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*Original Research Article*

# Antidiabetic Activity Assessment of *Syzygium jambos* Extract in Zebrafish Administered Alloxan

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ABSTRACT

Diabetes mellitus is a very common metabolic disturbance affecting the carbohydrate catabolic pathway. Not only carbohydrates but other macromolecules also fluctuate during this disease. Type 2 diabetes mellitus promptly originates because of the resistance of insulin functioning in the cells. The study assessed the anti-diabetic activity of the methanolic fruit extract of *Syzygium jambos* along with its wound-healing potential. Diabetes in the zebrafish was induced with 100-300 mg/kg of alloxan and various parameters were assessed, such as abnormality score assay, light and dark assay, acute and chronic toxicity, fasting and postprandial blood glucose level. After treatment of zebrafish with 1-7 mg/ml of methanolic fruit extract of *S. jambos*, most of the tested parameters were decreased. Also, wound healing was noted in the zebrafish in the form of fine regeneration after the administration of fruit extract (10µl/ml to 10mg/ml of extract).

***Keywords:*** Diabetes mellitus, *Syzygium jambos*, Zebrafish, Anti-diabetic, Wound healing.

## Introduction

Diabetes mellitus is a chronic metabolic-related complicated disorder in which the use of carbohydrates by the body is disturbed along with proteins and fat. Insulin deficiency or insulin non-functioning is the major cause of both acute and chronic conditions of this disease.[1] Globally, the incidence of diabetes mellitus is increasing.[2] WHO (World Health Organization) documented that millions of people are suffering from diabetes and the number will be expected to increase rapidly over time.[3] Usually, developing countries are dependent on plant medicines for their health needs.[4] Numerous plants are used in customary medicine systems.[5] The phyto-active components of medicinal plants possess anti-diabetic, anti-arthritic, anti-inflammatory and chemotherapeutic potencies.[6,7] The phyto-ingredients of plants are suitable alternative drugs to manage diabetes mellitus. Specifically, secondary active components such as phenolics, terpenoids, alkaloids, tannins and flavonoids aid in the reduction of blood glucose levels.[8] Several plants have been documented for their anti-diabetic efficacy.[9] The wound is physical damage and apt healing is indispensable for restoring damaged structures.[10] Sometimes, the wound may not be restricted to the surface but may be deeper, causing damage to vital structures such as tendons, muscles and blood vessels.[11,12] Wound healing generally occurs in four major phases and can be disrupted by many factors, such as lack of nutrition and diabetes mellitus.[13,14,15]

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The majority of people worldwide use traditional plant-infused medicines for healing wound because plants are considered potent healers with ease of availability and negligible toxicity.[16,17] Medicines marked for skin infection and wound treatment contain plant ingredients as the core along with a synthetic blend.[18,19] Presently, Zebrafish are used in the study of wound healing and to assess mechanistic mechanisms.[20] It is vastly used in experiments, in contrast to other animal models, because of its smaller size and ease of maintenance.[21,22] Moreover, the textural patterns of zebrafish are similar to those of human skin. These findings make zebrafish useful for the study of diabetes mellitus and wound healing.[23] *Syzygium jambos* (L.) of the Myrtaceae family are medium-sized shrubs distributed in tropical countries. The fruits are known as Malabar plum/rose apples and have a sweet flavor. The fruit has a centric, brownish, hard seed.[24] In traditional practices, it is applied for its anti-inflammatory and anti-pyretic effects.[25] In addition, it is used to cure respiratory complications, wound healing, sexually transmitted diseases and leprosy.[26] The glycosides and flavonoids of fruits have anti-inflammatory properties.[27,28] As a tonic, the fruit is a good healer for liver and brain-related diseases, along with improving diuretic function.[29] The fruit of *S. jambus* contains vital micronutrients.[30,24]

## Material and Methods

*Sample Collection*

*S. jambos* (L.) fruits were collected on July 2020 from the Yalagiri district, Tamil Nadu, India. The voucher number of the plant material is LS ID: IPNI 20013498-1.

*Extract preparation*

The collected fresh plants were dried and ground to a fine powder. The plants (200 g) were extracted with methanol solution (500 ml), for 1 h in a Soxhlet apparatus. The fruit extracts were dried at 50° C applying a vacuum rotary evaporator and stored for further use. In the methodology, approximately 750 ml of methanol was used.

