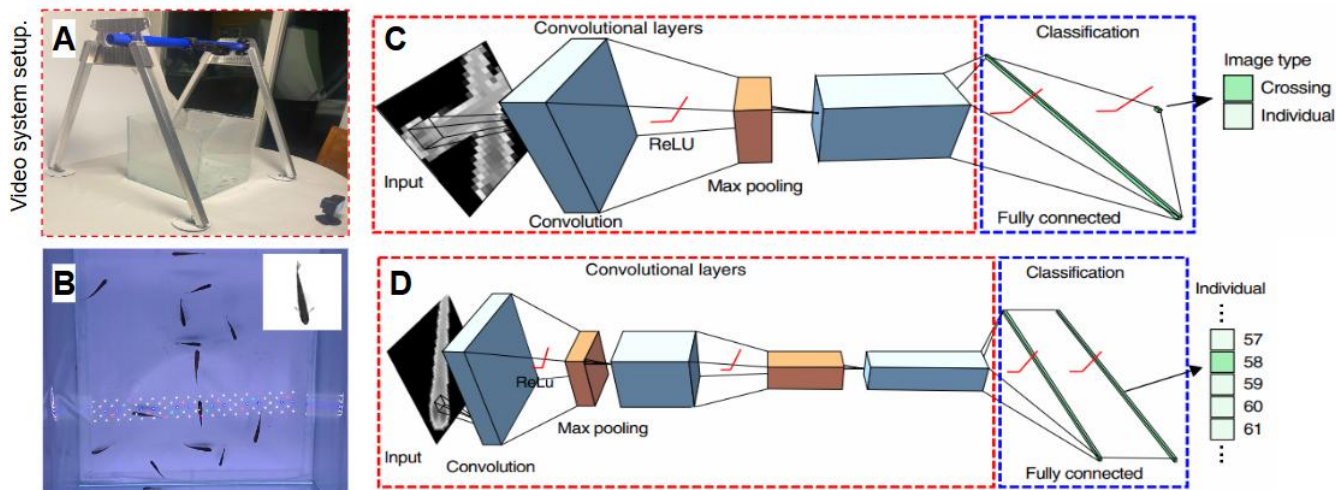


Figure 5: Experimental setup and AI crossing and identification models. (A) The video system setup. (B) Examples of grayscale image preprocessing. (C) Preprocessing crossings detector. (D) Individual identification model.

uptake, and lower hepatic glucose production. In concert, these three extracts form a comprehensive therapeutic strategy. Mulberry Leaf Extract mainly limits glucose entry from the gut, Bitter Melon Extract acts like insulin and improves glucose use in tissues, and Aloe Vera Extract supports  $\beta$ -cells and reduces inflammation. Using them together targets multiple steps of diabetes at once. This is why a three-herb group was included in addition to single-herb groups.

### 3.6 ZebraX AI Model Development

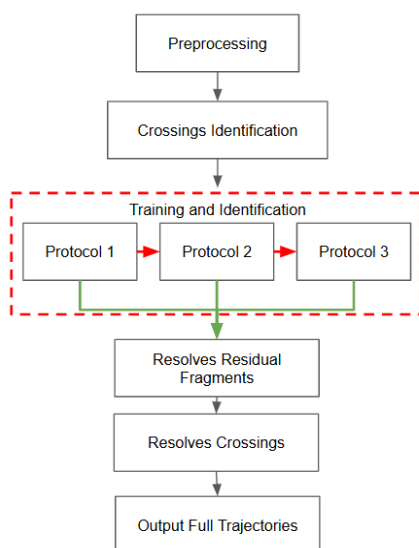


Figure 6: Schematic of process flow of the IDtracker model used for diabetic zebrafish model study.

The ZebraX system is an integrated platform that first employs the IDtracker.ai module to extract high-precision zebrafish trajectories from raw video data. These trajectory files are then analyzed by a built-in deep learning classifier to determine the health status of each fish. IDtracker is a powerful tool used to track complex zebrafish movements, including overlapping behaviors when fish interact or cross path. [13]. The AI can segment each fish into separate entities, referred to as blobs, and resolves overlaps, or “crossings,” by referencing preceding and subsequent frames. The AI model can fully trace fish trajectory from top-view videos. During preprocessing, a convolutional neural network (CNN) “crossing detector,” which distinguishes between single-animal blobs and crossings where two or more fish overlap. In Figure 5-6: The crossing-detector and identification Protocols are used to adapt to higher-difficulty videos. In Protocol 1, “image fragments” are intervals where crossings are not detected for each individual fish. “Global fragments” are the collection of image fragments with the same number of image fragments as the number of fish to be tracked. Protocol 1 select

