International Journal of Research in Pharmacology & Pharmacotherapeutics (IJRPP)

IJRPP |Vol.14 | Issue 1 | Jan - Mar -2025

www.ijrpp.com

DOI : https://doi.org/10.61096/ijrpp.v14.iss1.2025.57-66

ISSN: 2278-2648

Research

In Vivo Anti Diabetic Activity Of Helicteres Isora On Streptozotocin-Induced Diabetic In Rats

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Published on: 24 Jan 2025

Published by:
DrSriram Publications

Abstract

Helicteres Isora root is a well-known antidiabetic medicinal plant used for several traditional medicine aspects in different areas of the world, including India. This study includes phytochemical analysis, antidiabetic evaluation. The ***root*** of *Helicteres Isora* has been used in traditional health systems to treat diabetes mellitus in India. However, the antidiabetic activity of this medicinal plant is not scientifically validated and authenticated. The present study aimed to investigate the *in vitro* and *in vivo* anti-diabetic activity of *root* crude extract and solvent fractions of *Helicteres Isora.* The aim of this study was to investigate the antidiabetic activity and mechanism of action of Ethanolic extract prepared from *Helicteres Isora root.* Since this claim has not been investigated scientifically, the aim of this study was to evaluate the antidiabetic effect and phytochemical screening of Streptozotocin -induced diabetic Rats. The result demonstrated the beneficial biochemical effects of *Helicteres Isora root* extract by inhibiting α–amylase improving serum lipid profile levels. The root crude extract are effective in lowering blood glucose levels in diabetic and hypoglycemic rats. The claimed traditional use as antidiabetic has scientific ground.

Keywords: Diabetes mellitus, Herbal medicine, *Helicteres Isora root*, Streptozotocin, Anti diabetic activity.

INTRODUCTION

Diabetes Mellitus (DM)

Diabetes is one of the most common non-communicable diseases and a serious life-long condition appearing worldwide. The etiology of diabetes is a complex interaction of genetic and environmental factors. It is a heterogeneous group of metabolic disorders characterized physiologically by dysfunction of pancreatic beta cells and deficiency in insulin secretion or insulin activity and clinically by hyperglycemia or impaired glucose tolerance and other manifestable disorders. It is an endocrinological syndrome abnormally having high levels of sugar in the blood. This may be either due to insulin not being produced at all, is not made at sufficient levels, or is not as effective as it should be.

Type 1 Diabetes

In type 1 the pancreas stops producing insulin due to autoimmune response or possibly viral attack on pancreas. In absence of insulin body cells don't get the required glucose for producing ATP (Adenosine Triphosphate) units which results into primary symptom in the form of nausea and vomiting. In later stage, which leads to ketoacidosis, the body starts breaking down the muscle tissue and fat for producing energy hence, causing fast weight loss. Dehydration is also usually observed due to electrolyte disturbance. In advance stages, coma and death is witnessed.

Type 2 Diabetes

Increased fatigue: due to inefficiency of the cell to metabolize glucose, reserve fat of body is metabolized to gain energy. When fat is broken down in the body, it uses more energy as compared to glucose; hence body goes in negative calorie effect, which results in fatigue.

Polydypsia: As the concentration of glucose increases in the blood, brain receives signal for diluting it and, in its counteraction we feel thirsty.

Polyuria: Increase in urine production is due to excess glucose present in body. Body gets rid of the extra sugar in the blood by excreting it through urine. This leads to dehydration because along with the sugar, a large amount of water is excreted out of the body.

Polyphagia: The hormone insulin is also responsible for stimulating hunger. In order to cope up with high sugar levels in blood, body produces insulin which leads to increased hunger.

Weight fluctuation: Factors like loss of water (polyuria), glucosuria, metabolism of body fat and protein may lead to weight loss. Few cases may show weight gain due to increased appetite.

Blurry vision: Hyperosmolar, hyperglycaemia, nonketotic syndrome is the condition when body fluid is pulled out of tissues including lenses of the eye; this affects it's to focus, resulting blurry vision.

Irritability: It is a sign of high blood sugar of the inefficient glucose supply to the brain and other body organs, which make us, feel tired and uneasy.

Infections: The body gives few signals whenever there is fluctuation in blood sugar(due to suppression of immune system) by frequent skin infections like fungal or bacterial or UTI(urinary tract infection).