*Identification of Phyto-chemicals*
To note the phyto-ingredients in the methanolic fruit extract of *S. jambos*, LC-MS analysis was performed using reconstitute of dried extract in LC-MS grade methanol and water (50:50) (20 ml:20 ml).

*Experimental Zebrafish*
Zebrafish (adult) were taken for experimental purposes and maintained according to standard guidelines. Under light conditions, they were placed for 14 h in the dark and 10 h in the dark and fed twice daily with fish pellets.

*Induction of diabetes mellitus*
The zebrafish was induced with diabetes mellitus using 600 mg/kg of alloxan for 2 days and then given 2% and 10% glucose solution for 10 days. On the 7$^{th}$ day acute screening was performed and on the 14$^{th}$ day chronic toxicity screening was performed.

*Experimental Design in Zebrafish*
The zebrafish were taken and separated into 6 groups, each containing 20 zebrafish. The 10 µL/ml, 100 µl/ml, 1 ml/ml and 10 mg/ml of extract in varying dilutions and alloxan were dissolved in distilled water and fed to the zebrafish for three days for five weeks. Grouping of zebrafish was as follows: Group I: Control; Group II: Standard drug (Metformin); Group III: model; Group IV: diabetic zebrafish treated with extract dilution 1; Group IV: diabetic fish treated with extract dilution 2: Group V: diabetic zebrafish treated with extract dilution 3: and Group VI: diabetic fish treated with extract dilution 4.

*Determination of fasting blood glucose levels*
Zebrafish were euthanized by Sodium Pentobarbital and blood was collected in a sterile glass slide by laceration at the tail section to check blood glucose levels using a glucometer strip.

*Fin amputation*
Fin amputation was performed in diabetic zebrafish and the potency of fin regeneration was assessed after treatment of zebrafish with 1-7 mg/ml of methanolic fruit extract of *S. jambos*.

*Pancreas Immunohistochemistry*
The pancreas was taken from diabetic as well as treated zebrafish and histopathological study was performed to assess the changes occurring in the pancreas of diabetic zebrafish as well as the zebrafish which were subjected to fruit extract treatment. Using a microtome, thin sections of the pancreas were made and the tissue slice was placed gently on the glass slide and then stained with hematoxylin and eosin. Finally, the morphological alterations in the pancreas were noted and the images were captured. The diabetic and fruit extract treated zebrafish models were anaesthetized by applying tricaine (0.02%) and the caudal fin of the zebrafish was amputated with the help of a sterile scalpel.

*Light/dark assay and abnormality score assay*
Light and dark assay was performed to check the anxiety like behaviour of the zebrafish were noticed before and after treatment along with the standard control. Similarly, the abnormality score assay was performed.

*Blood glucose analysis*
The fasting as well as post-prandial blood glucose was checked for the zebrafish model from each group. The zebrafish were taken and the laceration was done at the tail section. The blood was collected in a sterile glass slide immediately using a glucometer device and the blood sugar level was checked.

*Histopathology of zebrafish pancreas*
The zebrafish were euthanized and the pancreas was taken out carefully and fixation was done for 24 h using Bouin. After fixation, the pancreas was treated with EDTA to remove the calcium (decalcification). This was done for 24 h and using ethanol (90%), the pancreas was dehydrated and then treated with xylene. Using paraffin pancreatic tissues were embedded and then in a microtome the pancreas was placed at the sample holder site and the thin tissue slices (5 µm) were made. The sliced pancreatic tissue was carefully placed on a sterile glass slide using H-E (hematoxylin-Eosin) stain the tissues were stained and placed under the microscope to notice the cellular abnormalities. Finally, using a digital camera the pancreatic tissue images were grasped and presented.[31]

Using 0.1% FA and 100% acetonitrile in water at a flow rate of 0.3 ml/min for 30 min, the extract was eluted in gradient mode. The NIST library was used to infer the structure and function of 54 distinct phyto-compounds. In diabetic studies, an abnormality score typically refers to a quantitative measure used to assess the severity or presence of abnormalities related to diabetes or its complications in individuals. This score plays a crucial role in clinical research and healthcare practice by providing a standardized way to evaluate disease progression, treatment effectiveness and overall patient health status. The abnormality score of adult zebrafish was analyzed at different dilutions of plant compounds. Assessing body weight before the onset of type 2 diabetes (T2D) is crucial for understanding its relationship with the development and progression of the disease. This assessment involves examining various factors related to body weight, such as BMI (Body Mass Index), waist circumference, body composition and others. The body weight was calculated before induction of diabetes in zebrafish.