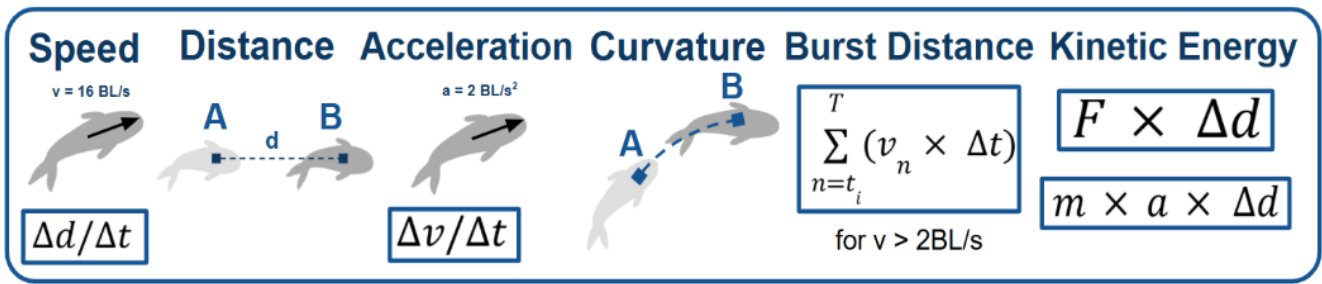


Figure 7: Six behavioral metrics used in ZebraX. Speed, distance, acceleration, curvature, burst distance, and kinetic energy were measured, with burst kinetic energy serving as the main indicator of diabetes severity.

the best global fragment to train the identification network. If the amount of remaining high-quality fragments is low, Protocol 2 is initiated. Protocol 2 retrains the network on the high-quality fragments and then selects more high-quality fragments. In Protocol 3, only the convolution layers are pretrained with high-quality fragments, fixing the parameters. Then, it trains the classification layers using those parameters. These protocols enhance the accuracy of the model.

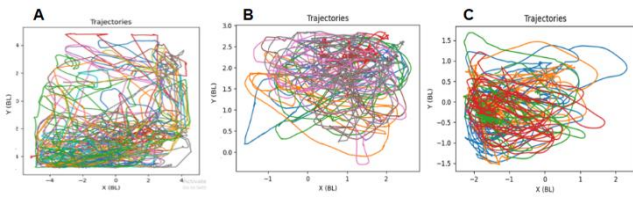


Figure 8: The trajectories of zebrafish (A) control group fish (B) slightly diabetic fish (C) heavily diabetic fish

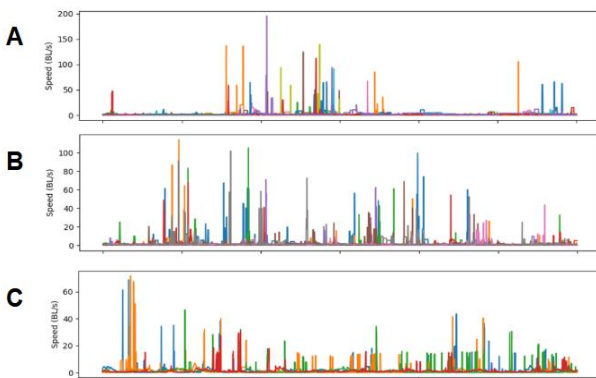


Figure 9: A comparison of the speeds of zebrafish with varying severities of diabetes over 60 seconds. From the x-axis, the maximum speed of more diabetic fish is far less than the maximum speed of control fish. Different colors represent the speeds of each fish. (A) control group fish (B) moderate diabetic fish (C) severe diabetic fish

Using the AI model results, a trajectory tool can generate speed, acceleration, distance from center,

and other trajectory graphs. The ZebraX system deepens and expands the functions of idtracker.ai, comprehensively tracking the movement data of zebrafish in top and side view videos (such as speed, acceleration, distance from the center, curvature, burst distance, kinetic energy, trajectory path Figure 8, etc.), and can accurately analyze the movement behavior of fish and their social interaction characteristics, providing key data support for behavioral research and potential treatment effect evaluation of diabetes models (Figure 9). In the preprocessing stage, ZebraX uses a CNN to analyze each frame of video. Its "crossing detector" can accurately distinguish between image blocks of a single fish and overlapping image blocks of multiple fish and solve the problem of fish crossing based on the information of the previous and next frames to generate high-precision movement trajectories. ZebraX can obtain the following key movement data: Speed - measured in body length per second (BL/s), eliminating the influence of body size differences; Acceleration - reflects the dynamic ability of speed changes during movement; Cumulative Movement - measures the overall movement activity of fish; Trajectory Path - analyzes the movement distribution of individuals in the experimental pool; Social Behavior - reveals the interaction pattern and consistency between multiple fish. The video is processed to

