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SHORT COMMUNICATION

Estimation of Anti-diabetic Activity of Polyherbal Seeds (Bitter Gourd and Black Pepper) on Zebrafish Model

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Abstract

Background: Diabetes mellitus is a chronic metabolic disorder. It affects 537 million people globally between the ages of 20 to 79, and 643 million individuals will have diabetes by 2030, and by 2045, that number will rise to 783 million. **Aim:** In this study, the Zebra zebrafish model was used for the estimation of anti-diabetic activity of polyhedral seeds (bitter gourd and black pepper). **Methods:** The bitter guard extraction was done by the acetone extraction method, and black pepper extraction was done by the Soxhlet extraction method using ethanol as a solvent. Diabetes was induced in the zebra fish by the immersion method. The fish were immersed in 2% glucose solution and 0% glucose solution on alternate days for 28 days. The blood glucose levels of diabetic zebra fish after diabetic induction were found to be 319±10.16 mg/dl. Then the zebra fish was divided into 5 groups. Group I was considered as a control group, Group II was considered as a disease control group, and Group III received a combination of Polypeptide-k (from bitter gourd seeds) and piperine (from black pepper) compounds at a dose of 0.1 g in 100 ml of water. Group-IV received Polypeptide-k only (from bitter gourd seed) at the dose of 0.1 g in 100ml. Group V received the Standard drug Metformin at a dose of 0.129 g in 100 ml of water. **Results:** Polypeptide-k and Piperine reduced the blood glucose levels of zebra fish after 28 days of treatment. Polypeptide-k-treated zebrafish reduced the blood glucose up to 163±9.55mg/dl, and Metformin treatment reduced the blood glucose up to 160±6.75 mg/dl in zebrafish. **Conclusion:** Based on the results, both the compounds have very good anti-diabetic activity, even though the combined treatment of both Polypeptide-k and Piperine shows very efficient anti-diabetic activity due to its synergistic effect.

Major Findings: Polypeptide-k from bitter gourd seeds and piperine from black pepper both have anti-diabetic activity by reducing the blood glucose level in zebrafish, and their combination product is more effective in reducing the blood glucose level due to its synergistic property.

Keywords: Anti-diabetic Activity, Bitter Gourd, Black Pepper, Piperine, Polypeptide-k, Zebra Fish

1. Introduction

Diabetes mellitus is a complicated metabolic disease that has a substantial impact on worldwide health and is defined by persistent hyperglycemia. It affects 537 million adults worldwide between the ages of 20 and 79. By 2030, 643 million people worldwide will have the disease, and by 2045, that number will rise to 783 million. With its escalating prevalence and associated complications, there is a pressing need for novel therapeutic approaches. Natural products, particularly polyherbal formulations, have garnered attention for their potential anti-diabetic properties, offering promising alternatives to conventional pharmaceuticals. Polyherbal formulations, derived from a combination of medicinal plants, have been integral to traditional medicine systems for centuries. Their diverse phytochemical composition often targets multiple pathways involved in glucose

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homeostasis, insulin sensitivity, and pancreatic function, making them attractive candidates for diabetes management[2]. In this study, polypeptide-K from bitter gourd and polypeptide P from black pepper were given as a combination and also given separately to evaluate the synergistic property of the compounds. Both of the seeds were used for the treatment of various diseases in traditional medicines such as *Ayurveda* and *Siddha* for several decades. Owing to its physiological similarities to humans, genetic tractability, and suitability for high-throughput screening, the zebrafish (*Danio rerio*) has become a promising model organism for drug development and disease modelling. Zebrafish possess conserved metabolic pathways and organ systems relevant to diabetes, making them well-suited for studying the efficacy of anti-diabetic agents[3]. Our investigation builds upon previous studies demonstrating the anti-diabetic effects of natural products in zebrafish models and contributes to the expanding body of evidence supporting the use of polyherbal formulations in diabetes management.

2. Materials and Methods

2.1 Materials

Acetone, Hexane, Potassium hydroxide, Sulfuric acid, Soxhlet apparatus, Ethanol, and the organism Zebrafish.

2.2 Methods

2.2.1 Collection and Maintenance of Zebrafish

Zebrafish blue striped (*Danio rerio*) at the age of 3 to 6 months (both male and female) were purchased from the aquarium at Kulathoor, Chennai. A circulating system that constantly filters and aerates the system water is used to keep zebrafish in an aquatic environment that is conducive to health. The room temperature or the tank temperature is generally maintained between 26-28.5 °C, and the lighting conditions are 14:10 h (light: dark). De-chlorinated water is used in the zebrafish system. The system water's pH should be monitored every day and kept between 6.8 and 7.5. Sodium bicarbonate should be used to raise pH levels when needed. Dry food (meal sizes range from 100 microns for larvae to 300/400 microns for adult fish) or live food (shrimp brine) can be fed to zebrafish[4], and the fish were kept at the Pharmacology laboratory, Karpagam Academy of Higher Education, Coimbatore, Tamil Nadu. The study period was 42 days (28 days for the induction of diabetes and 14 days for the treatment period) to evaluate the anti-diabetic property of the bitter gourd and black pepper.

2.2.2 Diabetes Induction

The method for inducing Type 2 Diabetes Mellitus (T2DM) in zebrafish involves exposure to 2% glucose and 0% glucose solutions on alternate days over a period of 28 days. Following this method, blood glucose levels were observed at the end of 28 days, and were found to be 319 mg/dl, which was four to five times higher than the normal state[5].

2.2.3 Preparation of Extract

The extract was prepared by a suitable extraction method (acetone extraction for bitter gourd and Soxhlet extraction for black pepper).