Normal Responses to Eating and Fasting[14]

1. In a fed state: there is increased insulin secretion, causing Glycolysis, glycogen storage, fatty acid synthesis/storage, and protein synthesis.
2. After an overnight fast: there is low insulin and high glucagon that can cause glycogen breakdown, hepatic Gluconeogenesis, and Lipolysis.
3. After a prolonged fast: there is extremely low insulin and low glucagon, this causes lipolysis to take over. Lipids are the main fuel source. Gluconeogenesis is minimized, as it causes nitrogen wasting, ammonia build-up, and loss of muscle mass.

Hormones- Hormones that raise blood sugar include glucagon, epinephrine and norepinephrine, cortisol, Growth hormone etc. These hormones are released due to *stress*. Thus during phases of stress like an infection, surgery or pregnancy diabetes control worsens and blood sugar rises.

Pathophysiology of type 1 diabetes[15]

In this condition the immune system attacks and destroys the insulin producing beta cells of the pancreas. There is beta cell deficiency leading to complete insulin deficiency. Thus is it termed an autoimmune disease where there are anti insulin or anti-islet cell antibodies present in blood. These cause lymphocytic infiltration and destruction of the pancreas islets. The destruction may take time but the onset of the disease is rapid and may occur over a few days to weeks. There may be other autoimmune conditions associated with type 1 diabetes including vitiligo and hypothyroidism. Type 1 diabetes always requires insulin therapy, and will not respond to insulin-stimulating oral drugs.

Pathophysiology of type 2 diabetes[16]

This condition is caused by a relative deficiency of insulin and not an absolute deficiency. This means that the body is unable to produce adequate insulin to meet the needs. There is Beta cell deficiency coupled with peripheral insulin resistance. Peripheral insulin resistance means that although blood levels of insulin are high there is no hypoglycemia or low blood sugar. This may be due to changes in the insulin receptors that bring about the actions of the insulin. Obesity is the main cause of insulin resistance. In most cases over time the patients need to take insulin when oral drugs fail to stimulate adequate insulin release.

Pathophysiology of gestational diabetes[17-18]

Gestational diabetes is caused when there are excessive counter-insulin hormones of pregnancy. This leads to a state of insulin resistance and high blood sugar in the mother. There may be defective insulin receptors.

Pathophysiology behind symptoms and complications of diabetes

- Polydipsia or increased thirst is due to high blood glucose that raises the osmolarity of blood and makes it more concentrated.
- Polyuria or increased frequency of urination is due to excess fluid intake and glucose-induced urination.
- Weight loss occurs due to loss of calories in urine.
- Polyphagia or increased hunger due to loss or excess glucose in urine that leads the body to crave for more glucose.
- Poor wound healing, gum and other infections due to increased blood glucose providing a good source of nutrition to microbes and due to a diminished immunity.
- Heart disease – this occurs due to changes in the large blood vessels leading to coronary, cerebral, and peripheral artery diseases, atherosclerosis, dyslipidemia etc.
- Eye damage – this is termed diabetic retinopathy and occurs due to damage of the fine blood vessels of the retina in the eye due to long term exposure to high blood sugar.
- Kidney damage – similar damage to small and large blood vessels of the kidneys. Initially there is proteinuria or increased outflow of protein and may lead to end stage renal disease (ESRD).
- Nerve damage – this can affect the arms and legs and is called stocking-glove numbness/tingling. It can also affect autonomic functions leading to impotence,erectile dysfunction, difficulty in digestion or gastroparesis etc.
- Diabetic foot – this occurs due to peripheral nerve damage as well as blood vessel affliction due to long term diabetes. Little trauma, sores and blisters go unnoticed due to lack of sensation and peripheral vascular disease impairs healing and allows infection.
- Diabetic Ketoacidosis is caused in type 1 diabetes where there is complete lack ofinsulin and reliance on fatty acids for energy. This uncontrolled lipid breakdown leads to formation of ketones and causes acidosis and ketonemia. This is a medical emergency.
- Non-Ketotic Hyper osmolarity – this is caused due to extreme rise of blood sugar. This is seen in type 2 diabetics. There is just enough insulin to suppress ketone synthesis. The high blood sugar leads to excessive concentration or osmolarity of blood which in turn leads to dieresis and collapse of the blood vessels and cardiovascular shock. This is a medical emergency.