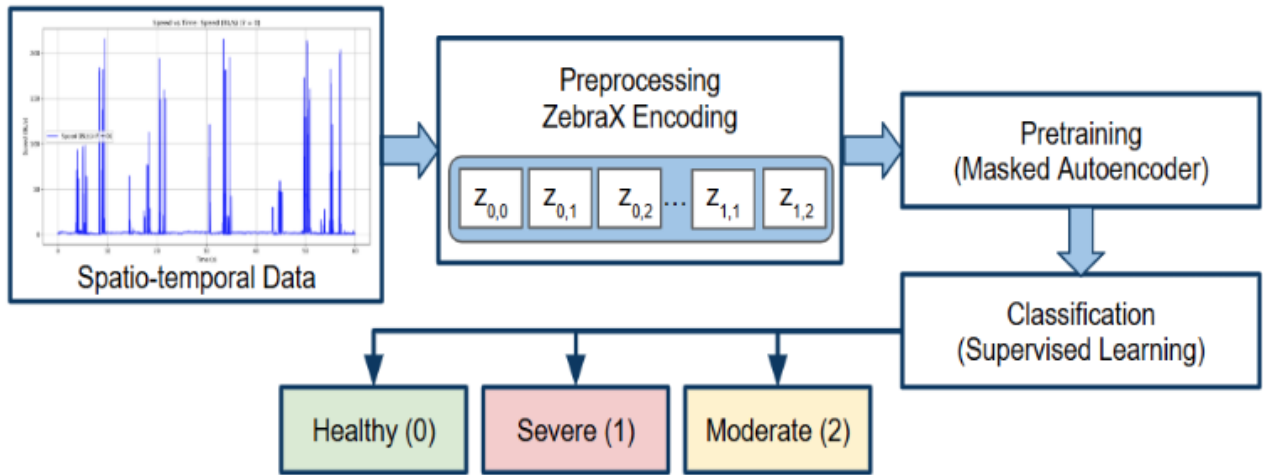


Figure 10: Encoding and classification process flow. Raw trajectory time-series data are encoded, pretrained, and classified into healthy, moderate, or severe diabetic states.

generate trajectory files of the fish, and the unique movement patterns of zebrafish are further revealed through smoothing and post-processing analysis. IDtracker is run on the provided zebrafish video, with a GUI for users to set the threshold through a slider, which separates the fish from the background and sets the target number of fish  $n$  to be identified and tracked. Then, IDtracker analyzes each frame of the video, performing all the tasks in the IDtracker algorithms, as described above. The result of IDtracker include the intermediate files such as the identified  $n$  number of fish, and the results in the form of trajectory files.

Set No.	X1	Y1	X2	Y2	X3	Y3
1	933.064	1354.959	1508.031	1193.066	885.782	1218.286
2	934.798	1355.196	1506.656	1190.638	885.356	1218.364
3	937.883	1355.922	1506.647	1189.965	895.322	1220.248
4	941.120	1356.412	1506.641	1189.109	905.109	1222.132
5	943.884	1356.947	1506.780	1188.699	914.985	1224.016
6	946.356	1357.577	1506.483	1187.619	924.861	1225.900
7	948.814	1358.032	1506.442	1186.098	933.682	1227.783
8	950.269	1358.431	1505.888	1186.088	944.614	1229.657
9	949.398	1358.435	1505.888	1184.947	954.491	1231.551

Table 1: The list 9 sets of coordinates of 3 zebrafish with their respective trajectories in X & Y axis.

An example of trajectory files is shown in Table 1, which lists 10 sets of coordinates of 3 zebrafish with their respective trajectories. In the second step, the above trajectory files (in .npy format) are fed into trajectory analysis tools and processed to generate the

distance, speed, acceleration, and other trajectory graphs. Postprocessing is then performed with increasing smoothing factors from  $\sigma = 0, 0.5, 1, \text{ to } 2$  in order to further reveal the patterns of zebrafish despite their fast movement. For example, zebrafish typically swim using "beat and glide" locomotion, with a quick and powerful swim phase, followed by a long gliding phase. These movement patterns turn out to be discernably different for zebrafish at different severities of diabetes.

