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Research Article

Extraction and Evaluation of Roots of Decalepis Hamiltonii For Antidiabetic Activity

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ABSTRACT
The aim of the present study is to evaluate the antidiabetic activity of various extracts of the *Decalepis hamiltonii* (F: Asclepediaceae) in alloxan induced diabetic rats. The fall of blood glucose levels after administration of aqueous, methanol and pet ether extracts at a dose of 200 mg/kg body weight were found to be 69.43 %, 62.04%, and 49.61%, respectively, after 4[hr] of oral administration. At the same dose the acute oral administration of aqueous extract showed significant decrease of blood glucose loaded normoglycemic rats. In vitro glucose uptake studies suggest that *D. hamiltonii* root extracts has direct insulin like effect which can enhance the peripheral utilization of glucose by rat hemi diaphragm.

Key Words: *Decalepis, D. hamiltonii,* anti diabetic, maceration, alloxan induced diabetes.

INTRODUCTION

Diabetes mellitus is a leading metabolic disorder affecting approximately 5% of the world's population. It has been proposed that approximately 57 million Indians will be affected by diabetes mellitus in the year 2025[1,2]. Management of diabetes with the agents devoid of any side effects is still a challenge to the medical system. This concern has led to an increase and demand for natural products with antihyperglycaemic activity having fewer side effects. *Decalepis hamiltonii* commonly called as maredu kommulu or barre sugandhi or maredu gaddalu or makali beru is an endangered climbing shrub belonging to the family Asclepiadaceae and its roots have been used in ayurveda, the ancient Indian traditional system of medicine to stimulate appetite, relieve flatulence and as a general tonic[3]. The plant is known to contain vanillin, salicylaldehyde, 2-hydroxy-4-methoxybenzaldehyde, bis-2, 3, 4, 6- galloyl-D-glucopyranoside, borneol, inositol, saponins, ketonic substances, sterols, amyrins and lupeols [4,5,6,7]. The roots have also been used as a substitute for *Hemidesmus indicus* in ayurvedic preparations of ancient Indian medicine[3].

MATERIALS AND METHODS

Plant material and Extraction procedure:

The roots of *D. hamiltonii* were collected from Forest Research Center, Dhulepalli, Hyderabad. The plant was authenticated by an expert taxonomist Dr.V.S.Raju, Department of botany, Kakatiya University, Warangal. The roots of *D. hamiltonii* were washed thoroughly with tap water, dried under shade, powdered to coarse particles and subjected to extraction. The extracts were prepared by using maceration technique, a process of extraction of a drug with a solvent with several daily shakings (or) stirrings at room temperature. This method is based on the extraction of active constituents present in the drug using various solvents ranging from non-polar to polar viz: petroleum ether, methanol and water. 100g each of the powdered plant materials were taken separately into maceration pots and three times the volume of petroleum ether was added to it, mixed well and the pots were closed. The pots were kept at room temperature for 24 hrs with frequent vigorous shaking and the drug to solvent ratio of 1:3 was maintained. After 24 hrs the contents were filtered through whatman no1 filter paper. The extraction of marc was repeated two more times with the same solvent for effective extraction. Each time before extraction with next solvent filtrate was concentrated by evaporation and dried in desiccator.

Chemicals

Alloxan, glibenclamide, and insulin were procured from Sigma-Aldrich Company (St. Louis, Missouri, USA). Glucometer kit was procured from Taidoc Technology Corporation, San-Chung, Taiwan. All the solvents and other chemicals were of analytical grade and were procured from standard sources.

