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IN-VITRO AND *IN-VIVO* ANIMAL MODEL FOR SCREENING ANTI-DIABETIC ACTIVITY OF *HELLENIA SPECIOSA* (J. KOENIG) S. R. DUTTA

R. Ramya * and R. Dhamotharan

Department of Biochemistry, J. B. A. S. College for Women, Teynampet, Chennai - 600018, Tamil Nadu, India.

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Correspondence to Author:
R. Ramya

Assistant Professor,
Department of Biochemistry,
J. B. A. S. College for Women,
Teynampet, Chennai - 600018,
Tamil Nadu, India.

E-mail: asmitalove28@gmail.com

ABSTRACT: Diabetes mellitus is one of the most common endocrine disorder which causes microvascular and macrovascular complications. Herbal medicines derived from plant extracts are used to treat various diseases because of its fewer side effects. The present investigation includes a screening of phytochemicals and evaluation of the *in-vitro* and *in-vivo* anti-diabetic activity of *Hellenia speciosa* leaves extract. The leaf extract was screened for various phytochemicals and then subjected to *in-vitro* anti-diabetic study using alpha-amylase and alpha-glucosidase enzyme. Inhibition of carbohydrate digestive enzymes amylase and glucosidase can effectively decrease the postprandial increase of glucose level. This is an important strategy in the management of diabetes. This was followed by the *in-vivo* study to evaluate the anti-diabetic effect of *Hellenia speciosa* leaves in normal and alloxan induced diabetic rats. The oral administration of crude leaf extract in diabetic rats for 28 days at a dosage of 300 mg/kg body weight exhibited a significant reduction ($P<0.05$) in fasting blood glucose level and remarkable increases in serum insulin level. The action of *Hellenia speciosa* was also compared with anti-diabetic drug metformin (350 mg/kg). An oral glucose tolerance test was also performed in the rat model. The study shows that the leaf is nontoxic and regenerates the toxic effect induced by alloxan. Based on the above results, it is evident that the leaves of *Hellenia speciosa* have an anti-diabetic effect and can be considered as an effective formulation for future studies.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia due to a relative deficiency of insulin or its resistance. This condition leads to disturbances in the metabolism of carbohydrates, proteins, and lipids [1].

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The presence of diabetes confers an increased risk of complications such as cardiovascular diseases, coronary artery disease, stroke, neuropathy, nephropathy, and retinopathy [2, 3].

Oral hyperglycemic agents are available for the treatment of diabetes; they are off less use because of its side effects. The use of medicinal based herbal formulations gained importance since they are less toxic and free from side effects when compared to synthetic drugs [4]. Medicinal plants have been used as a therapeutic aid for alleviating the ailments of humankind for thousands of years. At present several medicinal plants have gained

importance for the treatment of diabetes mellitus because of its bioactive compounds [5]. There is a great demand for research on herbal products with antidiabetic effects [6]. The use of plant extracts with antioxidant, anti-diabetic, and anti-hyperlipidemic effects are of great significance in the treatment of diabetes and related complications [7].

Ethnobotanical data indicate that there are more than 800 plants are used as traditional remedies for the treatment of diabetes [8]. The medicinal plant *Hellenia speciosa* is a member of the family Costaceae and is a newly introduced plant in India [9]. It is an ornamental, perennial, succulent rhizomatous herb grown up to 2.7 meters in moist, clayey soil under moderate shade [10].

The plant reproduces vegetatively by stem cutting, rhizomes or division of clumps. The leaves of the plant are oblong, thick, spirally arranged, flowers large white cone-like terminal spikes with bright red bracts [11]. The present study was conducted to investigate the anti-diabetic activity of *Hellenia speciosa* in alloxan-induced diabetic rats.