After movement trajectories were generated, the ZebraX system extracted six behavioral metrics to describe each zebrafish (Figure 7). These included: speed ( $v$ ) - instantaneous velocity ( $\Delta d/\Delta t$ ), normalized by body length per second (BL/s), distance ( $d$ )- total path length over a set time, acceleration ( $a$ )- rate of velocity change ( $\Delta v/\Delta t$ ), curvature- degree of turning along the swim path, burst distance: cumulative distance during high-speed episodes ( $v > 2 \text{ BL/s}$ ), kinetic energy-composite index from mass, acceleration, and distance. Together, these six metrics formed the feature vector used by the machine learning classifier. Among them, Burst Kinetic Energy was the most sensitive indicator which can effectively distinguishing

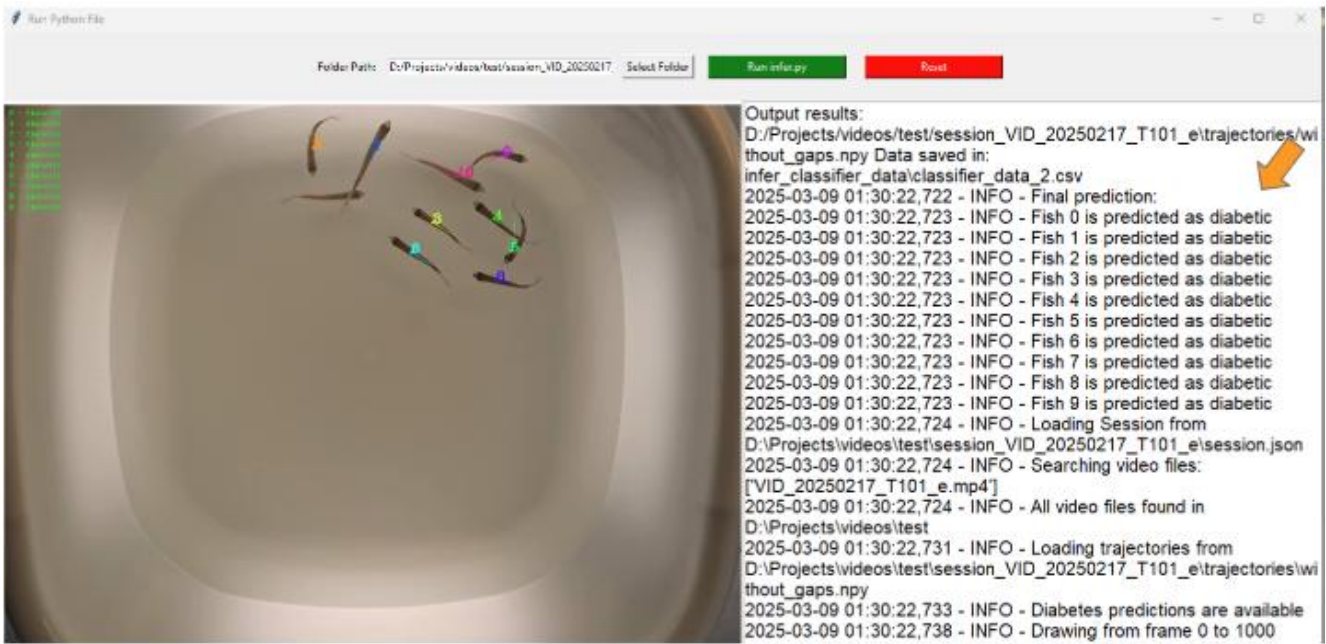


Figure 11: ZebraX AI Model Diagnosis. The program processes zebrafish trajectory data with the trained classifier and outputs whether each fish is predicted as healthy or diabetic; results are displayed in real time and saved as a CSV file for analysis.

healthy, moderate, and severely diabetic fish. The other metrics added important detail, especially for separating moderate from severe cases, resulting in a more accurate AI-based diagnosis. At its core, ZebraX can automatically classify the health status of zebrafish based on different behavioral data (Figure 10). The process extracts six kinematic metrics and encodes them into a structured format for deep learning analysis. An unsupervised Masked Autoencoder is first used to pretrain the model by reconstructing missing portions of the data. This step helps the system learn underlying movement patterns without labels, and improves its ability to capture zebrafish behaviors. The pretrained features are then passed to a Supervised Classifier that is fine-tuned on labeled data to classify zebrafish into three health categories: Healthy (0), Moderate (2), or Severe (1). This two phase design of unsupervised pre-training followed by supervised classification ensures robustness and accuracy of diagnosis.