*Statistical analysis*
All data were expressed as mean ± SD. All trials were done trice (n=3).

*Ethical approval*
This study has been approved by the ethical committee for the animal experimental study using zebrafish as 2084/PO/RcBt/S/19/CPCSEA.

## Results and Discussion

*LC-MS Profiling of S. jambos extract*
The LC-MS chromatogram revealed 54 different phyto-compounds in the *S. jambos* methanolic extract (Fig. 1). Clobenzorex is a chemical that showed an unprecedented rise, followed by proacacipetalin. Clobenzorex, (molecular formula $C_{16}$ $H_{18}$ $C_l$ N) (Asenlix, Dinintel, Finedal and Rexigen) is a stimulant drug of the amphetamine class used as a suppressant. Proacacipetalin (molecular formula $C_{11}$ $H_{17}$ N $O_6$) is a cyanogenic glycoside found in *Acacia terminalis* that has antioxidant activity. A bio-active compound N-(1-Deoxy-1- fructosyl) methionine (molecular formula $C_{11}$ $H_{21}$ N $O_7$ S) is a methionine derivative that mimics reducing the oxidative stress caused during diabetic nephropathy.[32] GYKI 52895, another bio-active compound was identified that inhibits the uptake of dopamine in cells.

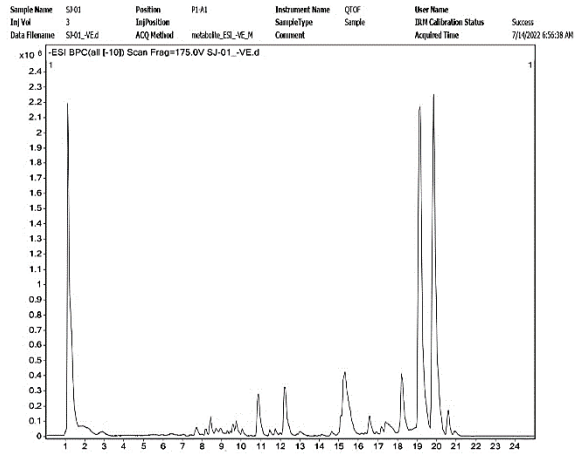


**Figure 1:** LC-MS chromatogram of *S. jambos* methanolic fruit extract

Myricetin (molecular formula $C_{15}H_{10}O_8$), a bio-active compound present in substantial amounts exhibits anti-oxidant and anti-diabetic activity. It can also prevent Alzheimer's disease and Parkinson's disease.[33] Another bio-active compound viz. 2',3'-Cyclic adenosine monophosphate acts as a signalling molecule in plants and is involved in metabolic pathways.[34] Retigabine (molecular formula $C_{16}H_{18}FN_3O_2$) is a pharmacological derivative.[35] Leukotrienes were found in trace amounts in the extract. It acts as an anti-convulsant and is used for treating partial epilepsy. It is a positive allosteric modulator and the first sodium-potassium opener for the treatment of epilepsy.[36] Phthalazine azelastine (molecular formula $C_{22}H_{24}ClN_3O$) is an antihistamine used in the treatment of allergic rhinitis and also prevents virus entry into cells, especially against SARS-CoV-2.[37] The compound known as flumethasone pivalate (molecular formula $C_{27}H_{36}F_2O_6$) is a glucocorticoid, a 3-oxo-Delta (1), Delta (4)-steroid, 11 beta-hydroxy steroid, 17 alpha-hydroxy steroid, 20-oxo steroid, a fluorinated steroid and a tertiary alpha-hydroxy ketone. It functions as both an anti-pruritic and anti-inflammatory agent. It is comparable to flumethasone in terms of functionality.[38] Alvimopan (molecular formula $C_{25}H_{32}N_2O_4$) is an opioid antagonist that is used to shorten the time that the upper and lower gastro-intestinal tracts heal after bowel resection surgeries involving primary anastomosis. B4 (molecular formula $C_{28}H_{44}N_2O_8S$), a di-hydroxy derivative of arachidonic acid is a putative mediator of inflammation and exhibits chemotactic and chemokinetic activity toward phagocytes both in *vitro* and *vivo*. It also has a unique stimulatory effect on the significant functional responses of phagocytes.[39]