Animals

Male wistar albino rats (150–200g) were purchased from Mahaveer enterprises, Hyderabad. Animals were maintained in an air-conditioned room at 22 ± 2 0 C and

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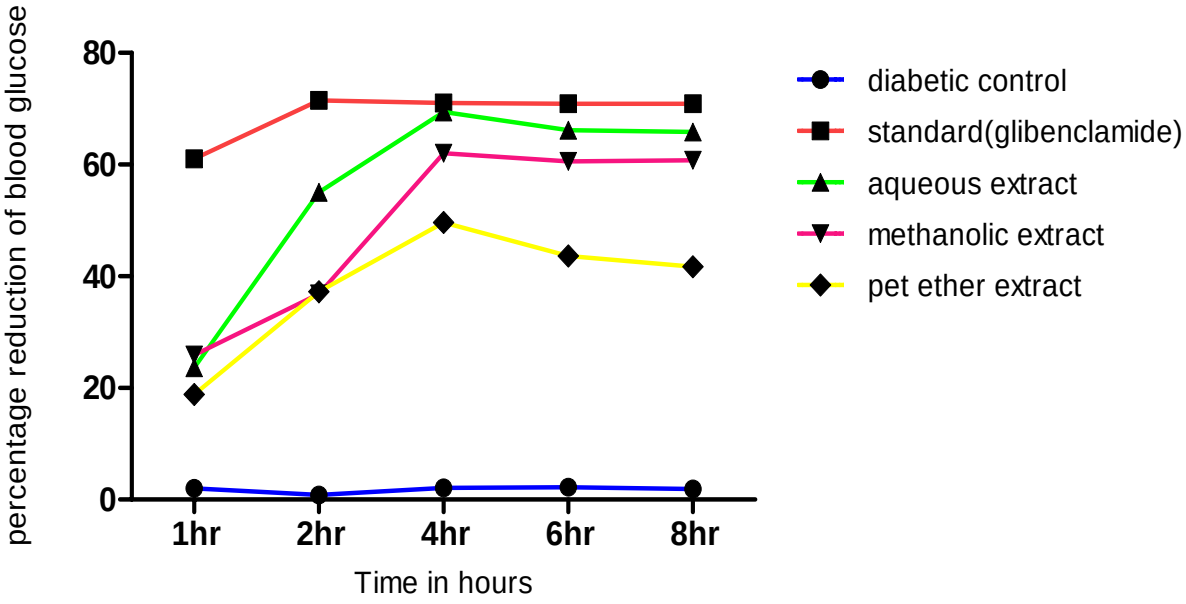

Figure 1: Percentage reduction of blood glucose of different extracts of *D. hamiltonii.*

relative humidity of 45–55% under a 12h light: 12 h dark cycle. The animals had free access to standard food pellets and water was available ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Vaagdevi College of Pharmacy, Warangal, (Registration No: 1047/ac/07/CPCSEA) and constituted in accordance with the rules and guidelines of the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India.

Induction of experimental diabetes and determination of the serum glucose level

Rats were deprived from food for 16 hours (fasted state) before the induction diabetes. Diabetes was induced in male wistar rats by a single intra peritoneal injection of aqueous alloxan monohydrate (120 mg/kg) solution and the serum glucose level determined by the glucose oxidase peroxidase method[9,10]. The rats showing a serum glucose level above 200 mg/dl (diabetic state) were selected for this study. Blood samples from the experimental rats were collected by retro-orbital plexus technique using heparinized capillary glass tubes. The collected blood samples were centrifuged at a speed of 7000 rpm for 15 min to get serum. Ten microliters of serum and 1ml of working reagent (GOD/POD) were mixed and incubated for 15 min at 370C. The UV VIS spectrophotometer (Elico SL 120) reading was adjusted to 0 by measuring the absorbance of blank (distilled water). The absorbance of sample (As) and standard Astd provided by manufacturer were measured against blank at 505 nm.

Glucose was estimated by using the formula:

$$\text{Glucose (mg/dl)} = \frac{\text{As}}{\text{Astd}} \times 100$$

Where as As = sample reading; Astd = standard reading.