MATERIALS AND METHODS:

Animals: Adult Sprague Dawley rats (100-140g) were purchased from Biogen, Bangalore, India were used for the study. All animals were housed 6/cage and kept in the animal house for one week for proper acclimatization under the controlled condition of illumination (12 h light / 12 h darkness), and the temperature is ranging 20-25 °C and fed on a standard pellet diet and water/ filtered water *ad libitum.*

Ethical clearance for the handling of experimental animals was obtained from Institutional Animal Ethics Committee (IAEC) constituted for the purpose and care of laboratory animals and taken as per the guidance of the Committee for Control and Supervision on Experiments on Animals (CPCSEA No.: 971/bc/06/CPCSEA)

Chemicals Required: The chemical and drugs used in the study were planted extract, Alloxan, Glucose, carboxymethyl cellulose (CMC), metformin, isoflurane (anesthetic agent) was purchased from Sigma-Aldrich, St. Louis, MO, USA. Animal restrainer (*e.g.*, Broom restraint, Plas Labs), Micrometer, glucometer (Accu-Check, Roche, Germany).

Plant Material: *Hellenia speciosa* was collected from Thrissur district of Kerela, identified and confirmed by the botanist Dr. S. Ravikumar, PG and Research Department of Plant Biology and Biotechnology, Presidency College, Chennai Voucher specimen no. PCMRDRM2017001.

Preparation of Plant Extract: Preparation of extracts was done according to the combination of the methods used by [12, 13]. About 1g of fleshy dried powder of *Hellenia speciosa* and plant materials were extracted with 20 ml ethanol 75%, acetone, chloroform, aqueous and petroleum ether (Merck, extra pure) for 1 min using an Ultra Turax mixer (13,000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman no. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evaporator at 40 °C to a constant weight and then dissolved in respective solvents. The dissolving rate of the crude extracts was approximately 100%. The solution was stored at 18°C until use.

Phytochemical Analysis of *Hellenia speciosa*: Phytochemical screening was carried out on the leaf extract of *Hellenia speciosa* using different solvents to identify the major natural chemical groups such as tannins, saponins, flavonoids, phenols, terpenoids, alkaloids, glycosides, cardiac glycosides, coumarins, and steroids. General reactions in this analysis reveal the presence or absence of these compounds in the leaf extracts tested [14, 15, 16].

***In-vitro* Anti-diabetic Activity of *H. speciosa*:**

Alpha-Amylase Inhibitory Assay: The alpha-Amylase inhibitory activities of the given plant samples were carried out according to the method and reported [17]. The starch solution (0.5% w/v) used as the substrate was prepared by boiling potato starch in distilled water for 15 min. The enzyme solution was prepared by dissolving 1 mg of porcine pancreatic α-amylase in 20mM phosphate buffer (100 mL, pH 6.9). The sample solutions were prepared in DMSO (dimethyl-sulfoxide) in different concentrations (10 to 100 mg/mL). The DNS solution (20 mL 96 mM 3,5-dinitrosalicylicacid, 12 g sodium potassium tartrate in 8 mL of 2 M NaOH and 12 mL deionized water) was used as the coloring reagent of reaction.

Three sets of experiments were conducted: test, blank, and control. A mixture of 1 mL of each of the test and enzyme solutions, in a test tube, was incubated at 25 °C for 30 min. Then, after taking out 1 mL from this mixture, 1 mL of the above-mentioned starch solution was added, and the mixture was incubated at 25 °C for 3 min. Finally, 1 mL of the DNS solution was added. The tube was then covered and heated in a water bath at 85 °C for 15 min. After cooling the tube, the reaction mixture was diluted with distilled water (9 mL). It was mixed well, and the absorbance was recorded at 540 nm. In case of blank, the DNS solution was added before the addition of the starch solution, while the rest of the method was the same as for the test. For control, all procedure was again the same except that plant extract was replaced by 1mL of DMSO. Acarbose, a well-known anti-diabetic medicine, was used as a positive control. The percentage inhibition was calculated by the formula

$$\% \text{ Inhibition} = [(A_c - A_s)] / A_c \times 100$$

Where, A_s = absorbance of the test sample, A_c = absorbance of control.

