Research Article

Preclinical Assessment and Scientometric Analysis of Habenaria edgeworthii in Experimental Type 2 Diabetes Mellitus

Sharayu Ingale[1*], Ms. Pranali V. Patil[2], Ms. Shreshtha U. Kadam[3], Ms. Shradha D. Shirdhone[4], Ms. Aishwarya Patil[5], Mrs. Parveen Nadaf[6], Ms. Supriya Shete[7], Sakshi Belamkar[8]

[1*]Dr. Shivajirao Kadam College of Pharmacy, Kasbe Digraj, Sangli

(Corresponding Author)

[2]Assistant Professor, Marathwada Mitra Mandal's College of Pharmacy, Thergaon, Pune

[3]Assistant Professor, Sarojini College of Pharmacy, Kolhapur

[4]Assistant Professor, Anandi Pharmacy College, Kolhapur

[5,6]Assistant Professor, Dr. J.J. Magdum Pharmacy College, Jaysingpur

[7]Assistant Professor, Ashokrao Mane College of Pharmacy, Peth Vadgaon

[8]Workflow Specialist, Tata Consultancy Services

Abstract

Introduction: One of the biggest worldwide health crises of the modern era is diabetes. A growing number of individuals are affected by this medical condition every year, which can lead to problems that affect lives. Medicinal plants and their products are still a valuable medicinal tool for curing human illness, plant-based antidiabetic treatments have been widely used since ancient times. In this area of study, the potential prevention of diabetes of *Habenaria edgeworthii* and its medicinal potency responsible for the hypoglycaemic activity have been studied.

Materials and Methodology: Acute oral toxicity was evaluated as per OECD guidelines. In vitro antioxidant activity was assessed using DPPH method. In-vitro methods such as alpha-amylase inhibitor activity, Alpha-Glucosidase Enzyme Inhibition Assay, Evaluation of glucose uptake by yeast cells were performed.

Oral Glucose Tolerance Test was performed. Animal models such as albino rats and zebrafish were used to examine in-vivo antidiabetic activity. The methods such as antidiabetic study using suitable animal model Low dose streptozotocin and High Fat Diet fed Rodent model and Inducing DM by glucose immersion method in zebra fish were used for in-vivo activity. Retinopathy was also studied in zebrafish. Physical, biochemical and other parameters were evaluated.

Results: The extract demonstrated significant in vitro DPPH radical scavenging activity. AOT showed that MEHE was safe up to 2000 mg/kg with no mortality observed. In vivo studies demonstrated a significant decrease in blood glucose levels was seen upon increasing the dose for *Habenaria edgeworthii* extract. In vitro, MEHE indicated effective inhibition of both the alpha-amylase and alpha-glucosidase enzymes. Also, percentage of glucose taken was considerable and exhibited dose-dependent action.

In-vivo studies in rats showed that there was also a notable decrease in the amount of glucose in the blood starting in the subsequent week of therapy. A significant decrease in blood glucose levels was seen upon increasing the dose for *Habenaria edgeworthii* extract, the mean levels of TC, TG, and LDL were also significantly lower after receiving a larger dose of the MEHE. histopathological examination was performed which showed good recovery of islet cells of the pancreas upon higher dose administration of MEHE. In zebrafish, the treatment group also demonstrated a subsequent drop in blood glucose level (BGL). In the retinopathy investigation, optomotor responses likewise showed positive outcomes in test groups.

Conclusion: The outcomes demonstrate that *Habenaria edgeworthii* has effective antioxidant and anti-diabetic properties, whereas body weight also had an influence.

Keywords: Diabetes Mellitus, *Habenaria edgeworthii,* blood glucose level, albino rats, zebrafish

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1. Introduction:

415 million individuals who are projected to have diabetes at current moment, an

extra 318 million people have low ability to tolerate glucose and a high chance of becoming diabetic in the future with changes in society and culture, The most prevalent kind of diabetes, the second type, is becoming more widespread. Up to 91% of persons with the condition in high-income nations have type 2 diabetes. According to IDF estimates, 193 million individuals have diabetes who are not diagnosed, increasing their risk of complications [1]. Every six seconds, a patient with diabetic condition passes away; this is a greater rate of death than that of many other illnesses [2]. All of the evidence that is currently available points out that diabetes is growing more widespread worldwide, mostly due to a rise in weight gain, adiposity caused on by a variety of factors. Prolonged hyperglycaemia can result in serious and often deadly side effects such as eye damage, kidney damage, and cardiovascular illness [3]. This research works mainly focuses on the type 2 diabetic condition.

Diabetogenic effects are attributed to selective destruction of pancreatic islet β-cells. This action results in the hormone insulin insufficient amount, a condition called hyper polydipsia, and polyuria in the animals, all symptoms typical of type one diabetes. Several animal species, such as the mouse, rat, and monkey, are susceptible to STZ's cytotoxic effects on pancreatic β-cells. Type II diabetes include elevated blood sugar levels and decreased insulin secretion or efficacy. In type 2 diabetes, insulin's incapacity to regulate gluconeogenesis leads to hyperglycaemia. The pancreas creates insulin when glucose is found in the diet, and gluconeogenesis is halted by the pathway's genes being lowered. Glucagon is activating the process of gluconeogenesis when there is no glucose present in the circulation. The pancreas β- and α-cells create insulin and glucagon, respectively [4].

In T2DM, there are three major abnormalities in the start of hyperglycaemias: increased gluconeogenesis in the liver, reduced insulin release, and altered insulin function.[5]

Medicinal plants in anti-diabetic treatments are also mentioned in many folklore medicines, including those from Chinese, Korean, and Indian cultures. [6]

Ashtavaraga is the classic group of eight medicinal plants in which four are orchids. *Habenaria edgeworthii* is one of the important orchids holding lot of medicinal properties. *Habenaria edgeworthii* is widely utilized in rejuvenating tonics such as "Chyavanprash," and the industrial need for raw material (*H. edgeworthii* tubers) is constant. [7]

2. Materials and Methods

2.1. Animals: In this study albino wistar rats weighing 180–220 grams of both sexes and aged between 8 and 12 weeks were selected and used.

2.2. Plant Material: The tuber samples of *Habenaria edgewothii* (Riddhi) were authenticated by Sanchomee Herboveda Pvt. Ltd., traded as “Manakarnika Aushadhalaya, Pune” having authentication letter number: **AD/233/02/44**

2.3. Preparation of plant extract: *Haberaria edgeworthii* tubers were grounded into a fine powder. The powder was exposed to methanol extraction using a soxhlet apparatus for 72 hours at a temperature between 60 to 70 ℃. Continued production of a dark brown solvent, guaranteeing the isolation of the highest phytoconstituents from the tuber powder. Following the extraction, the methanol was replenished at room temperature by evaporation. The extract was then kept both in a refrigerator and in an airtight container. After then, this extract was employed in further research study.

2.4. Drugs:

Metformin was used as standard drug (150 mg/kg). 200 and 400mg/kg Methanolic extract of *Habenaria edgewothii.* Streptozotocin (STZ) intraperitoneally three times at a dose of 20 mg/kg, diluted in a 0.1M cold citrate buffer (pH 4.5) to induce hyperglycemia.

2.5. Determination of Antioxidant activity by DPPH method [8]:

Specific 2,2-diphenyl-1-picrylhydrazyl (DPPH) methanol mixture was used to measure how rapidly the plant extractives could contribute hydrogen atoms. The violet/purple color that DPPH produces in a solution of methanol fades to yellowish tints in the presence of antioxidants. The 0.1 mM concentration of DPPH in methanol was prepared, and 2.4 mL in this solution was mixed with 1.6 mL extract diluted methanol at different doses (200-1000 µg/mL). The reaction mixture had been fully vortexed and

allowed to sit at ambient temperatures for thirty minutes. The mixture's absorbance at 517 nm was estimated using spectrophotometry. The proportion of the activity involved in DPPH radical scavenging was calculated following the formula below:

$$\%\text{DPPH radical scavenging activity} = \{(A_0 - A_1) / A_0\} \times 100$$

Where,

A_0 = absorbance of the control preparations,

A_1= absorbance of the standard or extractives

After that, the percentage of inhibition was plotted against concentration, and the curve's IC50 could be found. Each conc. ran three times.