Diagnosis[19]

Diabetes mellitus is characterized by recurrent or persistent hyperglycemia, and is diagnosed by demonstrating any one of the following:

- Fasting plasma glucose level ≥ 7.0 mmol/l (126 mg/dl)
- Plasma glucose ≥ 11.1 mmol/l (200 mg/dL) two hours after a 75 g oral glucose load as in a glucose tolerance test
- Symptoms of hyperglycemia and casual plasma glucose ≥ 11.1 mmol/l (200 mg/dl)
- Glycated hemoglobin (Hb A1C) ≥ 6.5%.

A positive result, in the absence of unequivocal hyperglycemia, should be confirmed by a repeat of any of the above methods on a different day. It is preferable to measure a fasting glucose level because of the ease of measurement and the considerable time commitment of formal glucose tolerance testing, which takes two hours to complete and offers no prognostic advantage over the fasting test. According to the current definition, two fasting glucose measurements above 126 mg/dl (7.0 mmol/l) are considered diagnostic for diabetes mellitus.
People with fasting glucose levels from 110 to 125 mg/dl (6.1 to 6.9 mmol/l) are considered to have impaired fasting glucose. Patients with plasma glucose at or above 140 mg/dL (7.8 mmol/L), but not over 200 mg/dL (11.1 mmol/L), two hours after a 75 g oral glucose load are considered to have impaired glucose tolerance. Of these two prediabetic states, the latter in particular is a major risk factor for progression to full-blown diabetes mellitus, as well as cardiovascular disease.

MATERIALS AND METHODS

Plant collection

The aerial part of *Helicteres Isora root* was collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. Madhava Chetty, K., Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference. The leaves of *Helicteres Isora root* were collected and authenticated by Department of Botany. After shade-dried (Temp<40°C.), plant material was grounded into a moderately coarse powder. The extract was made by maceration and the ethanolic extract was made by using soxhlet apparatus. The extract was allowed to dry. Both the extracts were preserved in the refrigerator till further use.[53]

***Invitro* antidiabetic activity of *Helicteres Isora root* leaves extracts**

Alpha-amylase inhibition assay

The a-amylase inhibition assay was performed using the 3,5-dinitrosalicylic acid (DNSA) method.50 The crude and solvent fractions of *Helicteres Isora root* were dissolved in buffer ((Na2HPO4/ NaH2PO4 (0.02 M), NaCl (0.006 M) at pH 6.9) to give concentrations ranging from 50 to 1000 mg/mL. A volume of 200 mL of a-amylase solution (Molychem) (2 units/mL) was mixed with 200 mL of the extract and was incubated for 10 minutes at 30 C. Thereafter, 200 mL of the starch solution (1% in water w/v) was added to each tube and incubated for 3 minutes. The reaction was terminated by the addition of 200 mL DNSA reagent (12 g of sodium potassium tartrate tetrahydrate in 8.0 mL of 2 M NaOH and 20 mL of 96 mM 3,5-DNSA solution) and was boiled for 10 minutes in a water bath at 85°C. The mixture was cooled to ambient temperature and was diluted with 5 mL of distilled water, and the absorbance was measured at 540 nm using a UV-visible spectrophotometer (Agilent Technologies). The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 mL of the buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using acarbose (Bayer) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The inhibition of a-amylase was expressed as percentage of inhibition and was calculated by the following equation: Inhibition (%) ¼ [(Ac -Acb) (As Asb) / (Ac -Acb)] × 100, where Ac is the absorbance of control; Acb is the absorbance of control blank; As is the absorbance of sample; and Asb is the absorbance of sample blank. The % a-amylase inhibition was plotted against the extract concentration and the IC50 values were obtained from the graph.[54]

Preliminary phytochemical screening of Ethanolic leaves extract of *Helicteres Isora root*

The Ethanolic leaves extract of *Helicteres Isora root* was used for testing preliminary phytochemical screening in order to detect major chemical groups.

Test for carbohydrates

Molisch's test: Dissolved small quantity of 300mg alcoholic and dried leaves extract powder of Pimenta dioica separately in 4ml distilled water and filtered. The filtrate was subjected to Molisch's test.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution.