Alpha-Glucosidase Assay: The alpha-glucosidase inhibition was determined using the modified method [18]. The α-glucosidase reaction mixture contained 2.9mM P-nitrophenyl-α-glucopyranoside (pNPG), varying concentrations (15 mg/mL to 75 mg/mL) of the given samples and 1.0 U/ml α-glucosidase in sodium phosphate buffer, pH 6.9. Control tubes contained only DMSO, enzyme, and substrate, while in positive controls, acarbose replaced the sample extract. Mixtures without enzyme, sample extract and acarbose served as blanks.

The reaction mixtures were incubated at 25 ºC for 5 min, after which the reaction was stopped by boiling for 2 min. The absorbance of the resulting p-nitrophenol (pNP) was determined at 405 nm using spectrophotometer (UV-Vis spectrophotometer UV-2450 (Shimadzu)) and was considered directly proportional to the activity of the enzyme. The IC_{50} values were determined from plots of percent inhibition versus log inhibitor concentration and were calculated by non-linear regression analysis from the mean inhibitory values. Acarbose was used as the reference alpha-glucosidase inhibitor. All tests were performed in triplicates. The percentage inhibition was calculated by the formula:

$$\% \text{ Inhibition} = [(A_c - A_s)] / A_c \times 100$$

Where, A_s = absorbance of the test sample, A_c = absorbance of control.

***In-vivo* Anti-diabetic Activity of Crude Leaf Extract of *Hellenia speciosa*:**

Oral Glucose Tolerance Test: [19] Fasted rats were divided into six groups according to their baseline glucose level.

Group 1: Normal control - distilled water,

Group 2: Vehicle control - 0.25% w/v (10 ml/kg) carboxymethyl cellulose,

Group 3: Metformin (500 mg/kg, p.o.),

Group 4, 5 and 6: *Hellenia speciosa* (200, 400 and 600 mg/kg) respectively.

The normal control received distilled water; the vehicle control received only carboxymethyl cellulose (P.O). Group 3 received metformin at a dose of 500 mg/ kg body weight. Group 4, 5 and 6 were administered with plant extract at different doe (200, 400 and 600 mg/kg) only in a vehicle respectively. After dosing the animals as specified a glucose load of 2 g/kg (P.O) was orally administered simultaneously. Blood samples were collected from puncturing the retro-orbital sinus just before drug administration and 15, 30, 90, and 120 min after loading glucose. Serum glucose level was measured immediately using glucometer (Accu-check, Roche, Germany).

Alloxan Induced Animal Model: [20] After one week of the acclimatization, rats were injected once with low-dose of alloxan (80 mg/kg) to induce partial insulin deficiency. The glucose value was noted using glucometer before alloxan injection. This is called Basal value.

After 48-96 h of alloxan injection, the rat's fasting blood glucose value (glucometer) were noted using tail flick method. The animals would display hyperglycemia and glucose intolerance.

Animals with similar degrees of hyperglycemia (mostly above 95 mg/dl) were considered, and according to their glucose value, animals were randomized and divided into groups as follows:

Group 1: Normal group

Group 2: Vehicle control (Untreated)

Group 3: Diabetic control (Untreated)

Group 4: Diabetic group + Metformin (350 mg/kg, p.o),

Group 5: Diabetic group + *Hellenia speciosa* (300 mg/kg)

Animal Blood Collection: Animals were kept fasting for 12 h on the day before glucose estimation. Then the animals tail were flicked, fasting blood glucose levels were noted on day 0 and every week (week 1, 2, 3 and 4) of the entire observation period.

Body Weight: Body weight of individual animals will be recorded from day 0 and every week (week 1, 2, 3, and 4) of the entire observation period.

Estimation of Glucose: The blood was collected from retro-orbital plexus under mild anesthesia from the overnight fasted rats and fasting blood glucose level was estimated.

Estimation of Insulin: The major function of insulin is to counter the concerted action of several hyperglycemia-generating hormones and to maintain low blood glucose levels. The assay was performed as Ultrasensitive rat insulin kit (catalog 90060).