2.6. *In-vitro* methods:

2.6.1. Determination of *Habenaria edgeworthii* alpha-amylase inhibitor activity [9]:

Assessing the reducing sugar (maltose equivalent) released throughout the test procedure allowed for measurement of α-amylase enzyme inhibition. Reduced units of chemical maltose released were used to express the inhibitory activity of the enzyme. The dinitro salicylic acid (DNS) method was modified to estimate the maltose equivalent. Different concentrations of the chosen plant methanolic extract were pre-incubated for 30 minutes with 1 U/mL of α-amylase, following that is the inclusion of 1 mL (1% w/v) in starch solution. The mixture underwent incubation at 37°C for 10 minutes. After adding 1 mL of DNS reagent a solution containing sodium potassium tartrate tetrahydrate 12 g, 8 mL of 2 M NaOH, and 96 mM of 3, 5-dinitrosalicylic acid to halt the reaction, the mixture was heated in a boiling water bath for five minutes. Two blanks were created: one devoid the amylase enzyme while the other without any plant extracts. The blanks were replaced with equal volumes of buffers (20 mM sodium phosphate buffer plus 6.7 mM sodium chloride, the pH 6.9 at 20 °C). The wavelength of the absorbance was taken at 540 nm. Acarbose was used as a positive control. By evaluating the inhibitory activity of α-amylase, which was calculated using the following formulae and expressed is a percentage of inhibition, the anti-diabetic efficacy was evaluated.

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

2.6.2. Alpha-Glucosidase Enzyme Inhibition Assay [10]:

Using a slightly altered version of the standard technique, the extract and fractions' α-glucosidase inhibitory activity was evaluated. The reaction mixture was preincubated to 37°C for 15 minutes in a 96-well plate. It comprised 20 µl of different extract concentrations (1000, 500, 250,100 and 50 µg/ml), total of 10 µl the enzyme alpha- glucosidase (1 U/ml), along with 50 µl, phosphate buffer (100 mM, pH = 6. 8). After adding the substrate (20 µl P-NPG (5 mM), the mixture was incubated at 37°C for an additional 20 minutes. Fifty microliters of 0.1M Na2CO3 were added to stop the reaction. At 405 nm, the level of absorption of the released p-nitrophenol was measured using Elisa Reader. As a standard, acarbose was utilized at various concentrations (0.1–0.5 mg/ml). Every experiment was conducted in triplicate, using the corresponding preparation of the control set devoid of the test substance. The formula was used to compute the % inhibition, which was used to express the results.

$$\%\ \text{Inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of extract}}{\text{Absorbance of control}} \times 100$$

2.6.3. Evaluation of glucose uptake by yeast cells [11]:

Commercial baker's yeast has been washed as well as a 10% (v/v) suspension had been made after centrifuged (3,000g, 5 min) was repeated in water that was distilled till the supernatant contents were clear. After combining different concentrations of extract (1000, 2000, 3000, 4000, and 5000 µg/ml) with one ml liquid glucose solution (10 mM), the mixture was incubated for an extra ten minutes at 37 °C. 100 µl liquid

yeast suspension was introduced to start the reaction, that afterwards was vortexed and incubated for an extra 60 minutes at 37 °C. Following a 60-minute duration, the tubes underwent a 2,500 × g centrifugation for five minutes, and the amount of glucose in the supernatant was estimated. The percentage rise of glucose absorption via yeast cells was calculated using the following formula,

The absorbance for the control reaction, which is devoid of the test sample but contains all other reagents, serves as control. At 540 nm, absorbance was measured, and every experiment was run in triplicate.

$$\% \text{ Increase in glucose uptake} = \frac{\text{OD of control} - \text{OD of extract}}{\text{OD of control}} \times 100$$

2.7. *In-vivo* methods in rats:

2.7.1. Acute oral toxicity (AOT) study with methanolic extract of *Habenaria edgeworthii* in rats [12]

Research was carried out to determine the acute oral toxicity for the methanol extraction of *Habenaria edgeworthii* within experimental animals, albino rats, in compliance with OECD guideline 423 (Acute oral toxicity-Acute toxic class technique). No death occurred when methanolic extract of *Habenaria edgeworthii* (MEHE) was given at maximum dose of 2g /kg. Thus, in the next investigation, 1/10th and 1/5th of the limit dosages was employed. The material may be deemed to have an LD_5o value more than 2000 mg/kg in accordance with OECD criteria. For the purposes of this investigation, two dosages of the methanolic extractive 200 mg /kg and a higher dose about 400 mg /kg of body wt. were chosen.

Animals were fasted for the overnight before the dose administration (food was withheld, but drink wasn't). Three experimental rats were given a suspension containing *Habenaria edgeworthii* orally with a dose of 2000mg /kg of animal's body weight. Oral gauge along with curved and ball-tipped at the insertion site was used to administer the experimental medication orally in a single dosage.

Every animal was continuously examined following the treatment, with particular focus on the first four hours post-dosage to search for any signs of toxicity. Over the following fourteen days, more behavioural and clinical signs of toxicity were seen. Changes in weight were calculated. Changes observed included skin, eyes, mucous membranes, fur colour change, anxiety, respiratory, circulatory, autonomic, and central nervous system inflammation, as well as somatomotor activity and behaviour patterns. We kept a watchful eye on the coma, piloerection, lethargy, salivation, convulsions, tremors, and diarrhoea.

2.7.2. Oral Glucose Tolerance Test (OGTT) [13]

Prior to OGTT research, the experimental animals were fasted for the whole night. Thirty Wistar albino rats total was split in five groups of six rats per group. 30 minutes following the test sample, the other groups were given 2g/kg of glucose orally, whereas Group I (normal control) was given simply distilled water. Glucose was administered to Group II (disease control) after distilled water. Group III was given glucose and regular treatment (150 mg/kg of metformin). Methanolic extract of *Habenaria edgeworthii* was administered to Groups IV and V at 200 mg/kg and 400 mg/kg, resp., in addition to glucose. All rats had blood drawn from their tail veins at 0,30,60,120, and 240 minutes following the injection of glucose. Test strips and a device called a glucometer were used to monitor the blood glucose levels (Control D).

2.7.3. Induction of type 2 diabetes mellitus in rats: [14,15]

The rats of eight weeks and weight of 150-180gms were used. Following eight weeks, six rats received regular rat chow, and 24 rats were given sufficient high-fat diet for 28 days induction period. The rats weighed between 230-260 grams in the last week of high fat diet course.

For this study, a rat model was fed a high fat diet plus given streptozotocin treatment (HFD/STZ) to induce type 2 diabetes. There were variations in the kind of diet, length of the HFD, and dosages of STZ injections, though. Rats given a high-fat diet acquire insulin resistant, yet their levels of blood sugar do not appreciably rise. Thus, the pairing of an IP dose of streptozotocin with a high-fat diet causes a mild risk of beta cell destruction, which impairs insulin secretion and causes glucose intolerance and insulin resistance to develop. As a result, through a shorter time span, the system helps to more nearly simulate the disease pathophysiology of developing type 2 diabetes.

High fat diet preparation:

An attempt was taken to produce the high fat diet pellets for a purpose of research study on causing diabetes by mixing the components specified below to form a rough dough. Dough was used to produce the pellets, which were then Roasted into a hot air oven at 50°C for 7-8 hours.

Material	Quantity (Per 100 gm)

Soyabean meal	7 gm
Casein	11 gm
Lard	25 gm
Sodium Cholate	0.3 gm
Refined wheat flour	40 gm
Calcium bicarbonate	1 gm
Fructose	15.7 gm

Composition of High fat diet

Streptozotocin Injection:

After a four-week period of high-fat diet (HFD) consumption, diabetes was produced by injecting streptozotocin (STZ) intraperitoneally three times at a dose of 20 mg/kg, diluted in a 0.1M cold citrate buffer (pH 4.5). Three IP injections of streptozocin were given to the animals in order to induce hyperglycaemia in the duration of 10 days. Throughout the STZ injection period, the rats were administered a 5% glucose solution for 4–8 hours to offset the hypoglycaemic effects generated by STZ. Three days after the rats' diabetes induced, samples of their blood were taken from their tail veins so a glucometer could measure their fasting blood glucose.

The rats received daily oral doses of both metformin and MEHE for a total of 28 days. For once in every week of the treatment period, the animals' fasting blood glucose levels have been documented.

