INTERNATIONAL JOURNAL OF PHYTOTHERAPY RESEARCH
ISSN 2278 – 5701

Research article

ANTI-HYPERGLYCEMIC EFFECT OF METHANOLIC EXTRACT OF *HELICANTHUS ELASTICUS* ON STREPTOZOTOCIN INDUCED DIABETIC RATS

M.S.Rajesh[*1], Dr. J Rajasekhar

1. Department of Pharmacology, Government College of Pharmacy, Bangalore-560027

2. Scientist, Research and Development, BI Pvt. Ltd, Bangalore.

Corresponding Author: M.S.Rajesh

ABSTRACT:

Natural products are an important source for managing the many pathological conditions from time immemorial. *Helicanthus elasticus* (loranthaceae) is an hemiparasite usuall grow on mango plant. In the present study an attempt is made to evaluate the anti-hyperglycemic activity and invitro α-Amylase activity of the plant. The methanolic extract of *Helicanthus elasticus* was tested for anti-hyperglycemic activity on Streptozotocin induced diabetic rats. Blood glucose levels were evaluated at 0, 30, 60,120 and 240 min in fasted and glucose loaded diabetic rats in acute studies and at 1st, 7th, 14th and 21stdayin sub-acute studies of Streptozotocin induced diabetic rats after extract administration at 200mg/kg body weight. The extract was studied for in-vitro alpha amylase inhibitory activity using acarbose as standard. In both acute and sub-acute studies the methanolic extract has shown statistically significant anti-hyperglycemic and glucose tolerance activity. Methanolic extract of *Helicanthus elasticus* possess statistically significant anti-hyperglycemic activity in both acute and sub-acute models of Streptozotocin induced diabetes and α-Amylase inhibition**.**

Key words: *Helicanthus elasticus*, Streptozotocin, Anti-hyperglycemic, alpha amylase, Loranthaceae.

INTRODUCTION

Interest in medicinal plants as a re-emerging health aid has been fueled by the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bio prospecting of new plant-derived drugs. Natural products have served as an important source of drugs since ancient times and about 67% of the today's useful drugs are derived from natural sources[1].India is endowed with a rich wealth of medicinal plants and play important role in lives of rural people, particularly in remote parts of developing countries like India with few health facilities. *Helicanthus elasticus*is hemi parasites belonging to the family loranthacea usually grow on mango tree. The leaves of *Helicanthus elasticus are* used to check abortion. It is also used in vesical calculi and kidney infections, leaves are used as poultices in sores and ulcers[2]. Our literature survey revealed that the partially purified extract has been used to test the Cytotoxicity and had reported good inhibition property.Diabetes is serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. The increasing number of ageing population, consumption of caloric rich diet, obesity and sedentary life style have led to a tremendous increase in the number of diabetics worldwide. About 2.5-3 % of the world's population suffers from this disease, a

proportion in developing countries, has reached more than 7%.[3, 4] Present studies was undertaken to investigate the type-1 antidiabetic activity of *H.elasticus* in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Plant material:

The plant *Helicanthus elasticus* (Loranthaceae) growing on *Mangifera indica* (Fam.Anacardiaceae) were collected from the regions of Western Ghats, Karnataka, India and from western ghats (Ambe ghat) of kholapur, Maharashtra. The plant was authenticated by Dr Siddamallaiah, Botanist and a vocher sample was deposited. The whole plant of *Helicanthus elasticus* was excised in to small pieces and dried under shade. The dried plant was pulvarized and extracted with methanol using soxhlet extraction apparatus. The methanol extract (HEM) was concentrated by using Rotavapour under vaccum pressure.

Experimental animals:

Albino Wister rats (200-250g) of either sex bred in the animal house of Drug testing laboratory, Palace road, Bangalore were procured and used in this study. The animals were fed on a standard pellet diet and had free access to ozonised filter water *ad libitum*. The animals were maintained in their respective groups under controlled conditions of temperature and humidity. All the studies were conducted in accordance with CPCSEA guidelines and the experiments were carried out with approval Institutional Ethics Committee (proposal No. GCP/CPCSEA/IAEC/2009-10)

Acute toxicity studies (OECD 425):

Acute toxicity studies were performed in mice based upon OECD guidelines 425. Extract up to 2g/kg body weight did not show any toxic reaction and 1/10th of this dose was used as therapeutic dose. The animals were observed continuously for the initial 6 hours and then again at 24h and 48h following drug administration. Mortality and general behavior of the animals were observed. The parameters observed were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion.