Determination of antihyperglycemic effect on diabetic rats

Diabetic animals were divided in to five groups; six animals in each group. Group I served as control. Remaining groups received the various extracts of *Decalepis hamiltonii* and glibenclamide. Group I, vehicle (distilled water; 10ml/kg); Group II, glibenclamide (10mg/kg)[11]; Group III, aqueous extract (200 mg/kg), Group IV, methanolic extract (200 mg/kg) and Group V, pet ether extract (200 mg/kg). All extracts were given orally while alloxan was given intraperitoneally. Rats were fasted overnight before the commencement of the study. The study involves the determination of serum glucose levels at 0, 1, 2, 4, 6, 8 and 24 hours after administration of all extracts.

Oral glucose tolerance test

The oral glucose tolerance test was performed on overnight fasting (16 h) normal rats. Selected rats were divided in to five groups; six animals in each group. Group I was kept as control receives distilled water, group II received glibenclamide (10 mg/kg), group III received aqueous extract (200 mg/kg), group IV received methanol extract (200 mg/kg) and group V received pet ether extract (200 mg/kg). All the groups were loaded with Glucose (5 g/kg, *po*), 30 minutes after the drug administration. Blood samples were collected from puncturing the retro orbital plexus at 0, 30, 60 and 120 min from control and experimental animals[12]. The plasma obtained after centrifugation was used for the determination of plasma glucose levels by a glucose oxidase peroxidase method.

Effect of D. hamiltonii extracts on glucose utilization by isolated rat hemidiaphragm

Overnight fasted albino rats were killed by decapitation and diaphragms were isolated quickly avoiding trauma

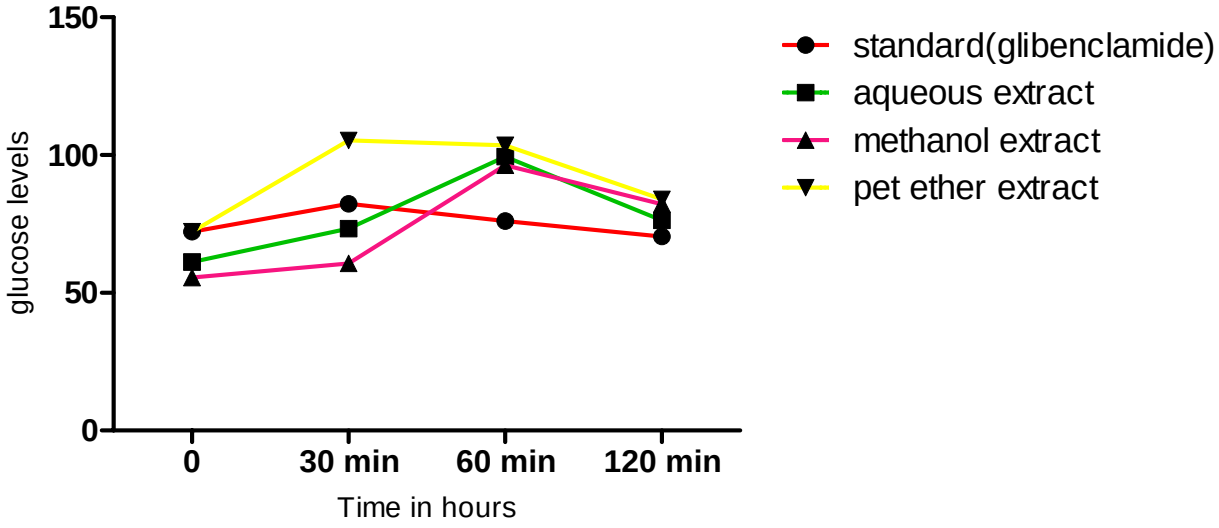

Figure 2: Glucose levels in oral glucose tolerance test of different extracts and glibenclamide