The trajectories of diabetic zebrafish. As the fish

becomes more diabetic, the movement becomes more clustered around the origin. (A) the control group fish, (B) slightly diabetic fish, and (C) heavily diabetic fish. The speeds of diabetic zebrafish. While the more diabetic zebrafish have more frequent episodes of swimming, the speeds during these episodes are much higher in control zebrafish. (A) the control group fish, (B) slightly diabetic fish, and (C) heavily diabetic fish. The video is processed to generate trajectory files of the fish, and the unique movement. The speeds patterns of zebrafish are further revealed through smoothing and post-processing analysis. The performance metrics were calculated based on its

AI Model Performance Metrics				
Model	Accuracy	Precision	Recall	F1-score
Healthy		97%	80%	88%
Diabetic		78%	97%	86%
Macro Avg		87%	89%	87%
Weighted Avg		89%	87%	87%
Accuracy	87%			

Table 2: ZebraX AI Model Performance Metrics in Detecting Health & Diabetic Zebrafish. (Precision, Recall, F1-score)

ability to classify zebrafish into healthy and diabetic categories. As shown in Table 2, the model achieved an overall accuracy of 87%. For healthy zebrafish, the precision was 97%, recall was 80%, and F1-score was 88%. For diabetic zebrafish, the precision was 78%, recall was 97%, and F1-score was 86%. The macro and weighted average F1-scores were both 87%, which also shows together that the model maintains balanced and consistent performance across both classes. These results validate the robustness of ZebraX in accurately detecting and analyzing disease-related behavior. To benchmark the ZebraX system, we compared its performance with several widely used AI-based tracking tools, including DeepLabCut and ToxTrac. While these tools have proven effective in specific experimental setups, they face limitations when handling high-density fish populations or overlapping behaviors. In multi-fish tracking tasks with 5–7 zebrafish in a confined space, ZebraX maintained an identity consistency rate of 87.0%, surpassing DeepLabCut (76.4%), and ToxTrac (67.9%). Additionally, ZebraX demonstrated superior resilience in handling occlusions and overlapping trajectories due to its custom-designed crossing detector and adaptive identification protocols. Unlike DeepLabCut, which requires manual keypoint labeling, ZebraX operates fully automatically, streamlining analysis in high-throughput studies. These improvements illustrate ZebraX’s advantage in real-time, multi-animal behavior monitoring for biomedical research.

### 3.7..The ZebraX AI Diagnostic Tool

To make the AI models usable in practice, a diagnostic software tool with a graphical user interface (GUI) was created (Figure 11). This tool represents the final stage

of the ZebraX pipeline, combining multi-animal tracking and behavioral classification into a single streamlined workflow.

As shown in the interface, the user first selects a zebrafish video for analysis. The software then processes the recording in real time, displaying the movements of each fish in the main panel. At the same time, the output console on the right logs the process and provides a final prediction for each fish. Based on quantified behavioral patterns, the program classifies each animal as either healthy or diabetic. By turning complex analysis into an accessible application, this tool enables fast, automated, and objective screening of diabetes-related behaviors in zebrafish.

## 4. Experimental Results

During the 21-day experiment, the average weight of the zebrafish in the diabetic group increased by about