*Investigation of type 2 diabetic acute toxicity in adult zebrafish fed S. jambos fruit extract*

Acute toxicity in individuals with type 2 diabetes typically refers to a situation where the body experiences a sudden and severe reaction due to the ingestion or exposure to a substance that adversely affects glucose metabolism or other physiological functions in individuals with diabetes type 2.[40] One of the most critical concerns in patients with type 2 diabetes is an accidental overdose of insulin or other glucose-lowering medications.[41] Insulin is essential for managing blood glucose levels. However, excessive intake can lead to dangerously low blood glucose levels (hypoglycemia).[43] Symptoms can range from mild shaking and confusion to seizures and loss of consciousness if not treated promptly.[42] Studying the effects of type 2 diabetes in zebrafish models using light and dark assays offers valuable insights into the effects of diabetes on behavior and physiology.[44] Zebrafish are increasingly utilized in research due to their genetic similarity to humans, rapid development and transparent embryos that allow direct observation of organ development and function.[45] The present study demonstrates that zebrafish behavior in response to light and dark cycles can be indicative of their physiological state. Typically, zebrafish exhibit increased activity in the light (scototaxis) and decreased activity in darkness (phototaxis). Changes in this behavior can indicate changes in metabolic regulation, stress responses and neurological function associated with diabetes. Stress and anxiety levels of adult fish treated with the plant compound at various concentrations (dilutions). The model spent the highest time on the dark side, whereas the control spent the least. Dilution 1 (10µL/ml) shows slightly similar activity to that of positive control drugs, as it spends less time on the darker side. The dilution of plant extracts 2, 3 and 4 exhibits gradually increased in the dark side compared with the control compounds (Fig. 2. a).

*Abnormality Score*

The results of the abnormality score of adult zebrafish revealed that dilution 1 showed an almost equal response to control drugs. Dilution 2, 3 and 4 exhibit slightly higher abnormality scores than the control and dilution 1 but lower abnormality scores than the model (Fig. 2. b). The model was performed in comparison to the control and all dilutions of plant compounds which indicated the highest therapeutic efficacy.[46,47]

*Assessment of body weight before type 2 diabetes*

The results of the assessment of the body weight before induction of diabetes indicate that: Dilution 2 and dilution 3 had the least weight among the treated groups, whereas dilution 1 was closely followed. The Control (0.68 g) and model (0.69 g) groups exhibited similar patterns of body weight but higher than the treated group at dilutions 1, 2 and 3 (0.55 g, 0.51 g, 0.52 g) (Fig. 2. c). Dilution 4 had the highest body weight (0.87 g).[48,49]

