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Research Article

Oral Glucose Tolerance Test (OGTT) in Normal Control and Glucose Induced Hyperglycemic Rats with *Hedyotis leschenaultiana* DC

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ABSTACT

The effect of ethanol extract of whole plant of *Hedyotis leschenaultiana* on Oral Glucose Tolerance was determined. Glibenclamide (600µg/kg) was used as reference drug for comparison. Ethanol extract of *Hedyotis leschenaultiana* whole plant was evaluated for Oral Glucose Tolerance Test (OGTT) in normal and alloxan induced diabetic rats. Blood glucose concentration was evaluated at 0, 30, 60, 90 and 120 minutes after treatment in both cases. The extract significantly ($p < 0.001$) reduced blood glucose level in alloxan induced diabetic (hyperglycaemic) rats orally at the dose of 150mg/kg and 300mg/kg body weight of ethanol extract respectively. These results suggest that the ethanol extract of *Hedyotis leschenaultiana* whole plant will be useful in the treatment of impaired oral glucose tolerance.

Keywords: OGTT, *Hedyotis*, Diabetes, Flavonoids

INTRODUCTION

The Oral Glucose Tolerance Test (OGTT) measures the body's ability to use a type of sugar called glucose that is the body's main source of energy. OGTT, a test of immense value and sentiment, in favour of using fasting plasma glucose concentration alone was seen as a practical attempt to simplify and facilitate the diagnosis of diabetes. Hyperglycemia is an important factor in the development and progression of the complications of diabetes mellitus[1,2]. Diabetes mellitus, one of the most common endocrine metabolic disorders has caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular diseases) complications[3]. The disease is rapidly increasing worldwide and affecting all parts of the world. Due to deficiency of the insulin people suffering from diabetes have high blood glucose level[4]. According to World Health Organization the diabetic population is likely to increase upto 300million or more by the year 2025[5].

The pharmaceutical drugs are either too expensive or have undesirable side effects. Treatment with sulphonylureas and biguanides are also associated with side effects[6]. Many indigenous Indian medicinal plants have been found to be useful to successfully mange diabetes and some of them have been tested and their active ingredients isolated[7,8] . This has led researchers to continue their search for the 'miracle drug' for treatment of diabetes from plants.

The genus *Hedyotis* finds a prominent place in different Indian system of medicine. The different ethnic communities in India have used different species of *Hedyotis* in the treatment of various ailments[9]. Taking into the consideration of the medicinal importance of whole plant of *Hedyotis leschenaultiana* DC have undertaken to evaluate the Oral Glucose Tolerance Test (OGTT).

However, no systematic attempts have been made to establish scientific basis of beneficial effects of *Hedyotis leschenaultiana* whole plant extracts. To our knowledge no report on the effect of *Hedyotis leschenaultiana* whole plant on experimental diabetic. This study was undertaken to evaluate the effects of ethanol extract of whole plant of *Hedyotis leschenaultiana* on Oral Glucose Tolerance Test in normal rats and diabetic induced rats.

MATERIALS AND METHODS

Plant material

Whole plant of *Hedyotis leschenaultiana* was collected from Kothagiri, Nilgiri Biosphere Reserve, Western Ghats, Tamil Nadu. With the help of local flora, voucher specimens (VOCB-4048) were identified and preserved in the Ethnopharmacology Unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin, Tamil Nadu for further references.

Preparation of plant extract for phytochemical screening and antidiabetic studies

The whole plant of *H. leschenaultiana* was shade dried at room temperature and the dried whole plant was powdered in a Wiley mill. Hundred grams of powdered *Hedyotis leschenaultiana* whole plant was packed in a Soxhlet apparatus and extracted with ethanol for 48hrs. The ehanol extract was concentrated in a rotary

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Table 1: Oral Glucose Tolerance Test in normal rats after treatment with *H. leschenaultiana* whole plant extract

