

# ANTI-DIABETIC ACTIVITY OF HELICTERES ISORA ROOT

Sama Venkatesh<sup>\*1</sup>, G.Dayananda Reddy<sup>1</sup>, B.Madhava Reddy<sup>1</sup>,  
M.Lakshman<sup>2</sup>.

<sup>1</sup>Department of Pharmacognosy, G.Pulla Reddy College of Pharmacy,  
Mehdipatnam, Hyderabad – 500 028. Andhara Pradesh.

<sup>2</sup>Department of Pathology, College of Veterinary Sciences, Acharya NG Ranga  
Agricultural University, Hyderabad – 500 038.

\*Corresponding Author,  
\*e-mail: venkateshsama@hotmail.com.

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## Abstract:

The different extracts of the roots of *Helicteres isora* (Family-Sterculiaceae) were tested for anti-diabetic activity, by glucose tolerance test in normal rats and alloxan induced diabetic rats. Aqueous ethanol and butanol extracts had shown significant protection and lowered the blood glucose levels to normal in glucose tolerance test. In alloxan induced diabetic rats the maximum reduction in blood glucose was observed after 3h at a dose level of 250 mg/kg of body weight. The percentage protections by aqueous ethanol and butanol extracts were 30 and 48% respectively. In long term treatment of alloxan induced diabetic rats, the degree of protection was determined by measuring blood glucose, triglycerides, cholesterol and urea levels on 0,3,5,7 and 10<sup>th</sup> day. Both the extracts showed a significant anti- diabetic activity comparable with that of glibenclamide. The histopathological studies during the long-term treatment have shown to ameliorate the biochemical damages caused by alloxan. These results indicate that the *Helicteres isora* root possess significant anti – diabetic activity.

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## INTRODUCTION

Diabetes mellitus is a major disease characterized by derangement in carbohydrate, fat and protein metabolism, affecting nearly 10% of the population. In the recent past many hypoglycaemic agents are introduced, still the diabetes and the related complications continue to be a major medical

problem not only in developed countries but also in developing countries. Many Indian medicinal plants are reported to be useful in diabetes<sup>1,2</sup>. However, search for new anti-diabetic drugs continue.

*Helicteres isora* Linn. (Family – Sterculiaceae) is a shrub or small tree available in forests from Bengal to Ceylon. In traditional the root juice is claimed to be useful in diabetes, empyema, and a favorite cure for snakebite<sup>1,3</sup>. From the roots betulic acid, daucosterol, sitosterol, isorin<sup>4</sup> were isolated. Cucurbitacin B and isocucurbitacin B were isolated and reported to possess cytotoxic activity<sup>5</sup>. The present study was undertaken to verify the claim and evaluate the anti-diabetic property of the roots *H. isora*.

## **EXPERIMENTAL**

### **1. Plant Material**

The roots of *H. isora* were collected from Srisailam forest. Andhra Pradesh, India and authenticated by Dr.S.T.Ramchandrachari, Principal and Taxanomist, Kamareddy Degree College, Kamareddy, India. A voucher specimen (HI/Rt/99) is being maintained in the laboratory of Phytochemistry and Pharmacognosy, G.Pulla Reddy College of Pharmacy, Hyderabad, India. The roots are dried in shade.

### **2. Preparation of extracts**

The dried root powder (5-kg) was extracted with 80% aqueous ethyl alcohol by maceration process for 3 days. The concentrated aqueous ethanol extract (113 g) was suspended in water and fractionated with butyl alcohol (4x500 ml) and yielded of butanol extract, (16.57g).

### **3. Test animals**

Male wistar albino rats (160 – 200 g) were used in the experiment. Animals maintained under standard environmental conditions, were fed with a standard diet (Hindustan Lever, India) and water ad libitum. The animals were fasted for 16h before experimentation but allowed free access to water.

### **4. Effect of *H. isora* extracts on glucose tolerance in rats**

Fasted rats were divided into 3 groups of six rats each. Group I served as a control, received distilled water. Group II – III received aqueous ethanol and butanol extracts respectively at a dose of 250 mg/kg body weight as a fine aqueous suspension orally. The rats of all groups were given glucose (2 g/kg body weight, p.o) 30min after administration of the drug. Blood samples were collected from the tail vein just prior to glucose administration and at 30 and 90 min after the glucose loading. Serum was separated and blood glucose levels were measured immediately by glucose-oxidase method<sup>6</sup>.

#### 5. Effect of the *H. isora* extracts on alloxan-induced diabetic rats

Male wistar rats (180-200g) were made diabetic by a single i.p injection of 120mg/kg body weight of alloxan monohydrate in sterile normal saline. The rats were maintained on 5 % glucose solution for next 24h to prevent hypoglycaemia<sup>7</sup>. Five days later blood samples were drawn from tail vein and glucose levels were determined to confirm the development of diabetes (350mg/dl). The diabetic rats were divided into four groups, each containing six animals. Controls rats (Group I) were given distilled water orally, while *H. isora* aqueous ethanol, and butanol extracts were given to groups II-III respectively, at a dose of 250 mg/kg, orally. Group IV received glibenclamide at dose of 10 mg/kg. Blood samples were collected from the tail vein just prior to and 1h, 3h and 5h after drug administration.

The effect of *H. isora* extracts was also tested for a prolonged treatment. The diabetic male wistar rats (160-180g) were divided into four groups of eight rats each. Group I served as diabetic control received distilled water instead of extracts. The rats of group II-III received aqueous ethanol and butanol extracts respectively at dose of 250 mg/kg body weight, as fine aqueous suspension, orally. Group IV received glibenclamide at dose of 10 mg/kg. The administration of extracts was continued for 10 days, once daily. Blood samples were collected through the tail vein just prior to and on days 1,3,5,7 and 10 after drug administration. The blood glucose, urea, total cholesterol, triglyceride levels were determined for all the samples.