2.8. *In-vivo* methods in zebrafish:

2.8.1. Acute oral toxicity (AOT) study of methanolic extract of *Habenaria edgeworthii* in Zebrafish [16]

Adult zebrafish weighing between 400 to 500 mg and aged between 100 to 150 days were selected and used for the research study. Acquired Zebrafish were placed in the glass tank with the proper aeration and clean water with the conditions like, based on these factors, zebrafish were typically kept in lab settings with low movement, mildly acidic to somewhat alkalinity (pH 6.5–8.0) the water around 28.5 °C, which are almost often regarded as the ideal temperature for proper growth, using daily 14h :10h the light: dark pattern.[17]

To evaluate the acute toxicity in adult zebrafish, a modification based on the suggestions put forward by Organization for Economic Cooperation and Development (OECD) that is 425 was implemented.

The two phases of the toxicity test were as follows: an oral limit test at 2 g/kg using the prescribed methods. The first test involved giving one animal two grams per kilogram of methanolic extract of *Habenaria edgeworthii* (MEHE) orally. The primary test was initiated if the death happened within 48 hours. The limit test was initiated if it continued to live. Five animals overall (including the test dosage animal) were tested in limit test, where 4 animals received treatment with 2 g /kg of MEHE. Within 48 hours, fatalities were noted. The primary test was initiated if there were 3 or more fatalities; if there were 3 or more living animals, The LD50 value cannot be calculated, and the administered MEHE is thought to be incredibly safe.

2.8.2. Induction of type 2 diabetes in zebrafish using glucose immersion method [18]

The 5-liter water tank had adult zebra fish that were given 0.25% glucose solution for first weeks, 0.5% for the second weeks, 1% for the 3rd week, and 1.5% glucose solution for the last weeks. This procedure was used during the induction stage on alternate days. After being submerged in the glucose solution for the first ten minutes, all zebrafish were observed for any indications of stress, such as trouble swimming or excessive gill movement. The fish was put to sleep in ice water that was cooled progressively from 15°C to 5°C for two to three minutes. When there is no more movement in the water, zebrafish are said to be anesthetized. The fish will be taken out of the water and given a tissue paper pat dry. The tail will be clipped right away, and a glucometer test strip (control D) will be placed immediately on the docked tail to obtain blood-glucose measurements.

For duration of 14 days daily, the zebrafish was given daily oral doses of metformin and a methanolic extract of *Habenaria edgeworthii*. For the length of the treatment period, the animals' fasting blood glucose levels have been documented.

Optomotor response model

2.8.3. Optomotor response model for diabetic retinopathy in zebrafish [19]

Following a month-long induction phase to assess each fish's optomotor reaction, about four to five were taken out of each tank. For this test, the fish were kept in a dark cabinet in a tiny bowl with a level bottom. A computer screen with a visual stimulus a big wheel with twelve wedges spinning at 1.04 revolutions per second was located underneath the bowl. The wheel turned thirty seconds in a clockwise direction. For every batch of fish, this procedure was carried out twice and captured on video. After that, the recordings were examined, and every six seconds, the fish's swimming behaviour was noted to see whether it was responding to the visual cue. For example, a score of 1 was recorded if one fish was swimming in the right direction, and a score of 2 if there were two fish, if two fish, a score of 2. After each week the blood glucose level of 1 fish in each group was tested and records were maintained.

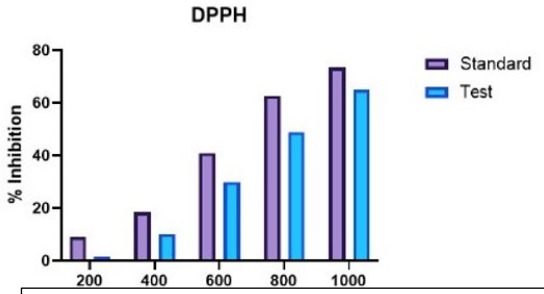
% Inhibition of DPPH radical scavenging activity of methanolic extract of *Habenaria edgeworthii*

2.9. Statistical Analysis:

Using GraphPad Prism 9.0, the statistical evaluation was conducted using a one-way ANOVA and Dunnett's test, with the provided values marked as Mean±SEM in the context of *in vivo* experiments. The significant criteria for statistical analysis were set at $P<0.05$.

3. Results:

The methanolic extract of *Habenaria edgeworthii* (MEHE) demonstrated significant in vitro antioxidant activity, showing 70.08% DPPH radical scavenging activity, comparable to ascorbic acid (79.31%). In enzyme inhibition assays, MEHE exhibited dose-dependent α-amylase and α-glucosidase inhibitory activity with IC_{50} values of 289.993 µg/mL and 965.129 µg/mL respectively. Additionally, glucose uptake by yeast cells increased significantly in a concentration-dependent manner, with an EC_{50} value of 805.19 µg/mL.



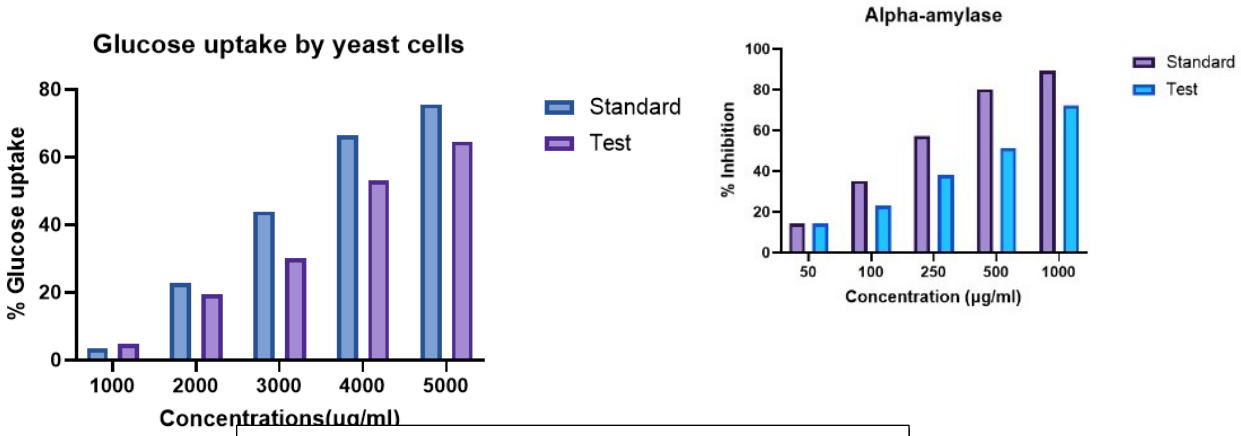
Testing of blood glucose level in zebrafish

3.1. DPPH Antioxidant activity:

The methanolic extract of *Habenaria edgeworthii* demonstrated significant in vitro DPPH radical scavenging activity. With an % inhibition of 79.31%, ascorbic acid showed notably stronger DPPH radical scavenging capability than the methanolic plant extract. On the other hand, the plant extract's % inhibition value was discovered was identified as 70.08%.

3.2. *In-vitro* Antidiabetic activities:

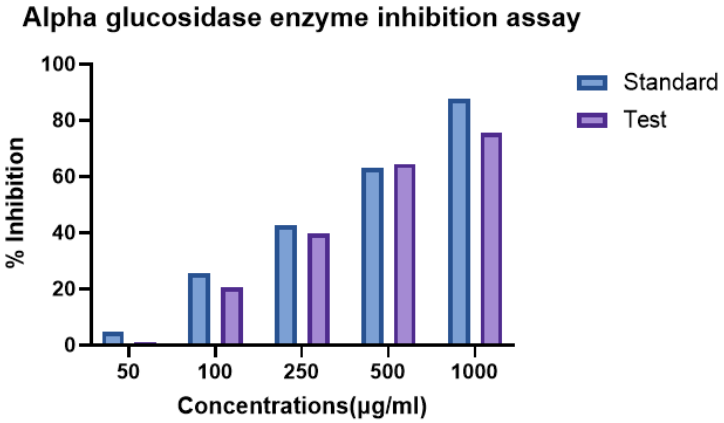
3.2.1. Alpha amylase enzyme inhibition assay:



% Glucose uptake by yeast cell

Methanolic solvent extract of *Habenaria edgeworthii* for doses about 50, 100, 250, 500 and 1000 µg/ml inhibited the alpha-amylase enzyme by 014.37%, 22.92%, 37.98%, 51.32% and 72.19%respectively, with IC50 value found to be 289.993 µg /ml. At doses of 50, 100, 250, 500 and 1000 µg/ml, Acarbose inhibited alpha-amylase by 14.37%, 35.32%, 57.14%, 80.41% and 89.30% respectively, with IC50 observed to be 212.753 µg /ml.