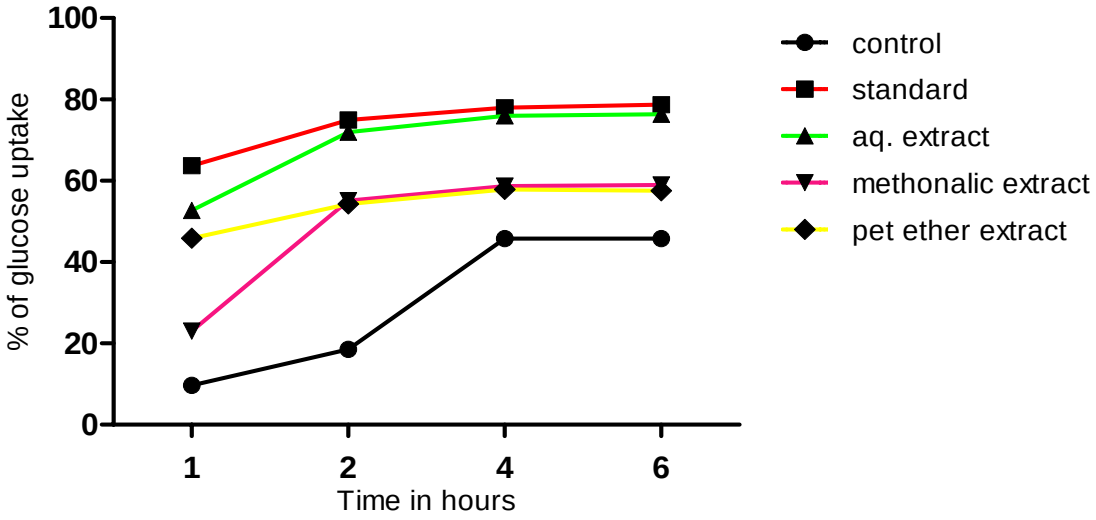

Fig3: Percentage of glucose uptake study of rat hemi diaphragm by different extracts and glibenclamide without insulin

and divided into two halves. The hemi diaphragms were then rinsed in cold Tyrode solution (without glucose) to remove any blood clots and were placed in a small conical flasks containing 2 ml tyrode solution with 2 g/l glucose and incubated for 30 min at 37°C in an atmosphere of 95% O_2/5% CO_2 with shaking at 140 rpm. Following sets of experiments were performed viz: tyrode solution with glucose: with insulin alone; with glibenclamide alone; with glibenclamide and insulin; with extracts alone; with extracts and insulin (Chattopadhyay *et al*., 1992). Animals were divided in to ten groups; three animals in each group. Animals were killed by decapitation and diaphragms were exposed to

Group I: tyrode solution with glucose (2 g/l) only and served as control.

Group II: tyrode solution with glucose (2 g/l) +Insulin (0.25 IU/ml).

Group III: tyrode solution with glucose (2 g/l) +glibenclamide (100µg/ml).

Group IV: tyrode solution with glucose (2 g/l) +Insulin (0.25 IU/ml) +glibenclamide (100µg/ml).

Group V: tyrode solution with glucose (2 g/l) +aqueous extract of *D. hamiltonii* (100µg/ml).

Group VI: tyrode solution with glucose (2 g/l) +Insulin (0.25 IU/ml) + aqueous extract of *D. hamiltonii* (100µg/ml).

Group VII: tyrode solution with glucose (2 g/l) +methanol extract of *D. hamiltonii* (100µg/ml).

Group VIII: tyrode solution with glucose (2 g/l) +Insulin (0.25 IU/ml) +methanol extract of *D. hamiltonii* (100µg/ml).

Group IX: tyrode solution with glucose (2 g/l) +pet ether extract of *D. hamiltonii* (100µg/ml).