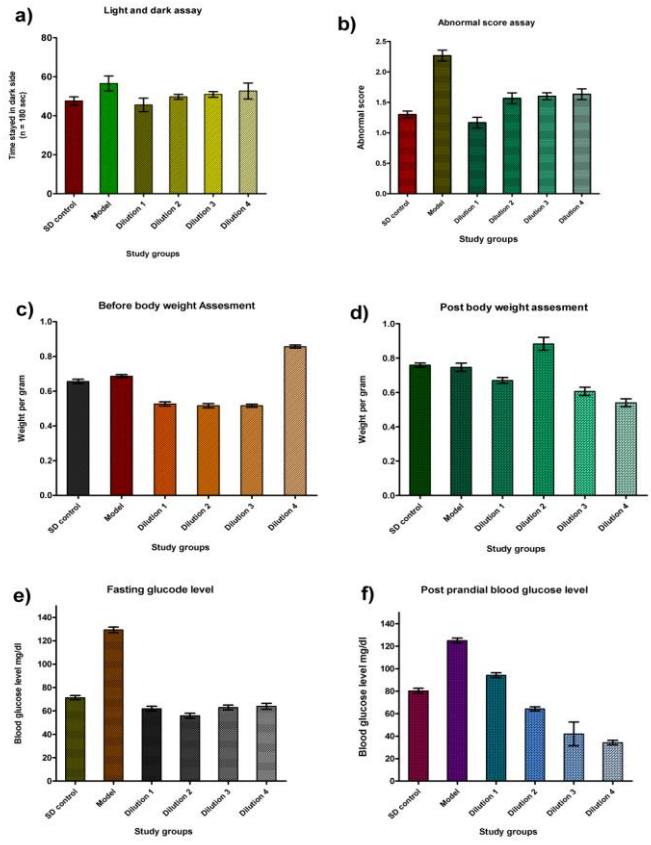


**Figure 2 (a-f):** Screening of type 2 diabetic chronic toxicity in adult zebrafish using the methanolic fruit extract of *S. jambos*

*Assessment of body weight after treatment in patients with type 2 diabetes*

Assessing body weight parameters before the onset of type 2 diabetes is essential for identifying individuals at risk and implementing preventive measures.[50] The assessment involves evaluating BMI, waist circumference, body composition and biomarkers associated with metabolic health to guide early intervention strategies and improve long-term health outcomes.[51] Body weight measurements were carried out to identify changes in weight in adult zebrafish, which were tested with plant extract at different dilutions. Dilution 2 had the highest weight (0.95 g) in comparison with the control (0.78 g). Compared with the other groups, control, model and dilution 1 groups were equally increased (Fig. 2. d). Dilution 3 and 4 have the least weight among the compound treated groups as 0.62 g and 0.58 g.

*Fasting blood glucose level*

Fasting blood glucose level (FBG) is a fundamental measure used in the diagnosis, management and monitoring of diabetes mellitus and related metabolic conditions.[52] The concentration of glucose in the bloodstream after an overnight fast typically for 8-12 h.[53] Fasting blood glucose levels were tested in diabetic-induced zebrafish to determine the therapeutic efficacy of the plant extract. Different dilutions of the plant crude extract were used. The model exhibited the highest glucose levels compared with dilution 2. The control (75 mg/dl), dilutions 1, 3 and 4 showed similar and lesser blood glucose levels than the model which

were 66 mg/dl and 67 mg/dl and the model had 134 mg/dl as blood glucose values (Fig. 2. e). Therefore, plant crude extract has significant therapeutic efficacy against type 2 diabetes.

*Postprandial glucose level*

Postprandial glucose level (PPG) refers to the blood glucose concentration after a meal.[54] Monitoring PPG levels is crucial in managing diabetes mellitus and assessing overall glycemic control.[55] In this test model dilution 1 has the highest glucose level compared with dilution 4, exhibiting the lowest blood glucose level, which indicates the significant anti-diabetic activity of the plant extract. The control and model groups had slightly higher blood glucose levels (81 and 129 mg/dl) than other dilutions. Dilutions 2, 3 and 4 exhibit blood glucose levels in the declining phase by exhibiting lesser blood glucose levels after a meal as 65, 55 and 38 mg/dl (Fig. 2. f).

Chronic toxicity typically refers to the toxic effects of a substance over time or with chronic exposure. In the context of type 2 diabetes, chronic toxicity refers to the long-term harmful effects or complications arising from the chronic management of diabetes and its associated treatments.[56] Many medications used to treat type 2 diabetes, such as certain oral hypoglycemic agents or insulin, can have potential side effects that manifest over time. These factors could include kidney or liver issues, cardiovascular problems and an increased risk of certain cancers.[57] Chronically elevated blood glucose levels can lead to various complications over time, affecting organs such as the kidneys, eyes, nerves and heart.[58] Chronic exposure to high glucose levels can contribute to diabetic nephropathy (kidney disease), retinopathy (eye disease), neuropathy (nerve damage) and cardiovascular diseases.[59] Overall, experiments involving light and dark conditions in diabetes research contribute to understanding the complex interactions between circadian rhythms, light exposure and metabolic health.[60] These findings may lead to strategies for optimizing treatment and management approaches tailoring to individuals with diabetes, considering their daily light exposure patterns and circadian biology.[61] In this study, the light and dark assay showed that the model spent the highest time on the dark side, whereas control and dilution 1 spent the least. Dilution 2 shows a similar activity as control, as it spends less time on the darker side (Fig. 3. a). Dilutions 3 and 4 show slightly longer time on the darker side and lesser than model as a significant result of the anti-diabetic effect of the plant extract.