Experimental induction of diabetes:

Adult albino Wister rats of either sex were made diabetic with a single intraperitoneal injection of 65[5] mg/kg body weight of Streptozotocin (Sigma Aldrich chemical company) dissolved in 0.1 M cold citrate buffer, PH-4.5, immediately before use. Streptozotocin injected animals exhibited massive glycosuria and hyperglycemia within few days. Diabetes was confirmed in STZ rats by measuring the fasting blood glucose concentration, on 4th day after the injection with STZ. Adult albino Wister rats with blood glucose levels more than 250 mg/dl were considered to be diabetic and were used in this experiment. The extract (HEM) at the dose of 200 mg/kg body weight was administered

orally after suspending in 5% Tween-80[6] solution. The blood samples were collected from tail vein[7] and the blood glucose levels were determined using Glucometer[8].

Experimental design:

Acute Studies

Effect of methanolic extract of *H.elasticus* (HEM) on Streptozotocin induced hyperglycemia:

After induction of diabetes the rats were divided into four groups of six animals each and screened for anti-hyperglycemic activity in overnight fasted diabetic rats. The blood samples were collected from tail vein and the blood glucose levels were determined using Glucometer.

Group 1: Normal control rats received 5% Tween 80.
Group 2: Diabetic control received 5% Tween 80.
Group 3: Served as std. control and received Glibenclamide at the dose of 2 mg/kg.
Group 4: Received methanolic extract of *H.elasticus* at the dose of 200mg/kg.

Oral glucose tolerance test and sub-acute treatment were carried out by including half the dose and double the dose of therapeutic dose.

Effect on glucose loaded diabetic rats:

In oral glucose tolerance test along with therapeutic dose, half the dose and double the dose of therapeutic dose were screened. Overnight fasted diabetic rats were divided in to six groups of six animals each as mentioned above and received the respective treatments. After 30min of drug administration the rats of all the groups were orally treated with 2 g/kg[9] of glucose. Blood samples were collected prior to drug administration and at 30, 60, 120 and 240 min after glucose loading. Blood glucose levels were measured immediately using Glucometer.

Group 1: Normal control rats received 5% Tween 80.
Group 2: Diabetic control received 5% Tween 80.
Group 3: Served as std. control and received Glibenclamide at the dose of 2 mg/kg.
Group 4: Received methanolic extract of *H.elasticus* at the dose of 100mg/kg.
Group 5: Received methanolic extract of *H.elasticus* at the dose of 200mg/kg.
Group 6: Received methanolic extract of *H.elasticus* at the dose of 400mg/kg.

Sub-acute treatment:

In sub-acute treatment, the methanolic extract of *H.elasticus* at the dose of 200mg/kg bodyweight, half the dose and double the dose of therapeutic dose were twice daily given for 21 days. Blood samples were collected from tail vein on 1st, 7th, 14th and 21st day of the treatment. Blood glucose levels were determined using Glucometer. On the last day of the treatment the animals were sacrificed with excess dose of ether anesthesia and blood was collected through retro orbital plexus for estimation of hematological parameters, piece of liver was extracted for glycogen estimation. The body weights of all the animals of all the groups were recorded before starting the treatment and at the end of the treatment period.

ISSN 2278 – 5701

In-vitro α-Amylase inhibition activity[9]:

The extract/Standard drug (Acarbose) sample (100 µl) was mixed with 100 µl of 0.02 mol/l sodium phosphate buffer (pH 6.9) and 100 µl α-amylase solution (4.5 units/ml/min) and pre-incubated at 25^0C for 10 min. Then, 100 µl of 1% starch solution was added and incubated at 25^0C for 30 min and the reaction was stopped by the addition of 1.0 ml dinitrosalicylic acid reagent (1 g 3,5-dinitrosalicylic acid in a solution containing 20 ml of 2 mol/l NaOH, 50 mL distilled water and 30 g Rochelle salt. The contents were dissolved and the volume was made up to 100 ml with distilled water). The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was then diluted 10-fold times with distilled water and the absorbance was measured at 540 nm. The readings were compared with the control (the sample was replaced by buffer) and α-amylase inhibition activity (%) was calculated.

% α-Amylase inhibition activity = (AControl – ASample) / AControl X 100

Where AControl: Control absorbance.

Asample: Sample/standard absorbance.

Statistical analysis:

Data obtained was analyzed using prism software and the results were expressed as Mean ± SEM, n=6. Statistical significance was determined by using One-way analysis of variance (ANOVA) followed by Dunnet's test.

RESULTS AND DISCUSSION:

Acute toxicity studies (OECD 425):

According to the guidelines the gross behavior and signs of toxicity were studied in animals but no change in behavior or any signs of toxicity were observed upto the maximum dose of 2000 mg/kg observed. The methanolic extract of *H. elasticus* did not produce any lethality up to the dose level of 2000 mg/kg.

Anti-hyperglycemic effect:

Acute effects after oral administration of methanolic extract of *H.elasticus* in overnight fasted diabetic rats are shown in Table 1. The fall was seen at 30min and remained up to 240min after administration of the extract which was statistically significant ($p<0.01$).