HOMA-IR: Insulin resistance was determined using the homeostasis model assessment index for insulin resistance (HOMA-IR) [21] using the following formula:

HOMA-IR index = [fasting glucose (mmol/L) × fasting insulin (μU/ml)] / 22.5

Statistical Analysis: Statistical comparison was done using one-way ANOVA followed by Dunnett post hoc comparison when more than two groups are involved. P value less than 0.05 was considered significant.

RESULTS:

Phytochemical Screening of *Hellenia speciosa*: Screening for phytochemicals of leaf extract of *Hellenia speciosa* is significant to find out the medicinal importance of the plant in the field of drug discovery. In the present study, the phytochemical analysis was performed with five different leaf extract (aqueous, ethanol, chloroform, acetone, and petroleum ether) of *Hellenia speciosa* **Table 1**.

TABLE 1: PHYTOCHEMICAL SCREENING OF LEAF EXTRACTS OF *HELLENIA SPECIOSA*

Phytochemicals	Leaf Extract				
	Aqueous	Ethanol	Chloroform	Acetone	Petroleum Ether
Tannins	-	+	-	+	-
Saponins	++	++	-	+	-
Flavonoids	+	+	-	+	-
Quinones	+	++	++	++	++
Glycosides	-	+	-	+	-
Cardiac glycosides	+	++	+	++	+
Terpenoids	+	++	+	++	+
Phenol	++	++	+	++	-
Coumarins	+	+	-	+	-
Steroids	+	++	+	++	+
Alkaloids	+	+	-	+	-
Anthocyanin	-	-	-	-	-
Betacyanin	+	+	+	+	-

++ = strong positive; + = positive; - = negative

***In-vitro* Anti-diabetic Activity of *Hellenia speciosa*:** Digestive enzymes have been targeted as potential avenues for modulation of blood glucose. The key enzymes for carbohydrate metabolism in the small intestine are pancreatic α-amylase and α-glucosidase which convert consumed polysaccharides to monosaccharides. In the present study, the inhibitory effect of leaf extract on alpha-amylase and alpha-glucosidase are compared with that of standard acarbose.

Alpha Amylase Enzyme Inhibition Assay: There was a dose-dependent increase in percentage inhibitory activity against alpha-amylase enzyme.

From the study, it was found that *Hellenia speciosa* exhibited maximum inhibition of 63.2% (150 mg/ml), and the IC_{50} value was calculated to be 111.2 mg/ml **Table 2**.

Alpha-Glucosidase Enzyme Inhibition Assay: There was a dose-dependent increase in percentage inhibitory activity against the alpha-glucosidase enzyme. The results of alpha-glucosidase inhibition study revealed that *Hellenia speciosa* exhibited maximum inhibition of 78.1% (75 mg/ml), and the IC_{50} value was calculated to be 47.84 mg/ml **Table 3**.

***In-vivo* antidiabetic Activity of Crude Leaf Extract *Hellenia speciosa* in Animal Model:**

Oral Glucose Tolerance Test: The effect of leaf extract of *Hellenia speciosa* (200 mg/kg, 400 mg/kg, and 600 mg/kg) on glucose tolerance test is shown in **Table 4**. The acute effect of *Hellenia speciosa* was evaluated by OGTT on overnight fasted animals. *Hellenia speciosa* significantly ($p< 0.05$) reduced blood glucose excursion at tested doses of 400 mg/kg and 600 mg/kg, respectively. Results suggested that there was a significant improvement in glucose tolerance in the experimental groups. Metformin also (500 mg/kg) significantly ($p<0.05$) lowered glucose excursion ($AUC_{0–120\ min}$). However, *Hellenia speciosa* at 200 mg/kg did not exhibit any significant blood glucose lowering when compared with vehicle control **Table 4**.