Treatment	Dose	Blood and urine glucose level (mg/dl)									
		0- hour Serum	urine	30 min Serum	urine	60 min Serum	urine	90 min Serum	urine	120 min Serum	urine
Group I	0.9% Saline	76.54±1.45	Nil	122.78±1.87**	Nil	137.34±1.46**	Nil	112.66±1.05*	Nil	73.54±3.56	Nil
Group II	150 mg/kg	72.56±0.96	Nil	112.51±1.58*	Nil	132.48±1.42**	Nil	101.22±1.07*	Nil	70.42±2.42	Nil
Group III	300 mg/kg	78.74±1.39	Nil	101.87±1.43	Nil	120.66±0.58*	Nil	91.43±1.68	Nil	67.38±2.19	Nil
Group IV	600 µg/kg	81.22±1.43	Nil	128.79±1.37*	Nil	136.52±1.39**	Nil	117.94±1.28	Nil	87.26±2.98	Nil

Values are mean ±SEM (N=5) *p<0.05; **p<0.01statistically significant compared to 0 min to other respective groups

Table 2: Oral Glucose Tolerance Test in diabetic induced rats after treatment with *H. leschenaultiana* whole plant extract

Treatment	Dose	Blood and urine glucose level (mg/dl)									
		0- hour Serum	urine	30 min Serum	urine	60 min Serum	urine	90 min Serum	urine	120 min Serum	urine
Group I	0.9% Saline	79.45±1.87	Nil	141.45±1.85*	Nil	123.59±1.58	NIL	96.27±1.58	Nil	71.42±2.86	Nil
Group II	0.9% Saline	237.26±5.96	(+++)	378.46±8.67***	(+++)	394.25±7.93***	(++++)	342.57±10.45***	(+++)	331.88±8.45***	(++++)
Group III	150 mg/kg	253.86±7.93	(+++)	386.67±6.09***	(+++)	398.45±8.45***	(+++)	312.56±8.12***	(+++)	267.78±6.98***	(+++)
Group IV	300 mg/kg	268.23±9.56	(+++)	369.49±9.11***	(+++)	391.67±8.13***	(+++)	301.66±9.23***	(+++)	232.82±8.54***	(+++)
Group V	600 µg/kg	276.44±8.63	(+++)	351.62±8.32***	(+++)	388.93±9.27***	(+++)	321.85±7.59***	(+++)	254.32±9.34***	(+++)

Values are mean ±SEM (N=5) *p<0.05; ***p<0.001, statistically significant compared to 0 min to other respective groups

evaporator. The concentrated extract was subjected to qualitative test for the identification of various phytochemical constituents as per the standard procedures[10,11] and concentrated ethanol extract was used for Oral Glucose Tolerance Test.

Animals

Normal healthy male Wistar albino rats (180-240g) were housed under standard environmental conditions at temperature (25±2^0 C) and light and dark (12/12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum*.

Acute Toxicity Study

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study[12]. The animals were kept fasting for overnight and provide only with water, after which the extracts were administrated orally at 5mg/Kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administrated was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/Kg body weight.

Induction of Diabetes in Experimental animal

Diabetes induced rats were administrated by the simple intraperitonal dose of alloxan monohydrate (150 mg/Kg)[13]. Two days after alloxan injection, rats screened for diabetes having glycosuria and hyperglycaemia with blood glucose level of 200-260 mg/100ml were taken for the study. All animals were allowed free access to water and pellet diet and maintained at room temperature in plastic cages.

Experimental design

In the present investigation, a total 45 rats were taken and divided into nine groups, the four groups for Oral Glucose Tolerance Test in normal rats (each group 5 rats) and five groups for Oral Glucose Tolerance Test in diabetic induced rats (each group 5 rats).

Oral Glucose Tolerance Test (OGTT) in normal rats

Normal rats were tested for the Oral Glucose Tolerance Test (OGTT) of the various concentrations of *H. leschenaultiana* extracts and standard drug glibenclamide. Albino Wistar rats of either sex weighing 150-200gm were divided into 4 groups consisting of 5 rats in each group.