Oral glucose tolerance test:

BGL at various intervals of time in diabetic animals are shown in Table2. All the values are compared with 0 hour reading of the respective groups. All the groups have shown significant increase in the BGL at 30 minutes after the administration of glucose load in contrast to 0 min reading. The methanolic extract of *H.elasticus* at the dose of 100mg, 200mg and 400mg has significantly decreased BGL when compared to diabetic control. HEM at dose of 200mg and 400mg treated group are on par with glibenclamide treated group showning significant decrease in BGL.

ISSN 2278 – 5701

Sub-acute treatment:

The values of BGL are shown in Table 3 and hematological parameters shown in Table 4. Methanolic extract of *H.elasticus* and Glibenclamide treated groups have shown significant decrease ($p<0.01$) in BGL. HEM is significant in reducing the blood glucose levels but there is no significant difference between the therapeutic dose and double the therapeutic dose while the effect of HEM at 100mg/kg is less significant than the therapeutic dose. The effect of extracts on parameters like cholesterol, triglycerides, liver glycogen and serum insulin shows significant effect ($p<0.01$) at the dose of 200mg and 400mg/kg body weight. At 100mg/kg body weight HEM is not significant however there is a marginal change in the values in contrast to the diabetic control.

α-Amylase Inhibition Activity:

HEM significantly inhibited the enzyme α-Amylase as compared to reference standard acarbose. The α-Amylase inhibition property is effective in suppressing the postprandial hyperglycemia because it prevents the breakdown of starch in to disaccharides that are acted upon by α-glycosidase to release glucose. The results are shown in Table 5.

Table.1: Effect of methanol extract of *Helicanthus elasticus* (HEM)in Streptozotocin induced diabetes on rats (Acute Model)

Treatment	Fasting Blood Glucose (mg %)				
	Prior to Drug Administration (0 min.)	30 min.	60 min.	120 min.	240 min.
Control	65.5 ± 4.80	58.5±4.06	65.75±1.62	58.75±2.57	61.25±1.77
Diabetic control	420.0 ± 32.1	428.3±24.03	431.0±20.57	426.0±4.34	429.3±7.58
Diabetic + Glibenclamide	336.8 ± 50.86	221.0±43.51**	193.5±29.67**	138.3±16.4**	125.3±38.5***
HEM 200	345.4±32.56	274.2±21.48	250.9±19.17*	200.6±19.89**	181.3±24.85**

Data are expressed as the mean ± S.E.M., n = 6 in each group. *P < 0.05, **P < 0.01 and ***P<0.001when compared to diabetic control group (one way ANOVA followed by Dunnet's test).

Table.2:Effect of methanol extract of *H. elasticus* on Oral Glucose Tolerance Test in diabetic Rats.

Treatment	Blood Glucose Levels mg/dl				
	0 min	30 min	60 min	120 min	240 min
Control	65.5±4.8	120.5±4.1	105.7±1.6	98.75±2.5	91.25±1.7
Diabetic control	412.5±3.1	574.0±3.7	534.3±16.7	479.8±20.0	433.8±10.1
Diabetic + Glibenclamide	345.0±14.6	588.0±1.2	447.0±20.9*	290.5±14.1**	250.8±4.9***
HEM 100	325.6±17.5	518.5±12.8	480.9±5.7*	401.5±12.5*	385.2±6.2*
HEM 200	352.6±20.4	536.7±15.7	412.3±13.7*	384.7±46.8**	301.7±7.9**
HEM 400	350.3±21.5	494.3±3.5	400.9±23.7*	385.3±12.7**	299.3±25.8**

Data are expressed as the mean ± S.E.M., n = 6 in each group. *P < 0.05, **P < 0.01 and ***P<0.001when compared to diabetic control group (one way ANOVA followed by Dunnet's test).

Table.3: Effect of methanol extract of *Helicanthus elasticus* in Streptozotocin induced diabetes on rats (Sub - Acute Model)

Treatment	Fasting Blood Glucose (mg %)			
	Day 1	Day 7	Day 14	Day 21
Control	75.8 ± 2.301	72.4±15.342	73.5±12.643	71.75 ± 2.052
Diabetic control	429.5 ± 15.67	398.6±14.768	345.9±15.246	330.5 ± 18.91
Diabetic + Glibenclamide	336.7 ± 16.8	226.3±19.452	134.7±9.142**	91.0 ± 26.8**
HEM100	342.1±10.890	298.2±13.253	245.2±8.109	165.4±5.367*
HEM200	365.3±12.562	258.4±4.287	155.7±16.860*	102.6±16.753**
HEM400	356.7±16.724	234.4±9.087	140.2±17.574*	95.6±14.965**

Data are expressed as the mean ± S.E.M., n = 6 in each group. *P < 0.05, **P < 0.01 and ***P<0.001when compared to diabetic control group (one way ANOVA followed by Dunnet's test).