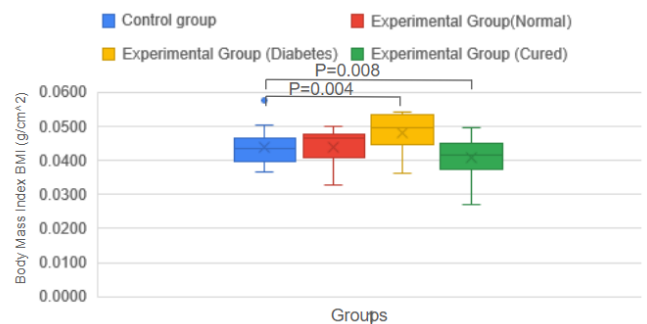


Figure 12: Zebrafish Body Mass Index in four groups

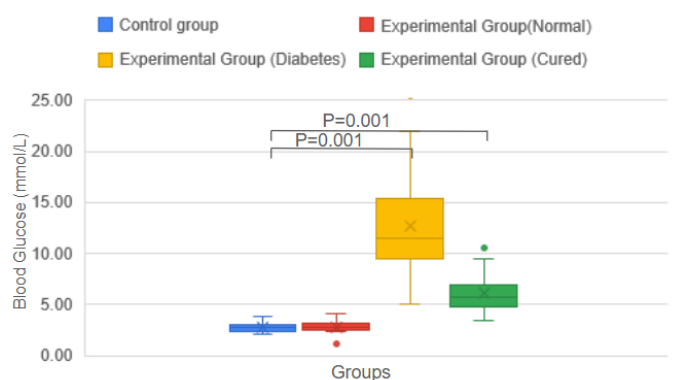


Figure 13: Zebrafish Blood Glucose level in four groups

15%, while the weight of the control group did not change significantly [14]. A visual comparison of body mass index (BMI) across all groups is shown in Figure 12. The blood glucose level of the diabetic group increased from 2.71 mmol/L at baseline to 12.73 mmol/L post-treatment. An unpaired two-tailed t-test confirmed that this increase was statistically significant ( $p < 0.01$ , 95% CI: [3.21, 3.91]), indicating successful induction of a diabetic model. Meanwhile, the control group remained stable at around  $4.6 \pm 0.2$  mmol/L, showing no significant changes. The impact of the herbal treatment on blood glucose levels is summarized in Figure 13. Following the 21-day experiment, the diabetic zebrafish were treated with 10 mg of mulberry leaf ( $C_6H_{13}NO_4$ ), 100 mg of aloe vera ( $C_{22}H_{21}O_9$ ) and 1 L of bitter melon extracts ( $C_7H_6O$ ) all purchased from Sigma-Aldrich where they offer high grade products [15] [16] [17]. blood glucose levels in the experimental diabetic group decreased significantly from 12.73 mmol/L to 6.08 mmol/L. The t-test yielded  $p < 0.05$  with a 95% confidence interval of the mean reduction: [1.38, 1.82 mmol/L], indicating a 52% decrease. The control group remained statistically unchanged at  $4.6 \pm 0.2$  mmol/L. These results indicate that mulberry leaf, aloe vera, and bitter melon extracts all have significant hypoglycemic effects, especially bitter melon extract. This statistical evidence supports the efficacy of the treatment and strengthens the reproducibility of our results. Moreover, the use of confidence intervals alongside p-values enhances transparency and reliability in evaluating treatment effectiveness. This is consistent with existing studies. For example, polyhydroxy alkaloids in mulberry leaves (such as 1-deoxynojirimycin) are its main hypoglycemic active ingredients, which reduce glucose absorption by

inhibiting  $\alpha$ -glucosidase activity, thereby reducing blood glucose levels [18]. In addition, flavonoids and polysaccharides in mulberry leaves have also been found to have blood glucose regulating effects. These findings provide strong support for natural treatments for diabetes.

To give a clear, step-by-step view of the experiment, images were used to show both the rise of diabetes and the recovery that followed herbal treatment. These visuals act as direct confirmation of the numerical data ( $1 \text{ mmol/L} \approx 18 \text{ mg/dL}$ ).

#### Phase 1: Diabetes Induction



Figure 14: Progression of blood glucose levels measured in zebrafish across control, moderate, and severe diabetic models over a two-week period.

The development of a diabetic state was tracked across three weeks. As shown in the top panel of Figure 14, control zebrafish maintained stable blood glucose within the normal range (0–100 mg/dL;  $\sim 0$ –5.6 mmol/L). After about one week on a high-glucose diet and immersion protocol, zebrafish showed signs of early to moderate diabetes, with blood glucose rising to 101–200 mg/dL ( $\sim 5.6$ –11.1 mmol/L). Over the next two weeks, the condition advanced to severe diabetes, with values consistently above 200 mg/dL ( $> 11.1$  mmol/L) and in some cases reaching 600–800 mg/dL ( $\sim 33$ –44 mmol/L). This stepwise pattern confirms the successful creation of a reliable hyperglycemia model.

#### Phase 2: Recovery with Herbal Treatment

The effects of herbal treatment were examined over



Figure 15: Blood glucose levels of zebrafish before and after four weeks of treatment.

four weeks. The bottom panel of Figure 15 shows severely diabetic zebrafish that began with glucose levels in the 300–600 mg/dL (~16.7–33.3 mmol/L) range. After four weeks of continuous exposure to the herbal extract mixture, blood glucose values dropped sharply, returning to the normal or mild range of 50–200 mg/dL (~2.8–11.1 mmol/L). These results provide strong visual evidence that the herbal treatment can reverse severe hyperglycemia and restore healthy glucose control in the zebrafish model.

Through the ZebraX model, there were significant behavioral differences between normal zebrafish and diabetic zebrafish. These results were obtained from 200 video recordings, generating approximately 1.44 million frames for analysis, as shown in Figure 16.

Specifically, the average swimming speed of zebrafish in the diabetic group decreased by about 30%, from approximate 2.5 cm/s to approximate 1.75 cm/s ( $p < 0.05$ ). In addition, diabetic zebrafish showed more irregular movement patterns, such as frequent sharp turns and pauses, and these abnormal behaviors may be related to neuromuscular dysfunction caused by high blood glucose. These behavioral characteristics can serve as potential behavioral indicators for diabetes and provide references for early diagnosis and intervention of the disease. Related studies have also shown that zebrafish will experience changes in

motor function under high sugar conditions, further supporting our findings.

## 5. Discussion

Among herbal remedies, bitter melon extract has the most significant blood glucose-lowering effect, followed by mulberry leaves and aloe vera. The plant extract synthesized from the three herbs has the best effect. It is the first known study to integrate both into a unified AI driven framework for diabetes research. This treatment method can also be applied to the health treatment of human diabetes, which is very valuable. Although our results are encouraging, there are still some limitations, mainly including the small sample size and short treatment duration. In this study, each group contained only 20 zebrafish, which is a small number of samples used for the early development stage. Later, the sample size will increase to conform current findings.