Phenols test: The extract was spotted on a filter paper. A drop of phosphomolybdic acid reagent was added to the spot and was exposed to ammonia vapours.

Test for flavanoids

Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added.

Lead acetate test: To 5ml of extract 1ml of lead acetate solution was added.

Test for tannins

Braemer's test: To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added.

Test for steroid/terpenoid

Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added.

Test for alkaloids

Draggendorf's test: A drop of extract was spotted on a small piece of precoated TLC plate and the plate was sprayed with modified Draggendorf's reagent.

Hager's test: The extract was treated with few ml of Hager's reagen.

Wagner's test: The extract was treated with few ml of Wagner's reagent.

Tests for Glycosides

Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution.

Test for Saponins

Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes.

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia.

Test for Amino acids

Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent.

Test for fixed oils and fats

Press small quantity of the petroleum ether extract between two filter paper.

Note: the results for the above experiments can be noted as follows.

- If the response to the test is high it can be noted as +++ which indicates that the particular group is present as the major class.
- If the response is average then note it as ++ indicates the presence in moderate quantity.

- If the response is very small then note it as + indicating the presence of only in traces.
- If no response is then negative.[55]

Acute oral toxicity study

In a research study when a drug is administered to a biological system there will be some interactions may happen. In most case these are desired and useful, but many effects are not advantageous. Acute, sub acute and chronic toxicity studies are performed by the manufacturers in the investigation of a new drug. Acute toxicity is involved in estimation of LD50 (It is the lethal dose (causing death) to 50% of tested group animals).

LD50 (median lethal oral dose)

LD 50 (median lethal oral dose) is a statistically derived oral dose of a substance that can be expected to cause death in percent of animals when administered by the oral route. The LD50 value is expressed in terms of weight of test substance per unit weight of animal (mg/kg). In this study acute oral toxicity study was carried out in Rats. The procedure was followed by using OECD 423(Acute toxic class method).The Rats are fasted overnight, prior to dosing. The three dose levels are administered by orally the help of oral feeding needle over the prior of 24 hours. After the drug has been administered, food may be with held for a further 3-4 hours in Rats. The purpose of sighting study is to allow selection of the appropriate starting dose for main study. The test substance is administered to a single animal in a sequential manner following from the fixed dose levels of 5, 50, 300 and 2000mg/kg. The interval between dosing of each level is determined by the mortality/onset, duration and severity of toxic signs over the period of 24 hours, special attention given during the first 4 hours. Four hours after the drug administration, provide the food and water for 14 days and daily observed some parameters such as food intake, water intake, mortality, onset, Duration and severity of toxic signs. The animal weight is recorded on weekly once. On the day fourteen all the animals are sacrificed, to isolate the organs and observe the histopathological changes. Based on the mortality result of sighting is decided and carried out with five animals per dose level (5 or 50 or 300 or 2000mg/kg).Based on the mortality result on 14th day of observation, the doses for *in vivo* study are selected.[56]

***Invivo* antidiabetic activity of *Helicteres Isora root* extract in Streptozotocin induced diabetic Rats.**

Prior to the experiment the rats were housed in a clean polypropylene cages (6 Rats / cages) for a period of 7 days under standard temperature (25 - 30ºC), relative humidity (45 – 55%), dark / light cycle (12 /12 hrs). The studies were performed with the approval of Organizational Animal Ethics Committee (OAEC) (DAEC/TNA/965/345/16). The animals were put in overnight fasting were deprived of food for 16 hrs but allowed free access of water.

Hypoglycemic Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g Rats)
Group II served as Positive control – Glibenclamide (2mg /kg)
Group III served as ethanolic extract of *Helicteres Isora root* – (200mg/kg)
Group IV served as ethanolic extract of *Helicteres Isora root* – (400mg/kg).
Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.