ISSN 2278 – 5701

Table.4: Effect of methanol and aqueous extracts of *Helicanthus elasticus* on Hematological Parameters in Diabetes induced rats

Treatment	Cholesterol (mg %)	Triglycerides (mg %)	Liver Glycogen (mg/kg)	Insulin (µU/ml)
Control	82.12±4.902	101.3±6.109	15.45±0.82	136.1±7.153
Diabetic control	104.4±5.508	121.3±3.537	8.34±0.75	62.6±6.720
Diabetic + Glibenclamide	83.20±4.246*	102.1±5.331*	14.05±0.49**	124.2±7.734**
HEM 100	95.4±5.547	113.6±3.780	9.97±0.79	75.4±7.643
HEM 200	88.8±5.647*	105.7±4.572*	12.85±0.88*	85.4±6.245*
HEM 400	84.1±2.653*	103.1±2.457*	13.11±0.77**	88.2±3.473*

Data are expressed as the mean ± S.E.M., n = 6 in each group. *P < 0.05, **P < 0.01 and ***P<0.001when compared to diabetic control group (one way ANOVA followed by Dunnet's test).

Table.5: Alpha-amylase inhibition assay of methanolic extract of *H. elasticus* (HEM)

Sr. No.	Sample	Conc. (µg/ml)	Mean Abs. ± SEM	% Inhibition
1)	Negative Control	Blank	0.847±0.078	--
2)	Acarbose	10	0.306±0.068	63.87
		50	0.218±0.012	74.26
		100	0.129±0.056	84.77
3)	HEM	10	0.385±0.026	54.55
	HEM	50	0.252±0.029	70.25
	HEM	100	0.156±0.074	81.58

CONCLUSION:

The result demonstrates that the HEM has good α-Amylase activity and also significant antidiabetic property. The extract is effective in managing the hyperlipidemia, glycogen content which is altered during the condition of diabetes mellitus. The previous studies that are reported for having antioxidant activity[10] and presence of active constituents like flavonoids[10] and other phenolic compousnds may be responsible for antihyperglycemic activity by either preventing the further damage to the pancreatic beta cell or by extra pancreatic action. Hence HEM can be developed as potential remedy in management of diabetes.

REFERENCES:

1. Newman, D.J., Cragg, G.M. and Snader K.M. 2003. Natural products as sources of new drugs over the period 1981-2002. J. Nat. Prod., 66(7), 1022-1037.
2. Kirtikar, K.R, Basu, B.D., 1999.Indian medicinal plants.Vol. III, International book distributors, Dehradun; p.732.
3. Annual Review of Diabetes 2000 by American Diabetes Association, reprint by Aramuc Scientific Comunications Pvt Ltd; Dahisar (W), Mumbai; 2000. (with grant from Panacea Biotec).
4. Type-2 Diabetes-The Indian Scenario; A Scientific Publication from Micro Labas Ltd, Bangalore; 2002.
5. Vats,V. Yadav,S.P, Grover J.K. Ethanolic extract of *Ocimum sanctum* leaves partially attenuates Streptozotocin-induced alterations in glycogen content and carbohydrate metabolism in rats. Journal of Ethanopharmacology., 90: 155-160 (2004).
6. Babu,V. Gangadevi,T. Subramoniam. Antidiabetic activity of Ethanol Extract of *Cassia kleinii* leaf in Streptozotocin–induced diabetic rats and isolation of an active fraction and toxicity evaluation of the extracts. Indian Journal of pharmacology., 35: 290-296 (2003).
7. Jayakar,B. Suresh,B. Antihyperglycemic and Hypoglycemic effect of *Aporosa lindleyana* in normal and Alloxan induced diabetic rats. Journal of Ethanopharmacology,. 84: 247-249 (2003).
8. Shanmugasundaram, E.R. Gopinath, K.L. Shanmugasundaram,K.R. Rajendaran, V.M. *J Ethnopharmacology,*.. 30: 265-279 (1990).
9. Sindhu, S. Nair, Vaibhavi Kavrekar and Anshu Mishra. In vitro studies on alpha amylase and alpha glucosidase inhibitory activities of selected plant extracts. European Journal of Experimental Biology,. 3(1):128-132 (2013).
10. Sunil Kumar, K.N. Saraswathy, A. Amerjothy, S. Susan, T. Ravishankar, B. Total Phenol Content and In Vitro Antioxidant Potential of Helicanthus elastica (Desr.) Danser-A Less-explored Indian Mango Mistletoe, J Tradit Complement Med,. 4(4):285-8 (2014).