The integrated ZebraX platform, which combines automated AI-based behavioral analysis with the zebrafish model, offers applications that reach well beyond diabetes research. These can be grouped into three major areas: pharmacology, biomedical diagnostics, and neuroscience. In pharmacology, ZebraX can be used for fast and low-cost screening of new drugs and natural products, including herbal therapies. By measuring treatment effects in an objective way, the system provides a reliable method for early-stage drug testing. For biomedical diagnostics, the ability to classify health states from subtle behavior changes shows promise for reducing human error and supporting disease prediction. This approach could be extended to complex conditions such as heart disease, cancer, and obesity. In neuroscience, multi-fish

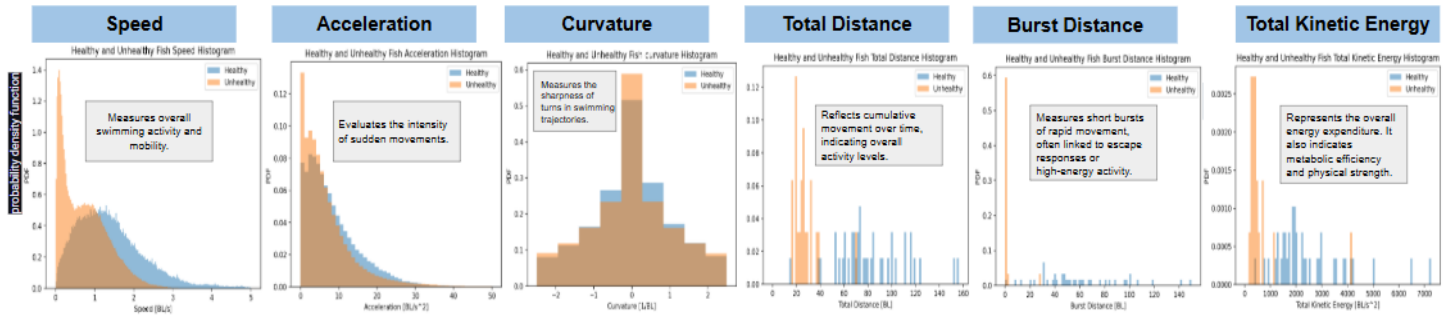


Figure 16: Behavioral Indicators Comparison of Healthy vs. Unhealthy zebrafish based on 200 videos (~1.44 million frames). The six behavioral metrics—speed, acceleration, curvature, total distance, burst distance, and total kinetic energy—exhibit significant differences between groups ( $p < 0.05$ ).

tracking and social interaction analysis allow detailed study of behaviors linked to disorders like anxiety, depression, and Alzheimer’s disease, where early symptoms are often behavioral. These applications highlight ZebraX as a flexible tool with impact across medicine with value far beyond diabetes.

## 6. Conclusion

ZebraX has been proven as a powerful AI-based solution for studying type 2 diabetes. ZebraX has successfully identified Glucose levels from Diabetic Zebrafish to Healthy Zebrafish. By analyzing zebrafish behavioral data with AI algorithms, ZebraX identifies specific behavioral indicators in the diabetic state, such as slowed swimming speed and abnormal movement trajectory. These behavioral changes provide new means for early diagnosis and efficacy evaluation of diabetes. The results also indicate that the diabetic fish went through treatment and within 28 days, their health has drastically improved. Therapeutic validation of natural treatments such as mulberry leaves, aloe vera, and bitter melon extracts was invented. The most significant effect was observed in blood glucose reduction, which decreased blood glucose by about 52% to 6.08 mmol/L ( $p < 0.001$ ). These findings establish zebrafish as a scalable model for diabetes research and demonstrate that the combination of artificial intelligence and natural therapies is a hope for

diabetes management and treatment. In summary, combining zebrafish models with ZebraX AI analysis provides new perspectives and methods for diabetes research and herbal treatment evaluation.

As the first study to present a novel AI-based approach to analyzing diabetes-related behavioral indicators in zebrafish and evaluating the therapeutic potential of herbal remedies, this study is currently ongoing. Future studies are planned to raise the sample size in order to strengthen the already obtained results. The work will also extend treatment durations to investigate the long-term efficacy and safety of herbal extracts. Furthermore, advanced artificial intelligence methodologies will be employed for unbiased detection and quantification of fluorescent signals, and assessment of the amount, distribution, and morphological changes of  $\beta$ -cells and further unravel mechanisms through which insulin acts in metabolic diseases.