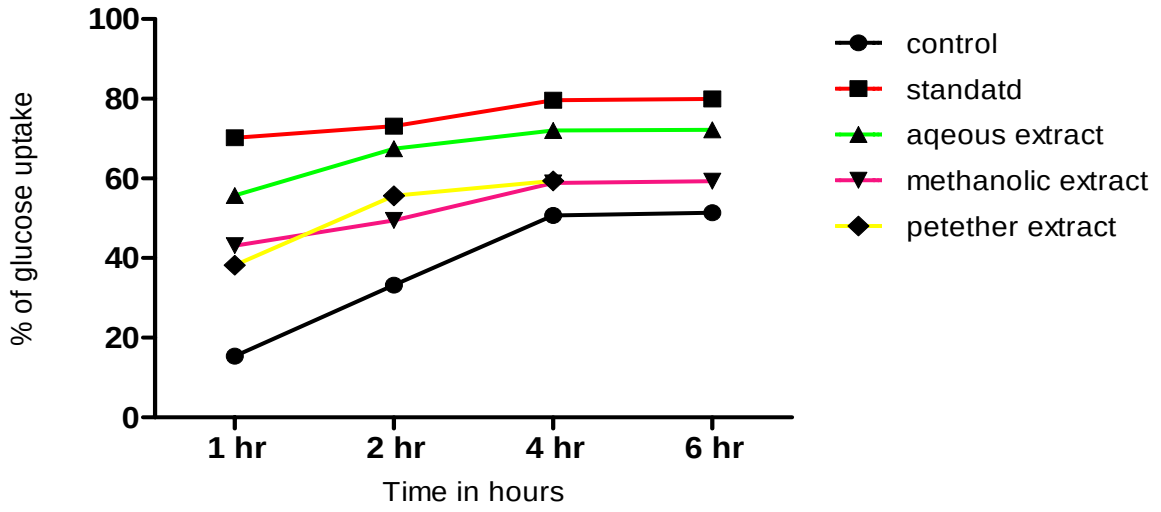

Fig4: Percentage of glucose uptake of rat hemidiaphragm by different extracts and glibenclamide with insulin

Group X: tyrode solution with glucose (2 g/l) +Insulin (0.25 IU/ml) +per ether extract of *D. hamiltonii* (100µg/ml).

Following incubation, the hemi diaphragms were taken out and weighed. The glucose content of the incubated medium was measured by glucose oxidase peroxidase method.

Glucose uptake was calculated at different time intervals

Statistical analysis

The statistical analysis of all results was carried out using one way ANOVA[13] followed by student-Newman-Keuls test using graph pad prism statistical software. Data were expressed as mean ± standard deviation (SD) (n=6). A value of $p < 0.05$, $p < 0.01$, $p < 0.001$ and was considered significant, very significant, and highly significant, respectively.

RESULTS

Assessment of antihyperglycemic activity in diabetic rats

Figure 1 depicts antihyperglycemic activity of a single oral administration of extracts of D. hamiltonii at a single dose of 200 mg/kg. Among the three extracts the aqueous extract produces significant fall of 69.43% fasting blood glucose level, after 4^{hr} of administration. The fall of blood glucose level for methanol and pet ether extracts were found to be 62.04%, 49.61%, respectively, after 4^{hr} of oral administration. However after 6^{hr} blood glucose level rises slightly as compared to that of 4^{hr}.

Effect on glucose tolerance test

The effects of various extracts of *D. hamiltonii* (200 mg/kg) on glucose tolerance are shown in Figure 2.

The supplementation of *D. hamiltonii* improved the glucose tolerance in the fasted normal rats. When the rats were first fed with glucose, the rate of increase in the blood glucose level was the same for normal, standard, aqueous, methanol and pet ether extract groups during the first 30 minutes. After that serum glucose level lowered very significantly ($p < 0.01$) at 60 min and high significantly ($p < 0.001$) lowered at 120 min in aqueous extract treated group as compared to normal control group. There was significant reduction ($p < 0.05$) in the pet ether extract group compared to control group. The methanol did not show any significant reduction.