Oral Glucose Tolerance Test Groupings were done as follows:

Group I served as control – Carboxy Methyl Cellulose (CMC) 0.5% (0.3ml\100g Rats)
Group II served as Positive control – Glibenclamide (2 mg /kg)
Group III served as ethanolic extract of *Helicteres Isora root* – (200mg/kg)
Group IV served as ethanolic extract of *Helicteres Isora root* – (400mg/kg).
All the groups of animals were fasted for 24h and blood samples were collected before drug or solvent treatment. The drug, extract and solvent, have been administered to different groups and 30mins later all the groups of Rats were treated with glucose orally at dose 10gm/kg body weight by using oral feeding needle. Blood samples were collected by the tail nipping method and glucose level checked by glucometer. After drug Administration blood samples have been collected different time intervals at 30, 60 and 120.[57]

Induction of diabetes to animals

A single dose (100 mg/kg b.w., i.p.) of Streptozotocin dissolved in sodium citrate buffer was used for the induction of diabetes in Rats after overnight fasting. After 1 hr of Streptozotocin administration, the animals were given feed and libitum and 5% dextrose solution was also given in feeding bottle for a day to overcome early hypoglycemic phase. The animals were stabilized for a week and animals showing blood glucose level more than 200 mg/dl were selected for the study.

Experimental design

Five groups of Rats six in each groups received the following treatment schedule for 14 days.
GROUP I - Normal control (normal saline 10 ml /kg, P.O)
GROUP II - Streptozotocin treated control (100 mg/kg, I.P)
GROUP III - Streptozotocin (100 mg/kg, I.P) + Standard drug Glibenclamide (2 mg/kg, P.O).
GROUP IV - Streptozotocin (100 mg/kg, i.p.) + EEHI (200 mg/kg, P.O)
GROUP V - Streptozotocin (100 mg/kg, i.p.) + EEHI (400 mg/kg, P.O)

Plant leaves extract, standard drug and normal saline were administered with the help of oral feeding needle. Group I serve as normal control which received normal saline for 14 days. Group II to Group V were diabetic control Rats. Group IV and Group V (which previously received Streptozotocin 100mg/kg) were given fixed doses of ethanol leaves extract (200 mg/kg, P.O, 400 mg/kg, P.O) of *Helicteres Isora root* and group III received standard drug Glibenclamide (2 mg/kg,P.O) for 14 consecutive days. (EEHI - Ethanolic extract of *Diospyros lotus Fruits*).[58]

Collection of blood samples

Fasting blood samples were drawn from retro orbital puncture of Rats at weekly intervals till the end of the study 1, 7, and 14 days.

Estimation of biochemical parameters Serum blood glucose

On 1,7, and 14 days fasting blood samples were collected and analyzed the blood glucose.

Blood glucose level

The blood glucose level test measures the amount of glucose in the blood sample obtained from the animals. The test is usually performed to check for elevated blood glucose levels which can be an indication of diabetes or insulin inhibition.

Statistical analysis

Statistical analysis was done by using GRAPHPAD PRISM 5.0.All the values of Biochemical parameters and body weight were expressed as Mean ± Standard Error Mean (SEM). The values were analyzed for statistical significance using one- way analysis of variance (ANOVA), comparison was done by using Dunnett's t test. P values < 0.05 were considered as significant, P values < 0.01 were considered as very significant, P values < 0.001 were considered as highly significant and ns were considered as not significant.[59]

RESULTS

Appearance and percentage yield of EEHI (Ethanolic Extract of *Helicteres Isora root*)

Table 1: a-Amylase Inhibitory Activities of the Crude Extract and Solvent Fractions.

	Percentage inhibition				
Concentration (mg/mL)	**Chloroform fraction**	**Ethyl acetate fraction**	**Aqueous fraction**	**Crude extract**	**Acarbose**
50	5.21 + 0.1	18.10 + 0.51	27.00 + 2.01	32.15 + 1.21	58.21 + 1.36
100	14.60 + 0.12	24.51 + 0.42	33.10 + 0.16	44.02 + 0.35	69.01 + 0.12
200	28.15 + 0.11	29.12 + 1.16	41.25 + 0.41	61.01 + 1.21	77.61 + 0.12
400	32.01 + 0.06	45.82 + 0.01	58.51 + 0.10	74.10 + 0.12	89.24 + 0.10
600	41.05 + 0.12	53.12 + 1.12	71.61 + 0.62	81.21 + 0.65	94.02 + 0.16
800	48.21 + 0.51	67.13 + 1.15	78.12 + 1.12	84.32 + 0.26	97.31 + 0.21
1000	56.12 + 0.41	73.29 + 0.23	85.32 + 0.10	89.02 + 0.29	98.05 + 0.01
IC50	34.12 + 0.32	24.28 + 0.75	16.42 + 0.81	10.72 + 0.90	4.51 + 0.35

IC50, half maximal inhibitory concentration.Each value of percentage inhibition of a-amylase is presented as means + standard error of the mean (SEM), n ¼ 3.