2.2.3.1 Bitter Gourd Extraction

In order to extract the polypeptide-k, the washed Bitter Gourd (*Momordica charantia*) seeds must be defatted using a 3:1 ratio of hexane to acetone (75 ml hexane to 25 ml acetone). The reason for using dry seeds is that, as a storage protein, polypeptide-K accumulates greatly when a seed is dried. Following de-fatting, the seeds were dried, ground into a powder, and dissolved in a 3:1 mixture of water and acetone (75 ml water to 25 ml acetone). Ammonium hydroxide is added to a mixture to bring the pH down to 9.5. After the supernatant was discarded, sulfuric acid was added to bring the pH to 3. The flocculent precipitate was collected. To remove oil, salts, and other unwanted particles, the dried bulk was pulverised and cleaned with water and acetone until it left a single spot in TLC[6].

2.2.3.2 Black Pepper Extraction

The solvent ethanol was utilised to extract piperine from black pepper seeds. Using a Soxhlet extractor, 20 g of powdered black pepper (95%) was extracted over the course of two to three hours. After filtering, the extract was concentrated at 60°C in a hot-air oven. In addition, 10 millilitres of a 10% potassium hydroxide solution were added to the filtrate and stirred continuously. After separating the remaining insoluble material, the alcoholic extract was filtered through a membrane filter and allowed to be kept overnight[7].

2.2.4 Administration of Extract to Zebrafish

The polyherbal extract (polypeptide-k + piperine) and polypeptide-k extract were administered to zebrafish for 14 days. The extract was not soluble in water, so the extraction was made in suspension by using 2% acacia solution. During the preparation of suspension, the extract was broken down to small particles, trituration was stopped when the tiny particles were able to be consumed by the fish, and then it was added to the 100 ml of water that contained zebrafish for treatment. The zebra fish were in contact with the extraction solution for 2 h as a treatment period[6].

2.2.5 Collection of Blood

Blood was collected from zebra fish by the tail oblation method. The fish are anesthetised by the addition of 6 drops of lignocaine then the anesthetised fish; float in the water were isolated separately. The blood was collected by cutting the tail using a surgical knife, and the blood was collected directly in the strip of a touch glucometer. The capillary tube in the strip accelerates the blood to the One Touch glucometer machine for the analysis of glucose[8,9].

2.2.6 Estimation of Anti-diabetic Activity

Anti-diabetic activity was estimated by measuring the blood glucose level of zebrafish using a One-Touch glucometer. The fish were divided into 5 groups, each group consist of 12 fish. Group I was considered as control group, Group II was the disease control group, which contained diabetes induced zebrafish. Group III received the treatment of polyherbal seed extracts, such as polypeptide-k from bitter gourd and piperine from black pepper, at a dose of 0.1 g in 100 ml of water. Group IV were treated only with piperine from black pepper at a dose of 0.1 g in 100 ml of water. Group V received the standard drug Metformin at a dose of 0.129 g in 100 ml of water (Table 1).

Table 1. Study the size of the zebra fish model

Groups	No. of Animals	Substance
GROUP-I	12	Control group
GROUP-II	12	Disease control
GROUP-III	12	Polypeptide-k from bitter gourd seeds and piperine from black pepper (0.1 g in 100 ml)
GROUP-IV	12	Polypeptide-k from bitter gourd (0.1 g in 100 ml)
GROUP-V	12	Standard group (Metformin 0.129 g in 100 ml)

Table 2. Change in blood glucose level during treatment

S. No.	Groups	Blood glucose level (before treatment)	Blood glucose level (after treatment)
1	Group I (control)	88±5.78	-----
2	Group II (disease control)	319±10.16	-----
3	Group III (polypeptide-k and piperine)	317.09±12.1	153±8.97
4	Group IV (polypeptide-k)	323.23±8.45	163±9.55
5	Group V (standard-metformin)	315±7.41	160±6.75

3. Results and Discussion

The findings of this study reveal promising insights into the anti-diabetic potential of compounds Polypeptide-k and piperine in zebrafish models. Our results demonstrate that when administered in combination, these compounds exhibit enhanced antidiabetic activity compared to individual administration. This was evidenced by a significant reduction in glucose levels, as measured using a one-touch glucometer. The individual administration of compound Polypeptide-k reduced the blood glucose level to 1.98-fold (323.23±8.45 mg/dl to 163±9.55 mg/dl), and the combined administration of polypeptide-k and piperine reduced the blood glucose to 2.1-fold (317.09±12.1 to 153±8.9). Standard drug metformin reduced the glucose level to 1.93-fold (315±7.41 mg/dl to163±9.55 mg/dl), but there was no change in the disease control group due to non-treatment. In the control group, the normal blood glucose level was found to be 88±5.78 mg/dl (Table 2). Furthermore, the method of blood collection from the tail fin of zebrafish proved to be effective for assessing glucose levels in response to the administered compounds. These results suggest a synergistic effect between compounds polypeptide-k and piperine, highlighting their potential for further exploration as antidiabetic agents.

4. Conclusion

In conclusion, our study demonstrates the enhanced antidiabetic activity of compounds polypeptide-k and piperine when administered together in zebrafish models. Through the measurement of glucose levels using a one-touch glucometer, we observed a significant reduction in blood glucose levels, indicating the potential of these compounds as effective antidiabetic agents. Moreover, the tail fin blood collection method proved to be a reliable technique for assessing glucose levels in response to treatment. These findings underscore the importance of exploring synergistic drug combinations for the management of diabetes and highlight the zebrafish model as a valuable tool for preclinical evaluation of anti-diabetic compounds.

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6. Author Contribution

Kannan Manivel formulated the principal research aims, B. Mohamed Bayas Khan performed the experiment and wrote the manuscript, S. Shyam Prakash statistically analysed the results. All authors have read and agreed to the published version of the manuscript.

7. References

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