TABLE 2: ALPHA AMYLASE INHIBITORY ACTIVITY OF *HELLENIA SPECIOSA*

Concentration (mg/ml)	% inhibition *H. speciosa*
30	8.3 ± 2.4
60	25.7 ± 2.3
90	47.4 ± 1.9
120	55.9 ± 3.7
150	68.4 ± 2.4

Values are expressed as Mean of triplicate measurements ± Standard deviation

TABLE 3: A ALPHA GLUCOSIDASE INHIBITORY ACTIVITY OF *HELLENIA SPECIOSA*

Concentration (mg/ml)	% inhibition *H. speciosa*
15	18.2 ± 2.1
30	34.5 ± 3.8
45	47.4 ± 4.1
60	58.2 ± 2.6
75	78.1 ± 1.4

Values are expressed as Mean of triplicate measurements ± Standard deviation

TABLE 4: EFFECT OF CRUDE LEAF EXTRACT OF *HELLENIA SPECIOSA* ON THE LEVELS OF OGTT IN CONTROL AND EXPERIMENTAL RATS

Time (h)	Normal Control	Vehicle Control	Metformin (500mg/kg)	*Hellenia speciosa*		
				200 mg/kg	400 mg/kg	600 mg/kg
0	97.5	99.6	101.5	100.5	99.3	100.1
30	98.2	340.6	262.7	330.4	298.2	276.3
60	97.6	268.2	196.5	298.1	275.2	201
90	98.6	225.6	180.4	245.2	200.2	195.4
120	97.3	193.7	140.3	190.3	180.3	176.1

Hypoglycemic Activity of Crude Leaf Extract of *Hellenia speciosa* in Alloxan-Induced Diabetic Rats: The efficacy of anti-diabetic plant materials can vary with time; this is customarily investigated through this sequence of acute studies. The typical measure of glucose homeostasis to be undertaken includes basal (fasting) or random blood/plasma glucose and insulin concentrations. After 28 days, changes in body weight, feed intake, blood glucose levels, and insulin levels were measured in the experimental and control groups.

Effect of Crude Leaf Extract of *Hellenia speciosa* on Body Weight: Table 5 shows the changes in body weight of control and experimental animals. Alloxan-induced diabetic rats displayed a significant ($p<0.05$) decrease in the final body weight when compared with normal control. Treatment with *Hellenia speciosa* extracts significantly ($p<0.05$) improved these variations and brought back the levels to near normal.

TABLE 5: EFFECT OF CRUDE LEAF EXTRACT OF *HELLENIA SPECIOSA* ON BODY WEIGHT IN CONTROL AND EXPERIMENTAL RATS

Groups	Initial body weight (g)	Final Body weight(g)
Normal control	131.62 ± 2.29	157.73 ± 2.23
Diabetic control	131.20 ± 1.04*	121.65 ± 1.69*
Diabetic vehicle control	131.88 ± 2.16	120.85 ± 2.94
Metformin (350 mg/kg)	131.27 ± 2.83	145.65 ± 2.86
Hellenia speciosa (300 mg/kg)	131.27 ± 1.22$^{\#}$	137.15 ± 1.61$^{\#}$

Values are expressed as Mean (n=6) ± Standard deviation *P<0.05 as compared to normal control and $^{\#}$P<0.05 compared to diabetic control

Effect of Crude Leaf Extract of *Hellenia speciosa* on Glucose Level, Insulin Level, and Homa IR Index: The blood glucose level of the diabetic group was significantly higher (P<0.05) than that of Normal. The results showed a significant (P<0.05) reduction in blood glucose in *Hellenia speciosa* (300 mg/kg) treated groups compared with the untreated diabetic group. The blood glucose of *Hellenia speciosa* treated groups was comparable with that of the metformin group at the end of the experiment **Table 6**.

TABLE 6 EFFECT OF CRUDE LEAF EXTRACT OF *HELLENIA SPECIOSA* ON GLUCOSE LEVEL, INSULIN LEVEL, AND HOMA-IR INDEX

Groups	Glucose level (mg/dl)	Insulin level (µU/l)	HOMA –IR Index
Normal control	81.17 ± 4.17	25.2 ±0.8	5.05 ±0.12
Diabetic control	158.83 ± 16.20*	13.2 ±0.9*	5.18±0.06*
Diabetic vehicle control	156.50± 12.0	13.8 ±0.6	5.33±0.38
Metformin (350 mg/kg)	156.50± 12.0	24.0 ± 1.3	7.22±0.74
Hellenia speciosa (300 mg/kg)	133.83 ± 8.86#	21.1 ±0.7#	6.96±0.50#