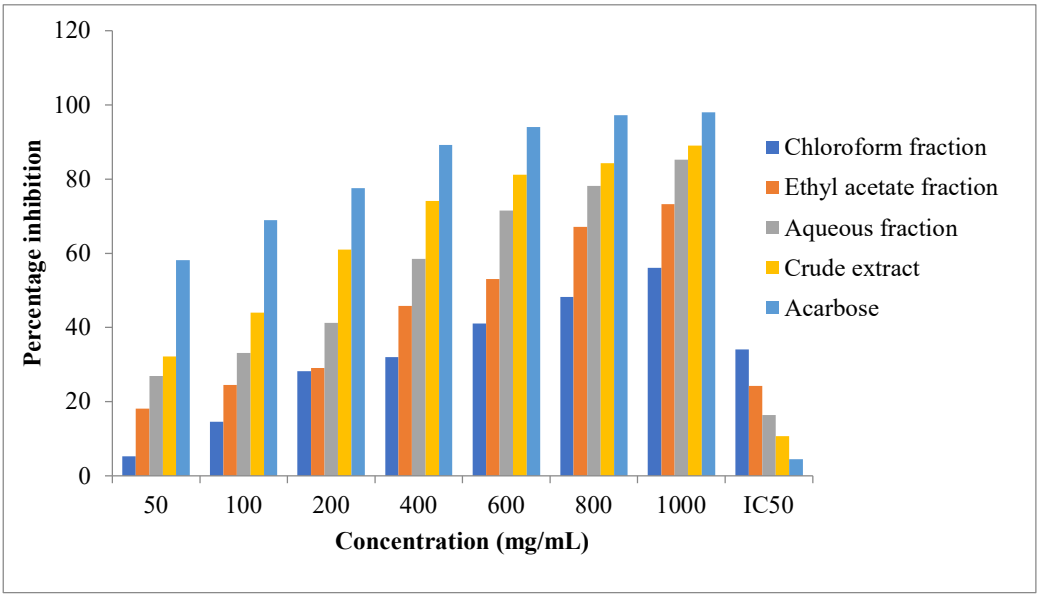

Fig 1: a-Amylase inhibitory activity of the ripe crude extract and solvent fractions of *Helicteres Isora root*

In Vitro a-Amylase Inhibition Activity of Crude Extract and Solvent Fractions *In vitro* a-amylase inhibitory study evaluating the percent of a-amylase inhibition as a function of extract concentrations and the IC50 values were calculated (Figure). Concentration dependent inhibitions were observed for various concentrations of the tested extracts and the standard. Among the extracts, the crude extract exhibited the lowest IC50 mg/mL and the IC50 values of water fraction, ethyl acetate fraction, and the chloroform fraction were 16.42 + 0.81, 24.28 + 0.75 and 34.12 + 0.32mg/mL, respectively. The standard positive control acarbose showed an IC50 of 4.51 + 0.35mg/mL (Table).

Minimum % Inhibition was found *Helicteres Isora root* leaves which resemblance to %Inhibition of positive control, So Ethanolic extract of *Helicteres Isora root* contain active constituents of antidiabetic.

Phytochemical studies

Table 2: Results of Ethanolic extract of *Helicteres Isora root*

Class of compounds	Tests performed	Results
Carbohydrates	Molisch's test Fehling's test	- -
Phenols	Phosphomolybdic acid test	+
Flavonoids	Shinoda test Lead acetate test	+ +
Tannins	Braemer's test	-
Alkaloids	Wagner's Mayer's Draggendrof's test	+ + +
Glycosides	Legal's test Brontranger's test	+ +
Saponins	Foam test	+
Sterols	Salkowski's test	-
Amino acids	Ninhydrin test	-
Terpenoids	Lieberman Burchardt test	+