3.2.2. Alpha glucosidase enzyme inhibition assay:



% Inhibition of Alpha-Glucosidase enzyme

Methanolic extract of *Habenaria edgeworthii* shown inhibitory activity against alpha-glucosidase, with IC50 values of 965.129 µg/ml. However, is efficacy was found to be lower than that of the Acarbose, which exhibited an IC50 value of 850.197µg/ml.

3.2.3. Glucose uptake by yeast cell:

Methanolic extract of *Habenaria edgeworthii* Showed 4.735% uptake of glucose at 1000 µg/ml and 64.64% at 5000 µg/ml concentration and 805.19 µg/ml was determined to be its EC50 value. The standard drug metformin showed 3.52% glucose uptake at 1000 µg/ml and 75.66% glucose uptake at 5000 µg/ml concentration and its EC50 value was found to be 789.309µg/ml When compared to metformin, the percentage of glucose taken was considerable and exhibited dose-dependent action.

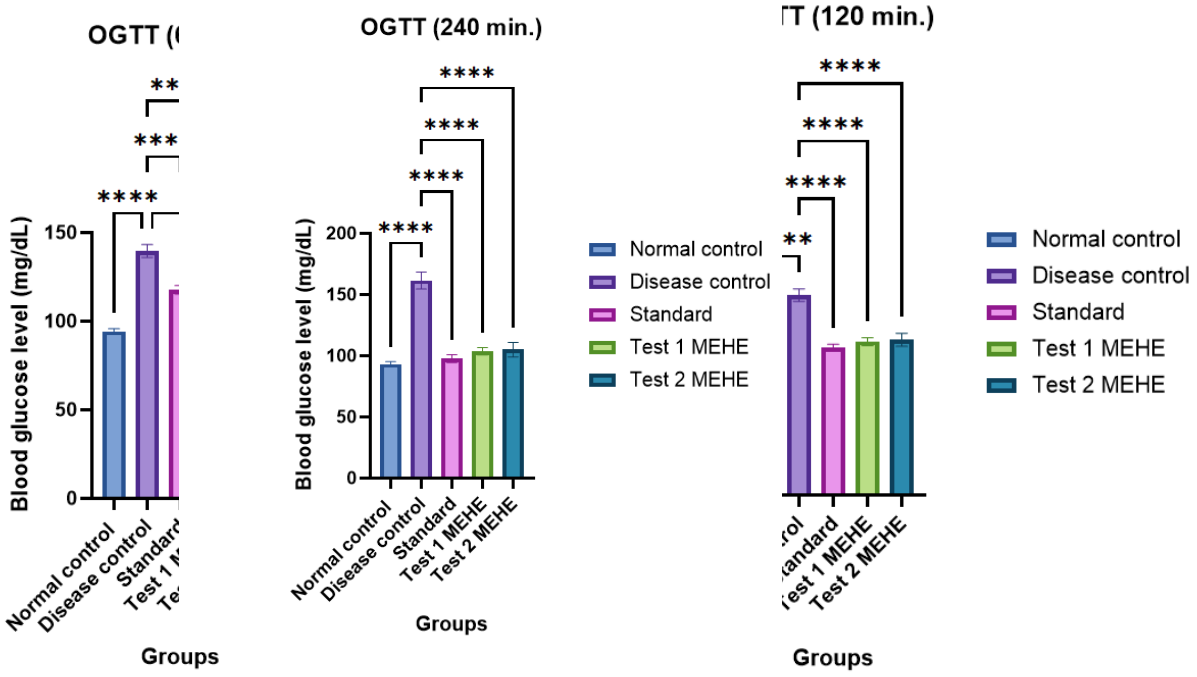
3.3. *In-vivo* studies in rats:

3.3.1. Acute oral toxicity study:

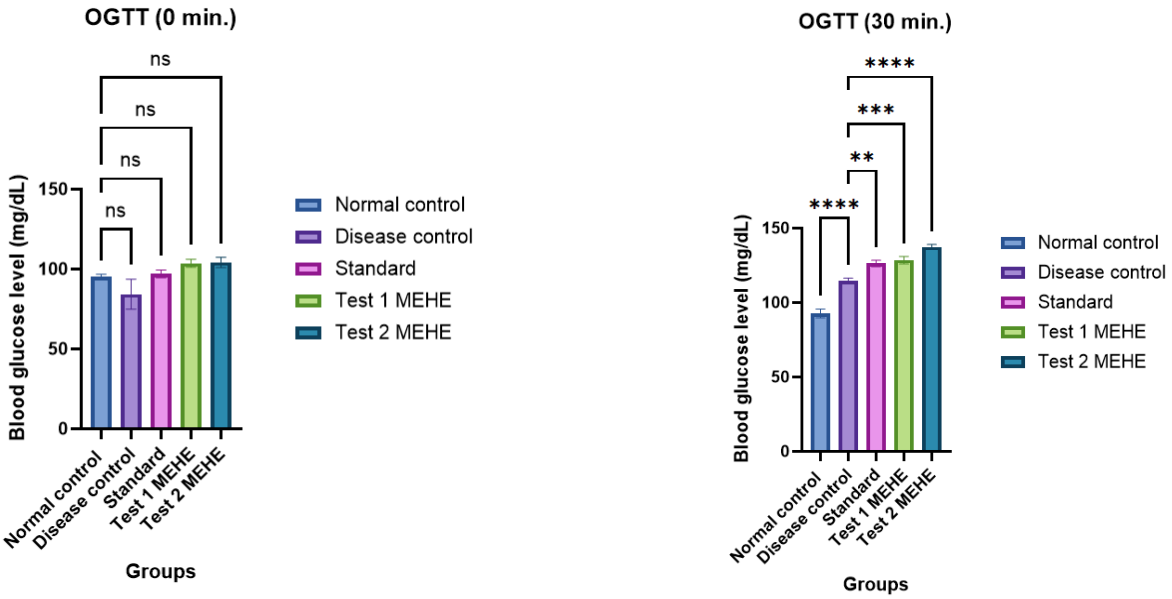
OECD guideline 423 was used to perform ethanolic extractive of *Habenaria edgeworthii* with a dose of 2000mg/ kg did not show any signs of mortality and toxicity in the animals. Similarly plant extract did not affect the gross behaviour and other parameters during the entire experimental period of 14 days.

3.3.2. Oral Glucose tolerance test:

The decrease in fasting blood sugar

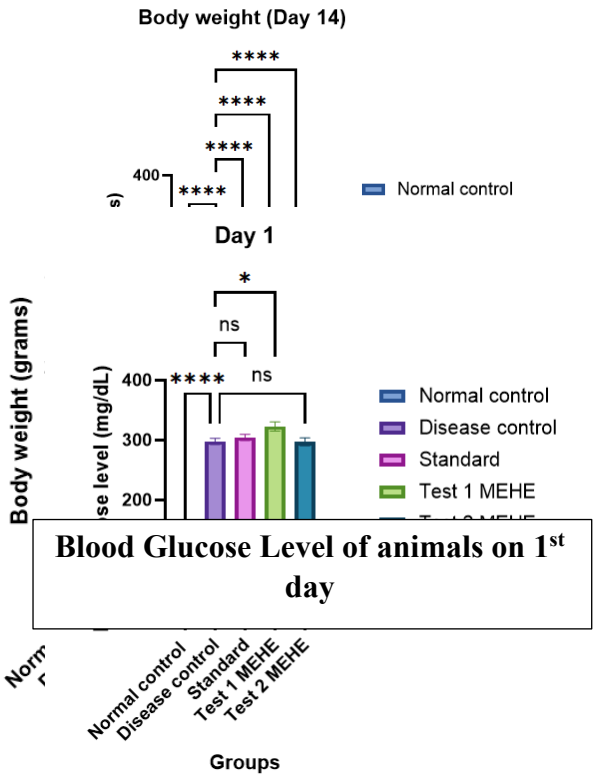


concentrations following metformin (150 *Habenaria* and 400 mg/kg) are shown in graph.



eageworthii (200 and 400 mg/ kg) at 60, 120, and 240 minutes effectively lowered the fasting blood glucose level. After the 30-minute glucose infusion (2 g/kg), the blood glucose levels in rats increased significantly in all experimental groups. Metformin and MEHE cause a noticeable drop in blood sugar

levels at 60, 120, and 240 minutes. At 240 minutes,



Blood Glucose Level of animals on 1st day

the standard group value was discovered to be 76.16 mg/dl. While the glucose level was lowered to 86.33 mg/dl and 80.83 mg/dl by the low and high doses of *Habenaria edgeworthii* methanolic extract, respectively, at 200 mg/kg as well as 400 mg/kg. Comparing same fasting blood glucose reading of 116.16 mg/dl in the disease control animal.