Effect on glucose uptake by isolated rat hemidiaphragm

The glucose uptake by rat hemi-diaphragm was significantly more in all the groups tested when compared to the control group (Figure 3 and Figure 4).

The aqueous extract has showed significant glucose uptake 76.39%, 72.19% at 6^{th} hour without and with insulin when compared to control group. The methanol and pet ether extracts has showed the percentage of glucose uptake at 6^{th} hour 58.98%, 57.58%, 59.25%, and 59.49% without and with insulin respectively.

There is no significant change in glucose uptake of extracts with insulin and without insulin.

DISCUSSION

Alloxan is widely used to induce diabetes in experimental animals. Alloxan induces diabetes by destroying β-cells of pancreas, through production of reactive oxygen species[14].

The present study was undertaken to evaluate the ant diabetic activity of various extracts of *Decalepis hamiltonii* in alloxan induced diabetic rats, among the three extracts aqueous (200mg/kg) and methanol (200mg/kg) extracts were showed significant antihyperglycemic effect. As it is evident from the results that maximum reduction in the blood glucose levels were observed after 4^{th} hour for aqueous extract and 6^{th} hour for methanolic extract.

The proposed mechanism of actions are, by promoting regeneration of β-cells or by protecting the cells in pancreas from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin

action and further affecting β-cells to release insulin and activate the insulin receptors to absorb the blood sugar. Regeneration of islet β-cells following destruction by alloxan may be the primary cause of the recovery. Categorically the study showed that aqueous extract has significant activity compared to other extracts. The order of activity is aqueous extract > methanol extract > petroleum ether extract.

Similar studies on antihyperglycemic effect of aqueous and cold extracts of leaves of *Terminalia catappa*, ethanolic extract of powdered bark of *Diospyros melanoxylon* and *Vinca rosea* extract was reported and the proposed mechanism was by regeneration of islet β-cells following destruction by alloxan[15,16,17] .

The estimation of glucose content in rat hemi-diaphragm is a commonly employed and reliable method for *in vitro* study of peripheral uptake of glucose. In earlier studies the hypoglycemic activity of *Ficus hispida* bark was attributed to enhanced uptake of glucose by peripheral tissues which were investigated by rat hemidiaphragm method[18] .

Studies on rat hemidiaphragm showed that aqueous extract has significant uptake at 1st, 2nd, 4th and 6th hours. The order of uptake was aqueous extract > methanol extract > petroleum ether extract. Interestingly activity is not significantly increased in presence of insulin. This may be because of insulin/extract interactions/complexation when given together. The complexicity has to be investigated in detail in future course of studies.

The aqueous extract of the plant shows significant oral glucose tolerance than the methanol and pet ether extracts, when compared with the control group. Oral administration of aqueous extract at 200mg/kg dose resulted in a significant fall in blood glucose level, 2 hours after a single dose of treatment in glucose loaded rats. Aqueous extract was effective in depressing the peak value of blood sugar at 60 min after glucose loading. The extract producing its hypoglycemic activity by a mechanism independent from the insulin secretion, it may be by inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption. Similar studies on aqueous extract of *Pterocarpus marsupium*, aqueous extract of *Spergularia purpuria, Momordica charantia* was reported and the proposed mechanism is by inhibition of endogenous glucose production or by the inhibition of intestinal glucose absorption[19,20].

CONCLUSION

An *in vivo* study on alloxan induced diabetic rat model reveals that aqueous and methanolic extract showed significant activity when compared to pet ether extract. Oral glucose tolerance test showed that aqueous extract exhibited significant activity compared to methanol and pet ether extract. The *in vitro* study on rat hemidiaphragm showed that aqueous, methanol and pet ether extracts showed glucose uptake activity. Presence of insulin does not influence the glucose uptake activity. The antihyperglycemic activity of aqueous, methanol and pet ether extract may be due to regeneration of pancreatic beta cells and enhanced peripheral glucose uptake by skeletal muscle.

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