+Present in moderate amount; -Absence

The phytochemical studies results revealed that the Molisch's test no characteristic observation indicated the absence of carbohydrates, by phosphomolybdic acid test Blue coloration of the spot indicated the presence of phenols. Shinoda test and Lead acetate test gave pink or red coloration of the solution indicated the presence of flavonoids Flocculent white precipitate also indicated the same. There is no dark blue or greenish grey coloration

of the solution indicated the absence of of tannins in the drug. No characteristic observation for steroids and dark pink or red coloration of the solution indicated the presence of Terpenoids. Orange coloration of the spot indicated the presence of alkaloids. Yellow or reddish brown precipitation indicated the presence of alkaloids. Pink to red colour solution indicates the presence of glycosides. No layer of foam formation indicates the absence of Saponins. If the response to the test is indicated table-1high it can be noted or which indicates that the particular group is present as the major class. If the response is average then note it as indicates the presence in moderate quantity and note it as indicating the presence of only in traces. If no response is then negative.

Table 3: Hypoglycemic Test

TREATMENT	DOSE mg/kg	BLOOD GLUCOSE LEVEL (mg/dl)		
		0 min	30min	1hr
CONTROL Carboxyme Thyl Cellulose (CMC)	0.5%	71.31±2.202	70.25±1.121	73.34±2.260
Positive Control Glibenclamide	2	72.19±2.112	53.42±2.541**	33.82±3.541***
Ethanolic Extract of *Helicteres Isora root*	200	70.29±0.195	60.18±1.512*	55.12±2.124*
Ethanolic Extract of *Helicteres Isora root*	400	71.03±1.100	49.13±2.129**	33.1+±0.414***

The glucose levels were analyzed by using glucometer and each value is the mean ± standard error (n= each group consist of 6 animals)($p<0.05$)*, ($p<0.001$)**& ($p<0.0001$)*** as compared to control & positive control group evaluated by one way, ANOVA followed by Dunnet 't' test. The hypoglycemic test results have shown Table No: which indicated ethanolic extract of *Helicteres Isora root* treated animals 200 & 400, significantly decreased in blood glucose level when compared to control and positive control.

***Invivo* antidiabetic study**

Table 4: Results of the effects of Ethanolic extract on blood Glucose levels

TREATMENT	BLOOD GLUCOSE LEVEL (mg/dl)		
	0 min	30min	1hr
Normal control 10 ml/kg P.O	80.25±2.105	73.0±0.101	70.1± 2.124
Negative control	265.1±2.43	258.1±1.026	261.0±1.01
Positive control (Glibenclamide 2mg/kg) P.O	250.12±1.120	131.20±2.1***	112±1.0***
EEHI 200 mg/kg P.O	256±2.1	241.2±1.154**	236.1±2.120**
EEHI 400 mg/kg P.O	261±2.05	168.0±2.68***	150.0±1.3***

(The values were expressed as Mean ± S.E.M. (n=6 animals in each group).

The experimental results have indicated on Table the negative control group glucose levels were significantly increased when compared to each other groups. All the groups of animals were affected in diabetes, which indicated blood glucose levels were slight changes in the blood glucose level for normal control group at 7th and 14th days. On day 7th glucose levels were significantly decreased Glibenclamide 2mg/kg treated group when compared with control group at 7th and 14th days. The Ethanolic leaves extract of *Helicteres Isora root* treated groups 200 & 400 mg/kg were dose dependent manner decreased when compared with control group but positive control have more anti diabetic activity at 7th day.

The Ethanolic leaves extract of *Helicteres Isora root* at the dose level 400mg/kg have equipotent activity when compared with positive control at 7th day. The Ethanolic leaves extract of *Helicteres Isora root* 200 & 400 mg/kg have been expressed dose dependent anti diabetic action when compared to control and positive control. On day 14th, Ethanolic leaves extract of *Helicteres Isora root* treated animals 200 & 400 mg/kg significantly decreased and maintain the blood glucose level when compared to control and positive control.