3.3.3. Body weight:

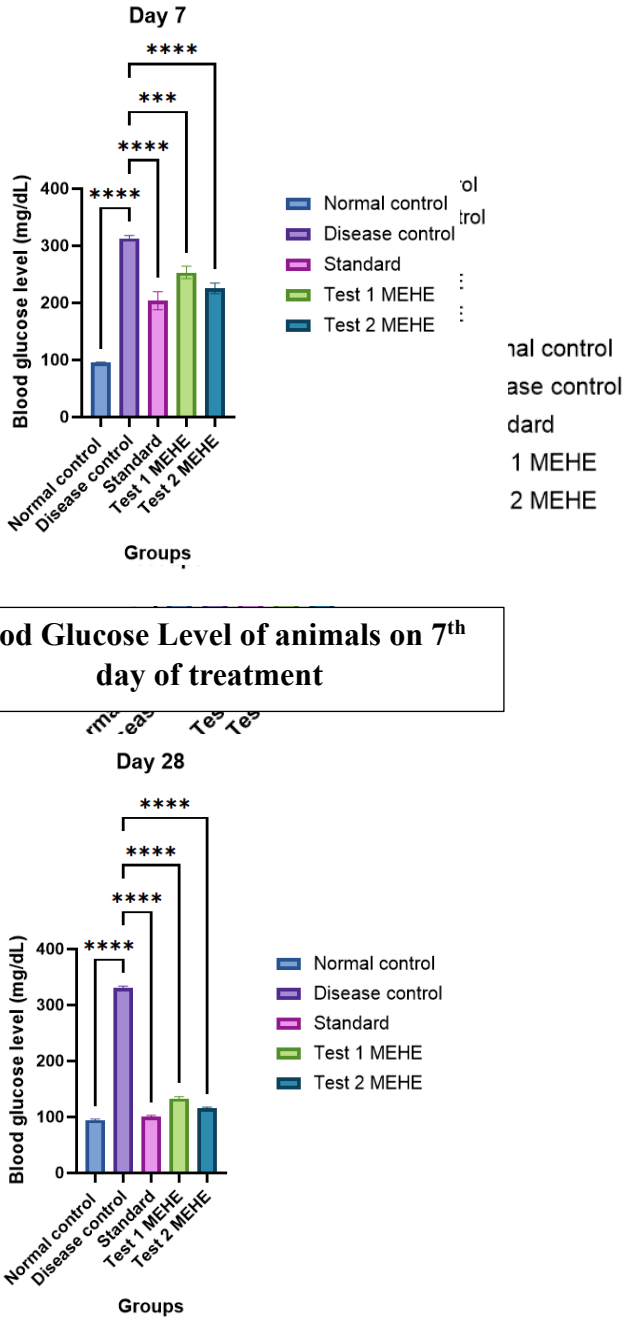
On days 14 and 28, there is a discernible decrease in blood sugar levels due to metformin and MEHE. The standard group value was found to be 251.6 grams on

Blood Glucose Level of animals on 14th day of treatment

the 28th day. At 200 mg/kg and 400 mg/kg, respectively, the low and high doses of Habenaria edgeworthii methanolic extract reduced the weight to 232g and 233g.

Body weight of animals on 14th day of treatment

3.3.4. Blood Glucose Level:



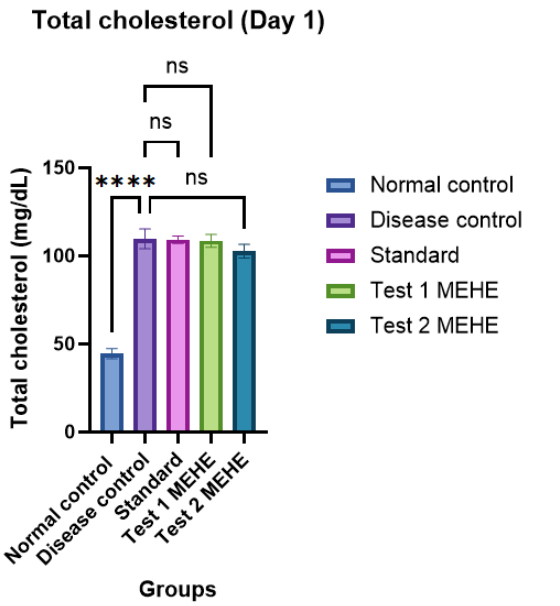
Blood Glucose Level of animals on 7th day of treatment

Blood Glucose Level of animals on 28th day of treatment

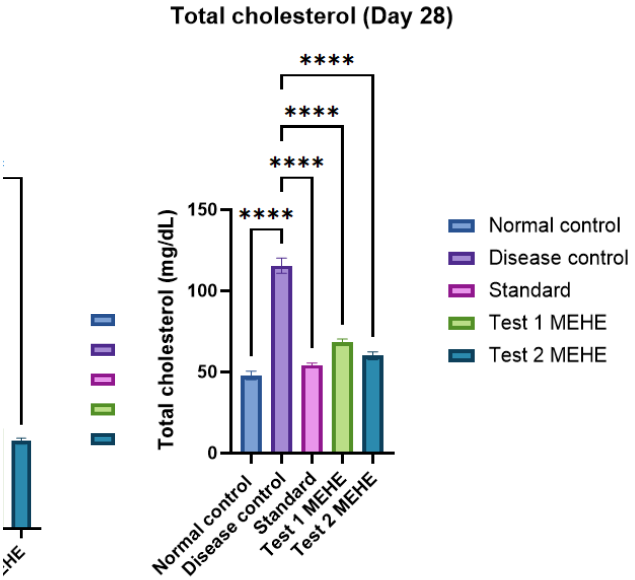
All diabetes-induced experimental groups had fasting blood sugar amounts that were significantly higher than those of the normal control group before beginning treatment. Last two weeks, the rats getting methanol extract of *Habenaria edgeworthii* showed a significant decrease in the level of fasting blood glucose as compared with the disease control group. There was also a notable decrease in the amount of BGL starting in the subsequent week of therapy.

Body weight of animals on 28th day of treatment

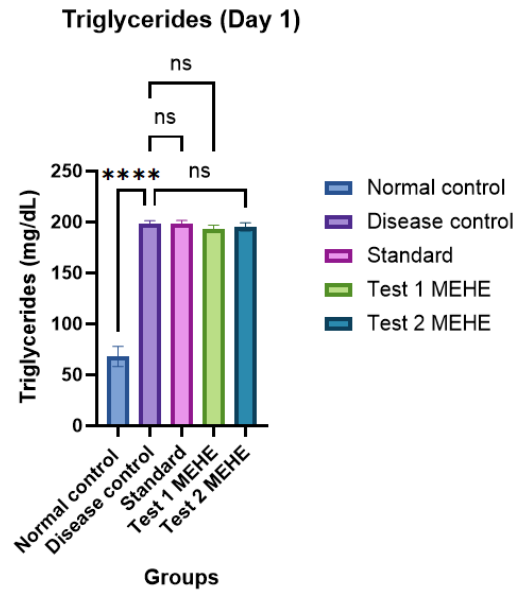
3.3.5. Lipid Profile:



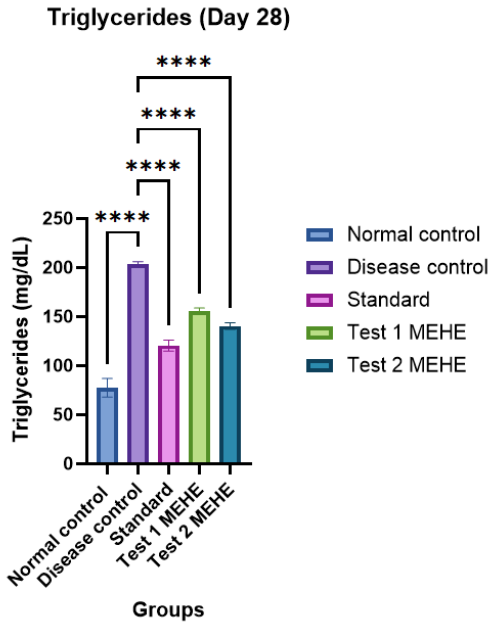
Effect of HFD+STZ on Total Cholesterol level



Effect of MEHE (test drug) on Total Cholesterol level



Effect of HFD+STZ on Triglyceride level



Effect of MEHE (test drug) on Triglyceride level

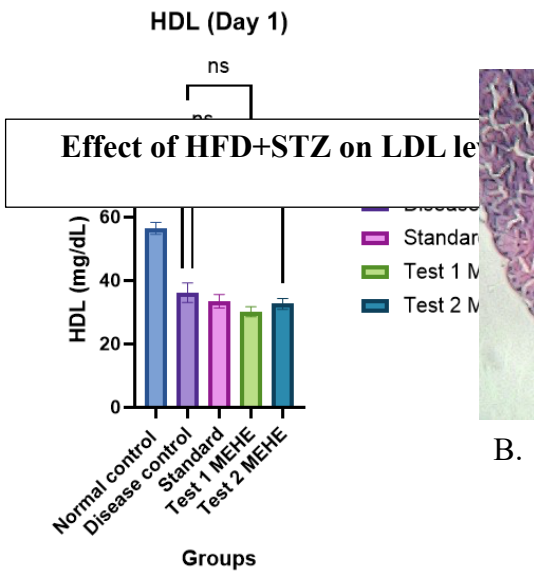
Treatment with methanolic extract of *Habenaria edgeworthii* significantly improved the altered lipid parameters in HFD-STZ induced diabetic rats. The higher dose (400 mg/kg) markedly reduced total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels compared to the disease control group. Additionally, a significant elevation in high-density lipoprotein (HDL) levels was observed in the treated groups. These findings indicate the hypolipidemic and cardioprotective potential of *Habenaria edgeworthii* in experimental type 2 diabetes mellitus.

MEHE treatment significantly ameliorated dyslipidemia associated with HFD-STZ induced type 2 diabetes. The higher dose (400 mg/kg) markedly reduced total cholesterol (TC), triglycerides (TG), and low-density lipoprotein (LDL) levels compared to the disease control group. Moreover, a significant elevation in high-density lipoprotein (HDL) levels was observed in treated animals. These findings suggest that *Habenaria edgeworthii* possesses substantial hypolipidemic activity along with its antidiabetic potential, thereby contributing to reduced cardiovascular risk in experimental diabetes.

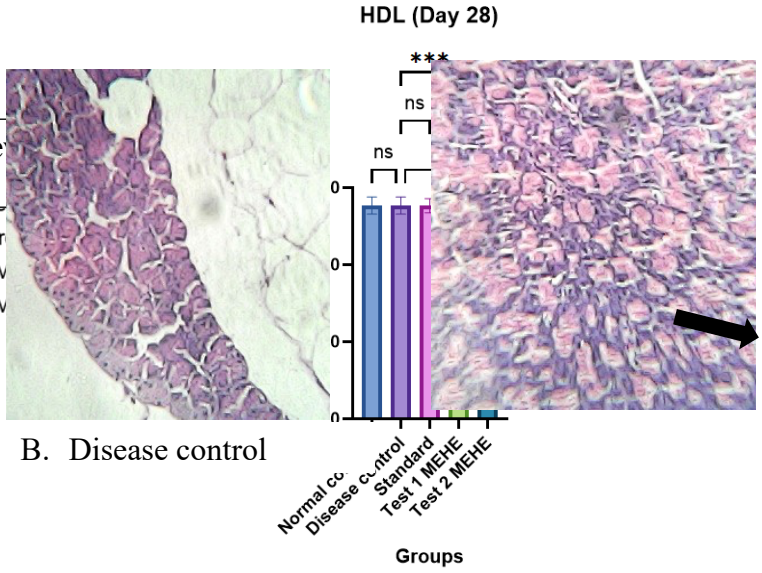
The findings indicate that, as compared to the Disease Control Group, the mean levels of TC, TG, and LDL were significantly lower after receiving a larger dose of the methanolic extract of plant *Habenaria edgeworthii*. Additionally, it is evident that the test drug-treated group had higher levels of HDL, or good cholesterol.

3.3.6. Histopathological Evaluation

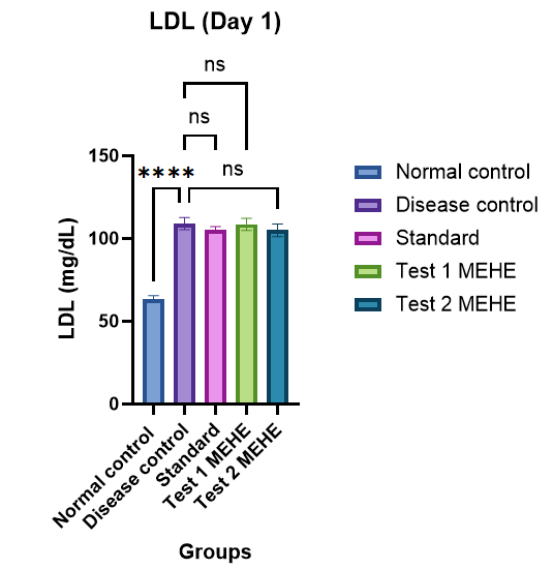
To observe the condition of pancreatic beta cells after the treatment of standard drug as well as test drug that is methanolic extract of *Habenaria edgeworthii*, the pancreas of animals was obtained and histopathological

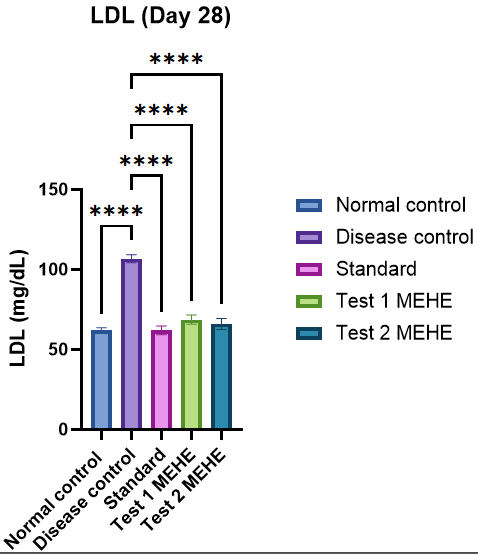


Effect of HFD+STZ on HDL level



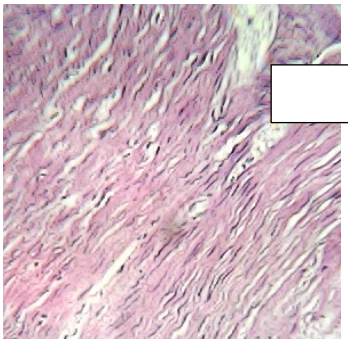
Effect of MEHE (test drug) on HDL level





Effect of MEHE (test drug) on LDL level

examination was performed. Recorded data is as below.