Groups:

Group I- Normal control received 0.9% saline

Group II- Ethanol extract of *H. leschenaultiana* whole plant (150mg/kg p.o)

Group III- Ethanol extract of *H. leschenaultiana* whole plant (300mg/kg p.o)

Group IV- Standard drug glibenclamide (600µg/kg p.o)

Oral Glucose Tolerance Test (OGTT) in diabetic induced rats

OGTT is carried out in the diabetic induced rats. The rats were divided into5 groups consisting of 5 rats in each group.

Groups:

Group I - Normal control received 0.9% saline

Group II – Diabetic control received 0.9% saline

Group III – Diabetic rats treated with 150mg/kg of ethanol extract of *H. leschenaultiana* whole plant

Group IV - Diabetic rats treated with 300mg/kg of ethanol extract of *H. leschenaultiana* whole plant

Group V - Diabetic rats treated with (600µg/kg) of Standard glibenclamide

After 60minutes of drug administration, the rats of normal and diabetic groups were orally treated with 2g/kg of glucose. The blood samples were collected through femoral vein at 0, 30, 60, 90, 120 minutes. Blood glucose level was estimated at various time intervals.

RESULTS AND DISCUSSION

Phytochemical screening of ethanol extract of *H. leschenaultiana* whole plant revealed the presence of alkaloid, catechin, coumarin, tannin, phenol, saponin, steroid, flavonoid, glycoside and xanthoprotein. Acute toxicity study revealed the non-toxic nature of the ethanol extract of *H. leschenaultiana* whole plant.

The blood glucose concentration of control and different doses of *H. leschenaultiana* (150 and 300 mg/kg) extracts were estimated 0, 30, 60, 90 and 120 minutes respectively are shown in Table-1. Drug treated rats suppress its rise in blood glucose level with 150mg/kg and 300 mg/kg as compared with vehicle control. Glibenclamide (600µg/kg) showed suppress in blood glucose at 3rd and 4th (Table-1).

For Oral Glucose Tolerance Test, the blood samples were analyzed for glucose content at 0, 30, 60, 90 and 120 minutes, respectively. The blood sugar levels of *H. leschenaultiana* (150 and 300 mg/kg) treated groups were found compared to be diabetic control and the effects were dose-dependent. Group III and IV glucose lowering efficiency between 90-120 minutes and were comparable to diabetic standard shown in Table-2.

The experiment showed that Glucose Tolerance Test (GTT) measures the body ability to use glucose, the body's main source of energy[14]. This test can be used to diagnose pre-diabetes and diabetes. Glucose lowering effects were found after oral administration of ethanol extract in rats. This may be due to the presence of hypoglycemic flavonoids, terpenes or saponins that also requiring further investigation.

The extracts have the properties to stimulate or regenerate the β cell for the secretion of insulin and are most effective for controlling diabetes by various mechanisms which may finally lead to improvement of carbohydrate metabolizing enzymes towards the re-establishment of normal blood glucose level[15]. This is the first study to show that the intraperitoneal administration of the ethanol extract of *H. leschenaultiana* whole plant cause rapid induction of hypoglycaemia in orally glucose induced hyperglycemic rats.

CONCLUSION

It is concluded that administration of ethanol extract of *Hedyotis leschenaultiana* whole plant promotes glucose tolerance. *Hedyotis leschenaultiana* whole plant is gaining much importance in diabetic control, since the phytochemical analysis has shown the presence of potent phytochemicals like flavonoids, glycosides, steroids, tannins, saponins and phenols. Several authors reported that flavonoids, steroids, terpenoids, phenolic acids are known to be bioactive antidiabetic principles[16]. Flavonoids are known to regenerate the damaged beta cells in the alloxan induced diabetic rats and acts as insulin secretagogues[17]. This evidence suggests that the whole plant of *Hedyotis leschenaultiana* could be beneficial for the protection of allevation of diabetic complications. Further studies need to be carried out to define the active principles present in the ethanol extract.

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