Table 5: Oral Glucose Tolerance Test

Treatment	DOSE mg/kg	Blood Glucose Level (mg/dl)						
		0 min	0.5hr	1hr	1.5hr	2hr	2.5hr	3hr

Control(CMC)	0.5%	65.01±2.120	141.1±2.103	185.2±0.111	174.1±11.02	151.1±0.203	148.2±1.140	125.1±0.10
Positive Control Glibenclamide	2	71.12±0.31***	105.2±1.101***	113.2±1.01***	95.12±2.120***	81.02±1.001**	76.01±0.102**	69.75±1.501***
EEHI	200	63.21±2.102*	122.1±2.002*	143.1±2.101*	132.1±0.125*	120.1±0.110*	116.02±0.10*	101.0±1.102**
EEHI	400	67.01±2.141*	112.1±0.136*	122±1.111**	101.0±1.021***a	91.10±1.102*** a	81.06±2.09*a	78.01±300***a

Oral Glucose Tolerance Test (OGTT) results have been expressed on Table. Half hour after the glucose treatment, all the groups of animal blood glucose levels were significantly increased. The blood glucose levels were significantly decreased for, Ethanolic extract of *Helicteres Isora root* 200 & 400 mg/kg when compared to control and positive control at 1hour and each and every ½ hour blood glucose levels (200 mg/kg were changes in the dose dependent manner extract treated group of animals compared to control and positive control but 400mg/kg produce the equipotent activity.

DISCUSSIONS

Invitro study is on the principle of Inhibition of α-amylase, enzyme that plays a role in digestion of starch and glycogen are considered a strategy for the treatment of disorders in carbohydrate uptake, such as diabetes. Pancreatic αamylase is a key enzyme in the digestive system and catalyses the initial step in hydrolysis of starch to a mixture of smaller. Sequential extraction was done according to increasing polarity order. Each extracts were tested for α-amylase inhibition to get the extraction with minimum IC50 value. As per the above mechanism all the extract have concentration dependent affinity towards the inhibition of α-amylase. Finally acarbose extract was observed as more active extract.

In this present study acute toxicity study was carried out in Rats. The procedure was followed by using OECD 423 (Acute Toxic Class Method). The acute toxic class method is a step wise procedure with three animals of a single sex per step. Average two to three steps may be necessary. The method used to defined doses (2000, 1000, 500, 50, 5 mg/kg body weight, Up-and-Down Procedure). Observe for signs for toxicity and were noted for 14 days. The onset of toxicity and signs of toxicity also noted. Hence, 1/10th (200mg/kg) and 1/5th (400mg/kg) of this dose were selected for further study. The principle involved in the Streptozotocin induced diabetes mellitus in Rats, Streptozotocin, a cytotoxic, diabetes induced chemical but wide variety of animal species by damaging the insulin secreting cells of the pancreas.

Literature sources indicate that the Streptozotocin induced Rats are hyperglycaemic. The treatment of lower doses of Streptozotocin (100mg/kg b.w.) produced partial destruction of pancreatic β-cells even though the animals become permanently diabetic.

Thus these animals have surviving β cells and regeneration is possible. It is well known that the sulfonylurea's act by directly stimulating the β-cells of the islets of Langerhans to release more insulin and these compounds are active in mild Streptozotocin induced diabetes. *Invivo* anti diabetic screening was performed for the confirmation of above mechanism of action was undergone the Ethanolic extract of *Helicteres Isora root* biological system (Which was already resulted for α-amylase inhibitory activity.

At the end of the Ethanolic extract of *Helicteres Isora root* (200 mg/kg p.o, 400 mg/kg p.o.) showed statistically significant decrease in blood glucose levels. So the Ethanolic extract of *Helicteres Isora root* showed antidiabetic activity. This work will be useful for further diabetes mellitus and its related diseases research worker to develop new entity for the treatment of diabetes mellitus.

CONCLUSION

The current research concerns first-time phytochemical and biological evaluations of *Helicteres Isora root*. The plant has a high reputation in traditional medicine as a hypoglycemic herb all over the world, and several previous articles have documented its antidiabetic activity. Compared to the plant growing in different areas, *Helicteres Isora root* had, in large part, similar phytochemical constituents and demonstrated similar biological activities, including *in vitro* and *in vivo* antidiabetic and antioxidant activities. The plant extract repaired the degenerative effect of STZ on the pancreatic and liver tissues, as demonstrated by the histopathological examination. The results also verified that inhibition of intestinal α-amylase by the extracts may contribute to the

antihyperglycemic activity. The results give scientific support for the use of the plant in folk medicine for the management of diabetes and its associated complications. *Helicteres Isora root* would be promising for further clinical studies in the management of DM. Further studies to find out the mechanism of this plant for its antidiabetogenic effect and there is a need for bioactivity guided investigation to isolate the lead compound responsible for the antidiabetic activity.

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