#### 6. Histopathological studies

Animals were sacrificed on 5<sup>th</sup> and 10<sup>th</sup> day during prolonged treatment. Pancreas, liver and kidney were removed, washed with cold saline and preserved in 10% formalin in buffered form. Blocks from tissues were routinely processed and embedded in paraffin. Thin sections were cut using rotary microtome and stained with hematoxylin and eosin for histomorphology evaluation.

## 7. Statistical analysis

The results are expressed as mean S.E.M. the significant of various treatments was calculated using students t-test and were considered statistically significant when  $P < 0.05$ .

## RESULTS

The extracts of *H. isora* have shown significant ( $P < 0.001$ ) increase in glucose tolerance. The results are given in Table 1. The blood glucose levels were reduced considerably within 60 minutes of the drug administration. The butanol and aqueous ethanol extracts reduced the glucose levels to normal. Maximum, effect was observed for butanol extract.

In alloxan-induced diabetic rats also, both extracts have shown considerable reduction in blood glucose levels. The results are shown in table 2. The reduction in glucose levels is significant ( $p < 0.001$ ) in the treated animals at 1h, 3h and 5h after drug administration. The maximum percentage reduction in blood glucose levels was found to be in butanol extract (48.86%), while aqueous ethanol showed (30%) blood glucose level. Treatment of the diabetic rats with glibenclamide (10 mg/kg) produced (29.77%) fall of blood glucose after 3h treatment.

The prolonged treatment of *H. isora* extracts on alloxan-induced diabetic rats produced consistent reduction in the blood glucose levels. Both the extracts have shown significant ( $p < 0.001$ ) reduction of blood glucose, urea, total cholesterol and triglycerides during the 10 days treatment period. However the butanol extract has shown maximum reduction (144.71 mg/dl on 10<sup>th</sup> day) and at a faster rate compared to aqueous ethanol extract (223.51 mg/dl)

## CONCLUSION

Helicteres isora root juice is claimed to be useful in diabetes. Results of anti-diabetic activity of H. isora root extracts established the scientific basis for the utility of this plant in the treatment of diabetes. The aqueous ethanol and butanol extracts have shown significant reduction in blood glucose levels in both glucose loaded and alloxan induced diabetic rats. The butanol extract produced maximum anti-diabetic activity and is higher than the hypoglycemic activity of glibenclamide in the diabetic rats. Therefore it is obvious that the fractionation with butanol has enriched the active principles.

In glucose loaded animals, the drug has reduced the blood glucose to the normal levels. It is possible that the drug may be acting by potentiating the pancreatic secretion or increasing the glucose uptake. Both aqueous ethanol and butanol extracts has reduced the glucose levels to 51% and 69% respectively, in prolonged treatment study. Hypercholesterolemia, hypertriglyceridemia, hyperurea have been reported to occur in alloxan diabetic rats <sup>8,9</sup> and a significant increase observed in our experiment was in accordance to these studies. Repeated administration of H. isora extracts had decreased the blood glucose, urea, total cholesterol and triglycerides significantly. Histopathological examination of pancreas, liver and kidney showed the recovery of damaged tissues when section of treated groups compared with diabetic control.

In conclusion, H. isora root butanol extract showed significant anti-diabetic effect in diabetic rats after oral administration. Thus the claim made by the traditional Indian systems of medicine regarding the use of root juice of this plant in the treatment of diabetes stands confirmed. Present efforts are directed to isolate the active constituents from butanol extract of H. isora roots and elucidation of mechanism of action.

## **ACKNOWLEDGEMENTS**

The authors wish to thank the All India Council for Technical Education, New Delhi for providing financial support. The authors also wish to thank the management of the college for encouraging and providing research facilities.

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**Table 1**Effects of *H. isora* extracts on oral glucose tolerance in rats<sup>a</sup>

Group	Treatment (dose / kg body weight)	Blood glucose (mg/dl)		
		Fasting	30 min	90 min
I	Glucose; 2g.	77.25 ± 0.907	146.90 ± 1.76	114.71 ± 1.60
II	Aqueous ethanol extract 250 mg + Glucose	78.99 ± 0.83	103.36 ± 1.33*	85.81 ± 1.13*
III	Butanol extract ; 250 mg + Glucose	79.03 ± 0.91	99.81 ± 1.17*	83.52 ± 1.02*

<sup>a</sup>Values are means ± S.E.M., ; n = 6

\*P&lt;0.001 VS group I

*H. isora* extracts were given orally 30 min before glucose loading**Table 2.**Effect of *H. isora* root extracts on blood glucose levels (mg /dl) of alloxan induced diabetic rats <sup>a</sup>

Group	Treatment	Blood glucose at different hours after the Treatment			
		0h	1h	3h	5h
1	Diabetic – untreated	390.10± 7.65	385.78± 8.12	376.21± 8.85	380.12 ±7.84
2	Diabetic rats treated with 250 mg/kg of a aqueous ethanol extracts	368.16± 8.59	270.10± 7.17**	256.39± 7.22**	267.59± 7.81**
3	Diabetic rats treated with 250 mg/kg of butanol extracts	396.84± 7.20	244.80± 6.28**	202.92±5.50**	222.29 ±5.31**
4	Diabetic rats treated with 10 mg/kg of gliconclamide.	382.08± 7.94	348.33±8.03*	268.31±7.40**	287.12± 7.37**

<sup>a</sup> Values are mean ± S.E.M. ; n = 6

\*P<0.01; \*\*P<0.001 compared with initial level of blood glucose of the rats (0h) in the respective group