Values are expressed as Mean (n=6) ± Standard deviation *P<0.05 as compared to normal control and #P<0.05 compared to diabetic control

The results from this study indicated that the plasma insulin level of the diabetic group was significantly (P<0.05) reduced when compared with the Normal group. The results show an increase in plasma insulin levels of diabetic rats treated with *Hellenia speciosa* (300 mg/kg). *Hellenia speciosa* significantly (P<0.05) attenuated alloxan-induced hypoinsulinemia. This increase in insulin and decrease in glucose is proved by calculating Homo IR index.

DISCUSSION:

Phytochemical Screening of Leaf Extract of *Hellenia speciosa*: Phytochemicals are non-nutritive plant chemical that has disease protective or preventive disease property. These phytochemicals either alone and/or in combination, have tremendous therapeutic potential in curing various ailments. Identification of phytochemical components could be used to explain some of the biological activity of plant extracts [22].

In the present study, the phytochemical analysis was performed with five different leaf extract (aqueous, ethanol, chloroform, acetone, and petroleum ether) of *Hellenia speciosa*. The ethanol and acetone leaf extract of *Hellenia speciosa* showed positive for tannins, saponins, flavonoids, quinones, glycosides, cardiac glycosides, terpenoids, phenols, coumarins, steroids, alkaloids, betacyanin except for anthocyanin. Similar findings were reported from the rhizomes of *Costus speciosus* [22, 23] and leaves of *Costus pictus* [24, 25]. The phenols and flavonoids present in the plant extract could protect living organisms against reactive oxygen species [26, 27].

Anti-diabetic Activity- *In-vitro* and *In-vivo* Studies of *Hellenia speciosa*: Modern lifestyle, advanced food habits, less physical work, mental workloads, and other parameters may be responsible for diabetes which was seen in high-income families. It is confirmed by a survey conducted by IDF [28] that, low-income groups were having the least diabetes prevalence when compared to the groups of increased income groups. It is expected to be the biggest economic burden of national health services, families, social health services, and countries to manage diabetes and its complications. For diabetes itself, it accounted 10.8% of gross expenditure on health worldwide in 2013. 90% of countries spend 5-18% of overall health expenditure for only to diabetes management [29].

Ethnobotanical information indicates that more than 800 plants are used as traditional remedies for the treatment of diabetes due to their effectiveness, fewer side effects, and low cost. Plant extracts or individual phytochemical or group of phytochemical has exhibited the many reactions or mechanisms to reduce the diabetes status [30]. The *in-vitro* study was carried out in *Hellenia speciosa* to exhibit its anti-diabetic activity. The plant extracts showed a dose-dependent increase in the inhibitory activity against alpha-amylase and alpha-glucosidase enzyme. The amylase inhibitory effect seen in *Hellenia speciosa* leaf extract in the current study is in agreement with [31]. Similar studies carried out in rhizome of *Costus sp*eciosus also showed the multiple effects of the extract in bringing the down the blood glucose level [32, 33].

To ascertain the medicinal importance of *Hellenia speciosa* in the treatment of diabetes, the *in-vivo* was designed to evaluate the anti-diabetic activity by oral glucose tolerance test and alloxan-induced diabetic model.

Oral Glucose Tolerance Test: The efficacy of anti-diabetic plant materials can vary with time; this is customarily investigated through a sequence of acute oral glucose tolerance test. In oral glucose tolerance test, the effect of the plant extract at different concentration was studied in normal and diabetic rats. In diabetic rats, the peak increase in blood glucose concentration was observed after 60 min, and it remained high over 120 min. *Hellenia speciosa* treated diabetic rats showed a significant decrease in blood glucose concentration at 60 min and 120 min interval. Supplementation of plant extract improved glucose tolerance in the normal fasted rats.