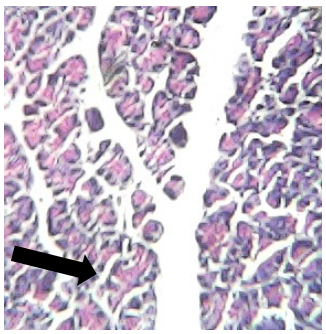


Histopathology reports of pancreas

the pancreas tissue collected from Wistar rats administered methanolic extract of *Habenaria*

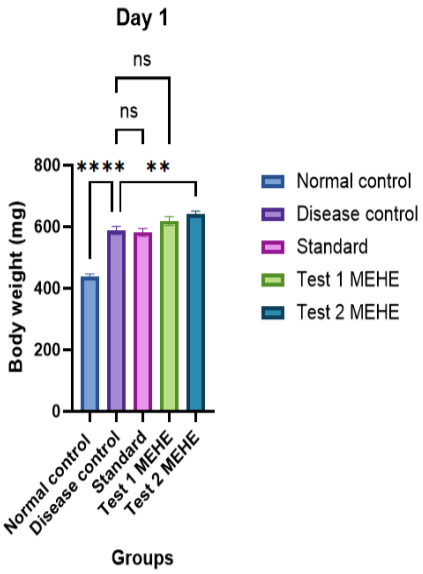
A. Normal control

D. MEHE 200mg/kg (T1)



edgeworthii after they developed diabetes

A. Normal experimental group- typical pancreatic islets that grow in number.
B. Disease induced group- Pancreatic tissue of rat induced diabetes with HFD+

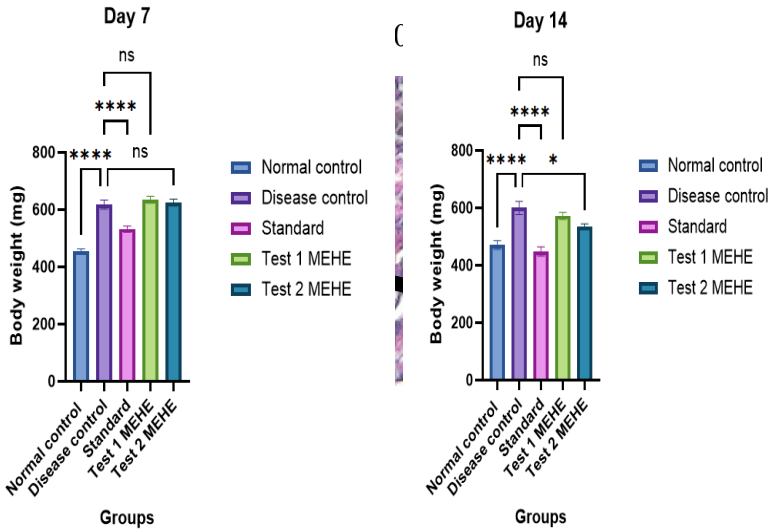


streptozotocin, arrow points to the thinning and degraded islets of pancreatic cells.

C. Standard drug treated group- The islet cells of the pancreas have recovered after their destruction by the course of the illness; the group treated with metformin drug is shown shows pancreaprotective

D. MEHE Test 1 (200mg/kg) ancreatic tissue of a group of animals treated with low dose of methanolic extract of *Habenaria edgeworthii* has partially regenerated islet function in the pancreas (arrow).

E. MEHE Test 2 (400mg/kg)- Pancreatic tissue



of this group's results indicates the recovery almost as same as standard drug treated group but less than the metformin's effect.

3.4. *In-vivo* studies in zebrafish:

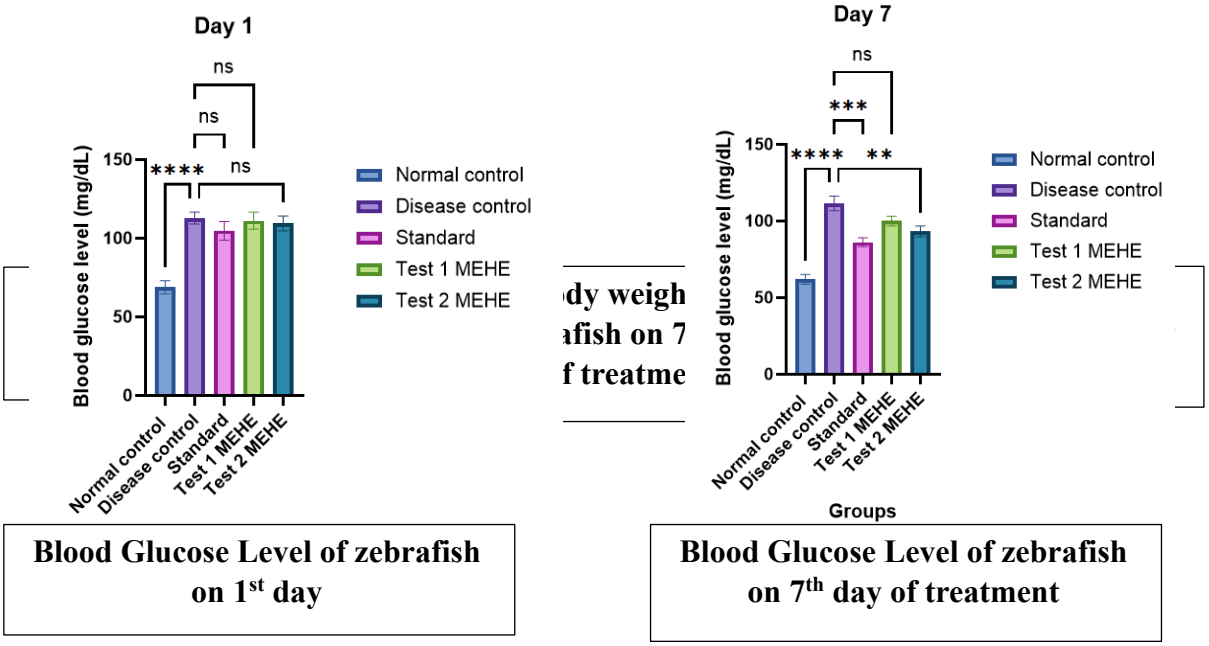
3.4.1. Acute oral toxicity study:

To evaluate the acute toxicity in adult zebrafish, a modification based on the suggestions put forward by Organization for Economic Cooperation and Development (OECD) that is 425 was implemented. The primary test was initiated if there were 3 or more fatalities; if there were 3 or more living animals, The LD50 value cannot be calculated, and the administered MEHE is thought to be incredibly safe. Methanolic extractive of *Habenaria edgeworthii* with a dose of 2000mg/ kg did not show any signs of mortality and toxicity in the animals.

3.4.2. Body weight:

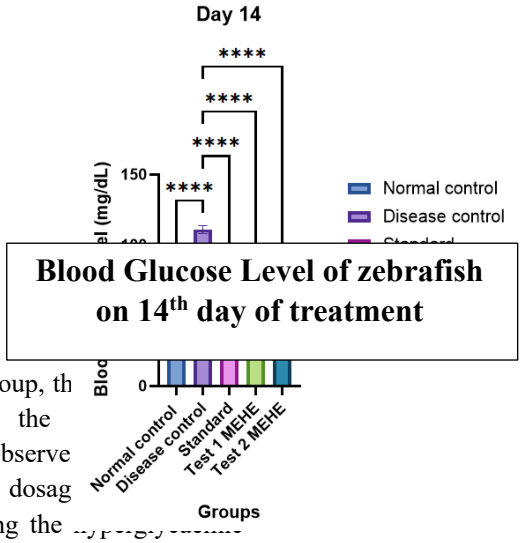
The induction caused the zebrafish to acquire weight both during and after. As soon as the therapy began, a zebrafish began to lose weight. In comparison to both the test as well as disease control groups, the standard group of zebra fish who received metformin treatment lost a significant amount of weight.