## 7. REFERENCES

- [1] Saeedi, Pouya., Petersohn Inga, Salpea Paraskevi, Malanda Belma, Karuranga Suvi, & Unwin Nigel. (2019). Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and

- 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition.
- [2] NCD Risk Factor Collaboration (NCD-RisC) (2024). Worldwide trends in diabetes prevalence and treatment from 1990 to 2022: a pooled analysis of 1108 population-representative studies with 141 million participants. *Articles* Volume 404, Issue 10467p2077-2093 November 23, 2024
- [3] Howe, K., Clark, M. D., Torroja, C. F., et al. (2013). The zebrafish reference genome sequence and its relationship to the human genome. *Nature*, 496(7446), 498-503
- [4] Barreiros, M., Barbosa F. Dantas Diego.,(2021). Zebrafish automatic monitoring system for conditioning and behavioral analysis. *Nature open access* 29 April 2021
- [5] Zhao, A.P., Li, S., Cao, Z., Hu, P.J.-H., Wang, J., Xiang, Y., Xie, D., Lu, X.: AI for science: Predicting infectious diseases. *J. Saf. Sci. Resil.* 5, 130–146 (2024).
- [6] Choi, T.-Y.; Choi, T.-I.; Lee, Y.-R.; Choe, S.-K.; Kim, C.-H. Zebrafish as an animal model for biomedical research.(2021) *Exp. Mol. Med.* 2021, 53, 310–317
- [7] Konadu, B.; Cox, C.; Speed, J.; Gibert, Y. Zebrafish model of Gestational Diabetes(2021). *FASEB J.* 2021, 35.
- [8] Sawaki R, Sato D, Nakayama H, Nakagawa Y, Shimada Y (2019) ZF-AutoML: An Easy Machine-Learning-Based Method to Detect Anomalies in Fluorescent-Labelled Zebrafish. *Inventions* 4(4) 2019.
- [9] Lubin A et al (2021) A versatile, automated and high-through-put drug screening platform for zebrafish embryos. *Biol Open* 10(9):15.
- [10] Feng, Y., & Martin, J. (2023). Unlocking the Potential of Zebrafish Research with Artificial Intelligence. *Medical & Biological Engineering & Computing*.
- [11] Salehpour A, Rezaei M, Khoradmehr A, Tahamtani Y, Tamadon A(2021) Which Hyperglycemic Model of Zebrafish (*Danio rerio*) Suits My Type 2 Diabetes Mellitus Research? A Scoring System for Available Methods. *Front Cell Dev Biol.* 2021 Mar 15;9:652061.
- [12] Abdullah, N., Sharif Foez, Azizan Nur. (2024). In-house device fabrication for multiple blood sampling of adult zebrafish, *Danio rerio*. *ScienceDirect*, 20 June 2024.
- [13] Romero-Ferrero F., Bergomi M., Hinz R., Heras F., Polavieja G.(2019). idtracker.ai: tracking all individuals in small or large collectives of unmarked animals, *nature methods* 14 Jan 2019.
- [14] Zang L., Maddison L., Chen W., (2018). Zebrafish as a Model for Obesity and Diabetes. *Frontiers, Cell Dev. Biol* 19 Aug 2018.
- [15] Veldman, M. B., & Lin, S. (2023). Effects of the timing of acute mulberry leaf extract intake on postprandial glucose metabolism in healthy adults: a randomised, placebo-controlled, double-blind study. *Nature*, 77, 468-473(2023).
- [16] Araya, F., Gutierrez H., Cuyul Iv., Pavez, L (2021). Effectiveness of aloe vera in patients with type 2 Diabetes Mellitus and pre-diabetes: An overview of systematic reviews. *ScienceDirect*,.Vol 15, Issue 6 Nov 2021
- [17] Kim, B., Lee, H., Kim, H., Lee, H., (2022). *Momordica charantia* (bitter melon) efficacy and safety on glucose metabolism in Korean prediabetes participants: a 12-week, randomized clinical study.

*Springer Food Science and biotechnology*, 32(5),  
697-704.

- [18] Chen, S., Xi, M., Gao, F., Li, Mi., (2023). Evaluation of mulberry leaves' hypoglycemic properties and hypoglycemic mechanisms. *Frontiers, Pharmacol* Vol 14, 2023.

## Declaration of Academic Integrity

The participating team declares that the paper submitted is comprised of original research and results obtained under the guidance of the instructor. To the team's best knowledge, the paper does not contain research results, published or not, from a person who is not a team member, except for the content listed in the references and the acknowledgment. If there is any misinformation, we are willing to take all the related responsibilities.

Names of team members: Jasper Shi

Signatures of team members: *Jasper Shi*

Name of the instructor: Karen Dennis

Signature of the instructor: *KAREN DENNIS*

Date: 08/16/2025