The results of OGTT revealed the potency of *Hellenia speciosa* leaf extract in the reduction of elevated blood glucose to normal in fasted animals. The results are in correlation with [34, 35]. The typical measure of glucose homeostasis at different time points, including basal (fasting) or random blood/ plasma glucose, indicated that *Hellenia speciosa* showed anti-diabetic activity at a maximum concentration of 600 mg/kg. Hence, half of the maximum drug concentration is selected for the 28-day dosing study of an alloxan-induced diabetic rat model.

Alloxan Induced Diabetic Model: In the present study following the alloxan treatment, the body weight, glucose level, and insulin level showed significant alterations in diabetic rats. Their alterations may be due to impaired insulin signaling in diabetes. Administration of alloxan has caused a significant elevation in glucose level when compared to control rats. Alloxan destroys the beta cells of islets of Langerhans and causes reduced insulin secretion indicating hyperglycemia [36].

Alloxan is responsible for decreased insulin, thereby inducing hyperglycemia. The cytotoxic action of a diabetogenic agent such as alloxan is mediated by reactive oxygen species [37]. The mechanism of hyperglycemia in diabetes mellitus involves excessive glycogenolysis and gluconeo-genesis and decreased utilization of glucose by tissues [38].

Body Weight: The results of the present study showed that there was a significant reduction in body weight of alloxan-induced diabetic rats when compared with diabetic treated and normal rats [39, 40]. Diabetes is accompanied by increased glycogenolysis, lipolysis, gluconeogenesis, and these biochemical activities result in muscles wasting and loss of tissue proteins. Supplementation of plant extract for 28 days showed a significant increase in body weight, which might be due to increased protein turnover, requiring an increased protein synthesis, which is thermogenically expensive [41]. It was reported that *Costus igneus* administration significantly increased body weight in alloxan-induced diabetic rats. This might be due to the phytochemical constituents present in the plant extract, which can recover the appetite and stabilize the feed intake.

Administration of crude *Hellenia speciosa* leaf extract improved the glucose control and decreased the protein catabolic effect, and thus energy expenditure drops toward normal [42, 43]. The body weight also found to be restored in metformin-treated rats. The phytochemical constituents present in the plant extract improved the body weight of diabetic rats.

Glucose Level, Insulin Level, and HOMA-IR: A significant increase in fasting blood glucose, decrease in fasting insulin was observed in rats with diabetes compared to normal rats. This condition was attributed due to hyposecretion of insulin by the beta cells. Treatment with crude leaf extract of *Hellenia speciosa* decreased the elevated fasting blood glucose significantly as compared to the diabetic group. However, fasting insulin was increased significantly as compared to the diabetic group. This indicates the effectiveness of the plant extract in decreasing the blood sugar level to normal.

The hypoglycemic effect of *Hellenia speciosa* may be due to its insulin secretory effect on the beta cells of Islets of Langerhans of the pancreas. Metformin-treated rats also showed significant glucose lowering effect. The results obtained in the present study is in correlation with Eliza and

Umamageswari [44, 45]. In 2009 [46] a study demonstrated that the aqueous and methanolic extracts of *Costus speciosus* were highly effective in bringing down the blood glucose level. Similarly, the normoglycemic effect of hexane, ethyl acetate, and methanol crude extracts of *Costus speciosus* in streptozotocin-induced diabetic rats was studied [47]. HOMA-IR stands for Homeostatic Model Assessment of Insulin Resistance**.** HOMA IR index was also calculated for the assessment of insulin resistance [48, 49]. The alloxan-induced diabetic rats showed a significant increase in HOMO-IR index. The present study showed that *Hellenia speciosa* is effective in normalizing blood glucose and insulin levels. This finding was in agreement with Rossetti [50].

CONCLUSION: The crude leaf extract of *Hellenia speciosa* effectively reversed the alloxan-induced changes in the blood sugar level and restored normal cell population in the liver. The results also suggest that the anti-diabetic activity of *Hellenia speciosa* is comparable to the standard drug metformin. Thus, the study highlights the use of *Hellenia speciosa* as a potential herbal formulation in the treatment of diabetes.

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CONFLICT OF INTEREST: The authors declare no conflict of interest.

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