3.4.3. Blood Glucose Level:



Blood Glucose Level of zebrafish on 1st day

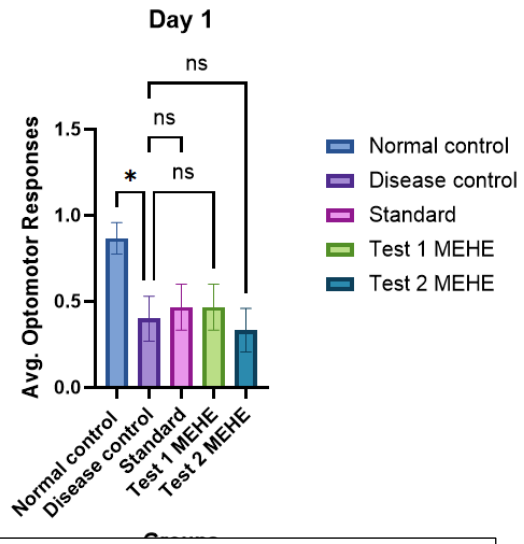
Blood Glucose Level of zebrafish on 7th day of treatment



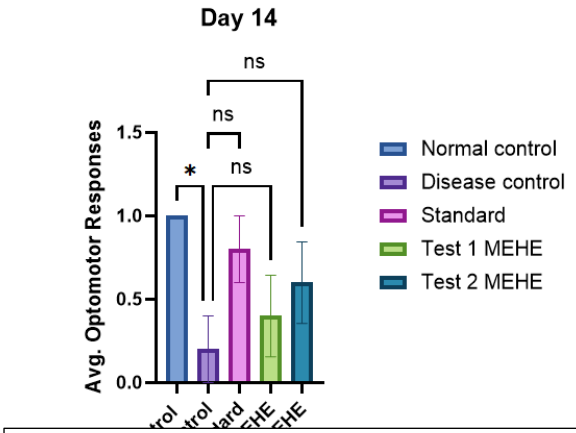
Blood Glucose Level of zebrafish on 14th day of treatment

We may conclude from the results that, with the exception of the Normal Group, th level rose noticeably in the zebrafish. Here, we can observe treatment, the higher test dosag good efficacy in overcoming the state.

3.4.4. Optomotor Responses Evaluation:

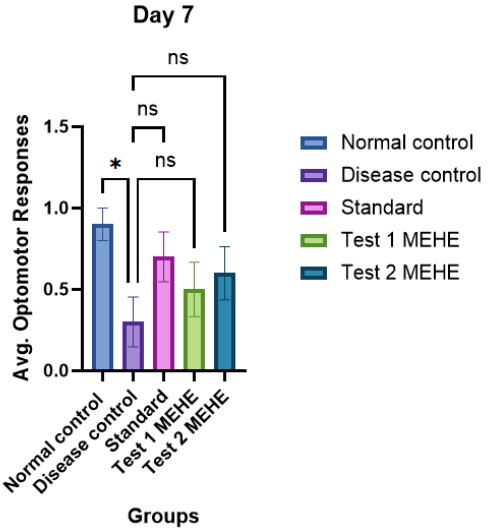


Optomotor responses of zebrafish on 1st day



Optomotor responses of zebrafish on 14th day of treatment

Research findings and statistical analysis reveal that since the zebrafish had developed diabetic retinopathy, there were relatively few optomotor responses upon the very initial day on therapy. The response progression was seen during treatment with the conventional medication metformin plus the test medicine, a methanolic extract containing *Habenaria edgeworthii.*



Optomotor responses of zebrafish on 7th day of treatment

4. Conclusion:

The goal of the current study was to assess anti-diabetic and retinopathy-preventive properties of the *Habenaria Edgeworthii* extract from plants utilizing both in vivo and invitro techniques. The test compound, *Habenaria Edgeworthii*, displayed influenced by dose inhibitory effects on invitro activity such as alpha amylase and alpha glucosidase activity, according to the overall results. In addition, dose-dependent findings demonstrated a modest percentage of glucose absorption by the MEHE in comparison to regular metformin.

Sighting to the in vivo part, the animals' body weight increased during the induction phase, causing them to become obese and diabetic, but they began to lose weight during their treatment phase. The chosen plant possesses anti-diabetic properties, as evidenced by the considerable drop in fasting blood sugar level that occurred with a higher dosage of MEHE (400 mg/kg). In addition, the test medication has been demonstrated to be advantageous in decreasing LDL, TG and total cholesterol while also raising HDL levels in the treated groups.

The second experimental model, involving diabetic zebrafish, likewise indicated the anti-diabetic benefits of MEHE since the higher test dosage treated groups had significantly lower blood glucose levels as well as body weight also had an impact. Ultimately, the optomotor response model was used to assess diabetic retinopathy. The test group's outcomes were favourable at the conclusion of the research.

So, it can be concluded from conducted research that the MEHE shows anti-diabetic activity.

References:

1. Atlas D. International diabetes federation. IDF Diabetes Atlas, 7th edn. Brussels, Belgium: International Diabetes Federation. 2015;33(2).
2. Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, Stein C, Basit A, Chan JC,

Mbanya JC, Pavkov ME. IDF Diabetes Atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes research and clinical practice. 2022 Jan 1;183:109119.
3. Kalra S, Jena BN, Yeravdekar R. Emotional and psychological needs of people with diabetes. Indian journal of endocrinology and metabolism. 2018 Sep 1;22(5):696-704.
4. Sami W, Ansari T, Butt NS, Ab Hamid MR. Effect of diet on type 2 diabetes mellitus: A review. International journal of health sciences. 2017 Apr;11(2):65.
5. DeFronzo RA, Bonadonna RC, Ferrannini E. Pathogenesis of NIDDM: a balanced overview. Diabetes care. 1992 Mar 1;15(3):318-68.
6. Alam S, Sarker MM, Sultana TN, Chowdhury MN, Rashid MA, Chaity NI, Zhao C, Xiao J, Hafez EE, Khan SA, Mohamed IN. Antidiabetic phytochemicals from medicinal plants: prospective candidates for new drug discovery and development. Frontiers in endocrinology. 2022 Feb 24;13:800714.
7. Hossain MM. Therapeutic orchids: traditional uses and recent advances—an overview. Fitoterapia. 2011 Mar 1;82(2):102-40.
8. Rahman MM, Islam MB, Biswas M, Khurshid Alam AH. In vitro antioxidant and free radical scaveng activity of different parts of Tabebuia pallida grow in Bangladesh. BMC research notes. 2015 Dec;8:1-
9. Bhutkar MA, Bhise SB. In vitro assay of alpha amylase inhibitory activity of some indigenous plants. Int. J. Chem. Sci. 2012 Jun 27;10(1):457-62.
10. Telagari M, Hullatti K. In-vitro α-amylase and α-glucosidase inhibitory activity of Adiantum caudatum Linn. and Celosia argentea Linn. extracts and fractions. Indian journal of pharmacology. 2015 Jul 1;47(4):425-9.
11. Pitchaipillai R, Ponniah T. In vitro antidiabetic activity of ethanolic leaf extract of bruguiera Cylindrica L.–glucose uptake by yeast cells method. International Biological and Biomedical Journal. 2016 Dec 10;2(4):171-5.
12. Matsuura N, Aradate T, Sasaki C, Kojima H, Ohara M, Hasegawa J, Ubukata M. Screening system for the Maillard reaction inhibitor from natural product extracts. Journal of health science. 2002;48(6):520-6.
13. Chemicals DO. OECD Guideline for testing of chemicals. The Organisation for Economic Co-operation and Development: Paris, France. 2005 Apr:1-3.
14. Guo XX, Wang Y, Wang K, Ji BP, Zhou F. Stability of a type 2 diabetes rat model induced by high-fat diet feeding with low-dose streptozotocin injection. Journal of Zhejiang University. Science. B. 2018 Jul;19(7):559.
15. Vatandoust N, Rami F, Salehi AR, Khosravi S, Dashti G, Eslami G, Momenzadeh S, Salehi R. Novel high-fat diet formulation and streptozotocin treatment for induction of prediabetes and type 2 diabetes in rats. Advanced biomedical research. 2018 Jan 1;7(1):107.
16. Ferreira DQ, Ferraz TO, Araújo RS, Cruz RA, Fernandes CP, Souza GC, Ortiz BL, Sarquis RS, Miranda JC, Garrett R, Carvalho JC. Libidibia ferrea (jucá), a traditional anti-inflammatory: a study of acute toxicity in adult and embryos zebrafish (Danio rerio). Pharmaceuticals. 2019 Nov 30;12(4):175.
17. Del Vecchio G, Mazzei A, Schiavone R, Gomes AS, Frangelli G, Sala T, Fantino S, Brocca MG, Barca A, Rønnestad I, Verri T. Rearing Conditions and Automated Feed Distribution Systems for Zebrafish (Danio rerio). Applied Sciences. 2022 Oct 29;12(21):10961.
18. HIDAYAH N, Fairuz F, Khatib A. DEVELOPMENT OF INDUCED DIABETIC MODEL USING ZEBRAFISH.
19. Joseph Fratello, “Retinopathy in Hyperglycemic Zebrafish”, 2018. https://edspace.american.edu/jf9359a/wp content/uploads/sites/1322/2018/10/Retinopathic-and-Hyperglycemic-Zebra-Fish.pdf