*Abnormality score assay*

Abnormality scores are designed to quantify deviations from normal physiological or bio-chemical parameters associated with diabetes.[62] These parameters could include blood glucose levels and HbA1c (glycated hemoglobin), insulin resistance indices, lipid profiles and markers of diabetic complications like nephropathy or retinopathy.[63] Abnormality scores were tested at several dilutions of the plant extract. In dilution 1, similar activity was noted to that of the positive control. The model performed in contrast to standard control. Dilution 2, 3 and 4 yielded moderately higher abnormality scores (52.1 sec, 53.4 sec and 53.7 sec) than control and dilution 1, which was 51.9 s but lesser than model (63.4 sec) (Fig. 3. b) which indicates significant therapeutic efficacy.

*Body weight assessment at postprandial*

In diabetic studies, body weight is a critical component that provides valuable insights into various aspects of the disease, its progression, management and associated complications.[64] Weight measurements were performed to monitor the increase or decrease in weight in adult zebrafish treated with the plant extract. Dilution 4 exhibited the least body weight (0.41 g) among the other treated groups, whereas control (0.56 g) and dilution 2 (0.56 g) exhibited similar and slightly higher weights than dilution 2. The model exhibited higher body weight than the control (Fig. 3. c). Dilution 1 exhibits the highest body weight among other groups as 0.69 g.

*Fasting glucose levels in chronic toxicity*

Fasting blood glucose levels are a cornerstone of the diagnosis and management of diabetes mellitus. As a key indicator of glycemic status, it guides clinical decisions to optimize treatment outcomes and reduce the risk of diabetes-related complications.[65] Regular monitoring of FBG levels is essential for maintaining overall health and quality of life among individuals with diabetes.[66] The model had the highest glucose level, whereas dilution 4 had the lowest glucose level (35 mg/dl) (Fig. 3. d). The control exhibited the second highest blood glucose level (102 mg/dl), while dilution 1 indicated blood glucose levels of 61 mg/dl. Dilutions 2 and 3 exhibited slightly lower glucose levels (52 and 50 mg/dl) than the control, model and dilution 1.

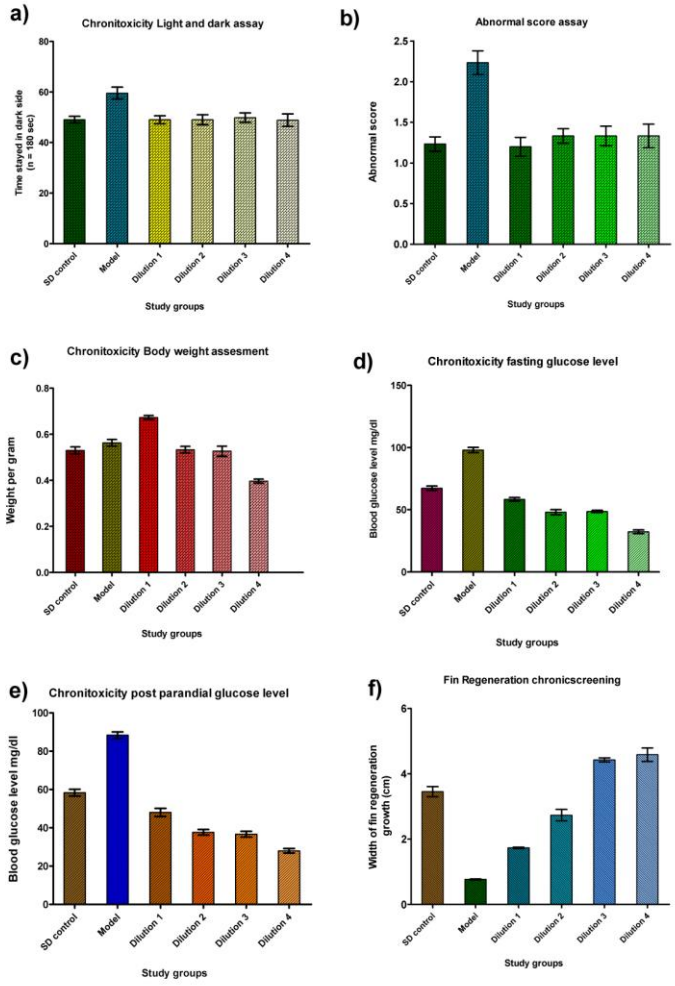


**Figure 3 (a-f):** Zebra fish subjected to light and dark assay, abnormal score assay, body weight assessment, fasting blood glucose, postprandial blood glucose, and fin regeneration

*Postprandial glucose level in chronic toxicity*

Postprandial glucose level measurements play a pivotal role in diabetes management by providing insights into the immediate glycaemic response to meals.[67] Monitoring PPG levels, along with FBG and HbA1c, guides healthcare providers in optimizing treatment strategies and reducing the risk of diabetes-related complications, ultimately enhancing the quality of life for individuals with diabetes.[68] The model had the highest glucose level, whereas dilution 4 exhibited the lowest blood glucose level. The dilution 1 exhibits a lower blood glucose level (51 mg/dl) than control (and mod1 mg/dl) (Fig. 3. e). Dilutions 2, 3 and 4 exhibit a declining phase by exhibiting lower blood glucose levels (40, 39 and 30 mg/dl) after a meal as a result of the significant therapeutic efficacy of the plant extract.

*Chronic fin regeneration*

Chronic fin regeneration refers to the ongoing or prolonged process by which fish can regenerate their fins after injury or amputation.[69] This phenomenon is particularly noteworthy in scientific research because of its potential implications for understanding tissue regeneration and wound healing in vertebrates.[70] The fin regeneration study was conducted to identify the wound-healing capacity of fish with diabetes after treatment with the plant extract. In the amputated day experiment, each dilution had the potential to heal the wounds. The fin regeneration model exhibits a lower regeneration capacity compared to dilutions 4 and 1, which yield the highest regeneration capacity (Fig. 4). Other dilutions, such as 2 and 3 have shown minimal amount regeneration. The chronic screening model possessed the lowest fin regeneration capacity compared to the positive control. Dilutions 1, 2, 3 and 4 exhibited higher wound healing capacity than the control (Fig. 4) and other treated groups because of the significant therapeutic efficacy of the plant extract.

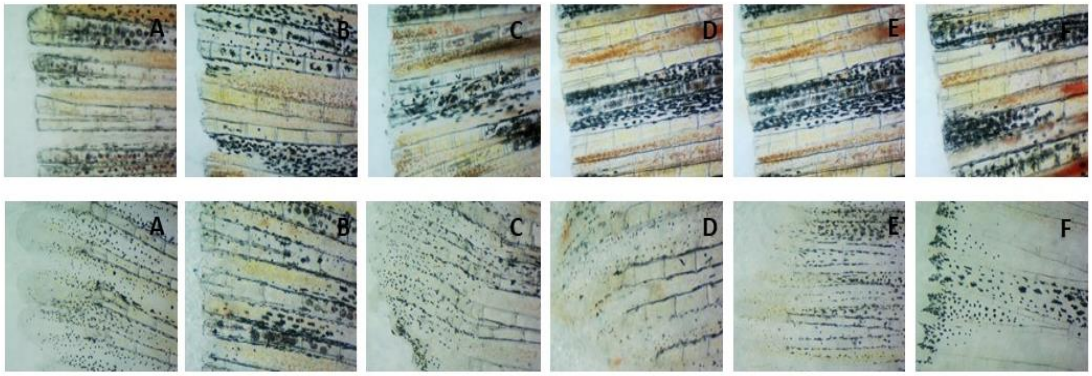


**Figure 4:** Chronic fin regeneration in adult zebrafish treated with *S. jambos* methanolic fruit extract

*Pancreatic histopathology*

Pancreatic histopathology involves microscopic examination of pancreatic tissue to assess structural changes, cellular abnormalities and pathological features indicative of various diseases and conditions affecting the pancreas.[71,72]

*Acute Screening*

In this study, the positive control indicated no significant changes, indicating healthy cells; the model indicated pancreatic islet cell hyperplasia and few cells exhibited inflammation and degeneration. Dilution 1 indicates pancreatic islet cell hyperplasia and dense inflammatory infiltration in the pancreas. Dilution 2 indicates pancreatic islet cell hyperplasia and few cells exhibit inflammation and degeneration. Dilutions 3 and 4 exhibited mild or no islet cell hyperplasia and degeneration similar to control due to the plant extract's significant effect (Fig. 5). In chronic screening control, drug indicates no significant changes, which indicates healthy cells; model indicates pancreatic islet cell hyperplasia and few cells exhibit inflammation and degeneration. Dilutions 1 and 2 indicate pancreatic islet cell hyperplasia and dense inflammatory infiltration. Dilutions of 3 and 4 indicate very little presence of degenerative and inflammatory cells because of compound treatment which is comparable to the control.

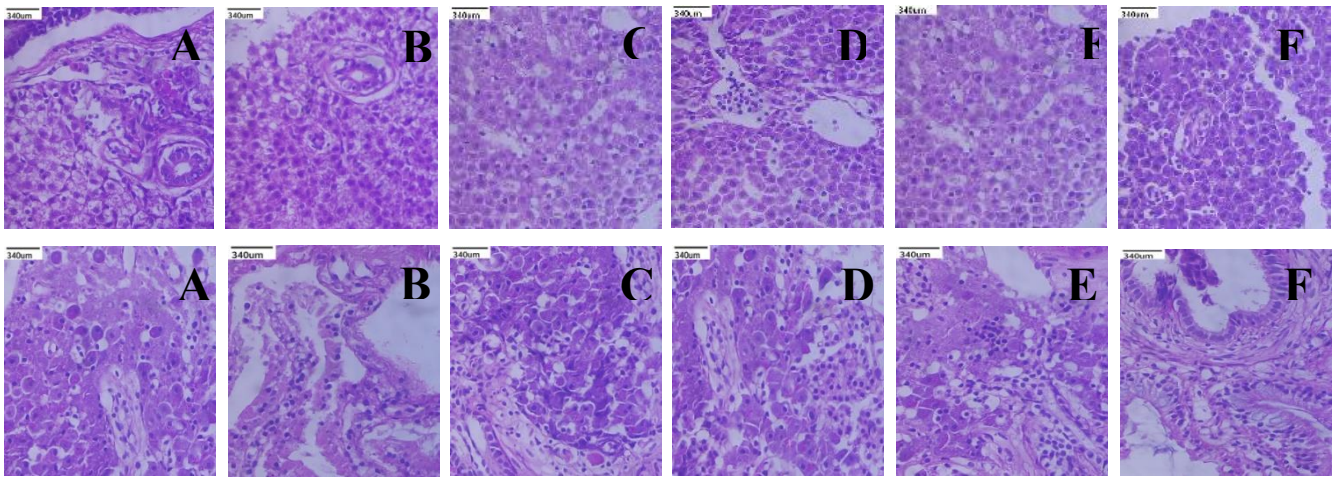


**Figure 5:** Pancreatic histopathological views of acute and chronic screening in diabetic induced adult zebrafish treated with *S. jambos* methanolic fruit extract

*Mortality rate*

In studies on diabetes, mortality rates play a crucial role in assessing the impact of diabetes on overall health outcomes and mortality risk.[73-77] The table presents of the mortality rate of adult zebrafish from the start to the end of the study. There is no mortality in control and dilution 1, while dilution 2 and dilution 4 exhibit a single mortality rate. The model exhibited the highest mortality rate of 50% of the total population (Table 1).

**Table 1:** Mortality rate of adult zebrafish treated with *S. jambos* methanolic crude fruit extract

| | Mortality rate |
|---|---|
| **Study group** | **Death** |
| Model | 5 |
| Control | 0 |
| D1 | 0 |
| D2 | 1 |
| D3 | 0 |
| D4 | 1 |

## Conclusion

The methanolic fruit extract of *Syzygium jambos* decreased various assessed parameters in the diabetic zebrafish model. The study also authenticated the wound-healing properties of the fruit extract identified through fin regeneration in a diabetic model of zebrafish. Overall, the study showed, the antidiabetic efficacy of the fruit extract of *Syzygium jambos*. Studies (*in vivo*) and gene expression in diabetes are needed.

## Conflict of Interest

The authors declare there is no conflict of interest.

## Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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