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Antidiabetic and Antioxidant Activities of *Decalepis hamiltonii* Wright & Arn.

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Abstract

The aim of study was to evaluate antidiabetic, hypolipidemic and antioxidant activities of the ethanolic extract of the roots of *Decalepis hamiltonii* in experimentally induced diabetic rats. Alloxan (150mg/kg, intraperitoneal) was used to induce hyperglycemia. Administration of ethanolic extracts (100, 200 and 400mg/kg, oral) once daily up to 28days to diabetic rats reduced blood glucose and glycosylated hemoglobin, and increased insulin levels significantly. Triglycerides (TG), total cholesterol (TC), VLDL and LDL levels were significantly decreased, whereas, HDL levels were increased. The extract also significantly reduced reactive thiobarbituric acid levels (TBARS), super oxide dismutase (SOD) and increased the levels of reduced glutathione (GSH) and catalase (CAT) when compared to the diabetic-control animals. *D. hamiltonii* was assessed for *in-vitro* antioxidant activity by DPPH (2, 2-diphenyl-1-picrylhydrazyl), hydrogen peroxide and reducing power model. Significant i*n-vitro* antioxidant activity was observed in all the models. From the results, it is evident that alcoholic extract of the roots of *D. hamiltonii* can be effectively used as antidiabetic, hypolipidemic and antioxidant.

Key words: antidiabetic, antioxidant, hypolipidemic,decalepis.

Introduction

Diabetes mellitus is a metabolic disorder of multiple aetiology characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism due to deficient action of insulin on target tissues resulting from defects in insulin secretion, insulin action or both (Chait and Brunzell, 1994). It has been suggested that a total of 300 million people around the world will have diabetes by the year 2025 and the global cost of treating diabetes and its complications could reach US$ 1 trillion annually (Chakrabarti *et al*., 2002). The high prevalence and severity of the disease is quite alarming in most of the developing countries. In India alone has more than 40 million diabetic individuals which represent nearly 20% of the total diabetes population of the world and it is estimated to rise to almost 70 million by 2025 (Hoskote *et al*., 2008). Although different types of oral hypoglycemic agents are available along with insulin for the treatment of diabetes mellitus, none offers complete glycemic control (Jiang *et al*., 2003). And there is increasing demand by the patients to use the herbal preparations with antidiabetic activity.

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D.hamiltonii Wight & Arn. commonly called as maredu kommalu or baree sugandhi or makali beru is an endangered climbing shrub belonging to the family Asclepiadaceae. Its roots have been used in Ayurveda, to stimulate appetite, relive flatulence and as a general tonic (Nayer *et al*., 1978), demulcent, diaphoretic, diuretic and tonic. It is useful in the loss of appetite, fever, skin disease, diarrhoea, nutrition disorders, blood purifier, flavouring principle (Wealth of India, 1990).

The phytochemical constituents present in *D.hamiltonii* roots are ellagic acid (Srivastava *et al*., 2007), volatile oil (0.68%), which contain 2-hydroxy, 4-methoxy benzoic acid (96%) (Nagarajan and Rao (2003)), Salicyaldehyde(0.018), benzaldehyde (0.017%), methylsalicylate (0.044%), benzyl alcohol (0.016%), 2-phenyl ethyl alcohol (0.081%), ethyl salicylate (0.038%), p-

anisaldehyde (0.01%), and vanillin (0.45%) (Nagarajan *et al.*, 2001), ketone, resinol, sterols, saponins, tannins, inositol, fatty acids (Murthi *et al.*, 1942) α-amyrin, β-amyrin acetate, and lupeol.(Nagarajan *et al*., 2003)

According to Gayathri and Kannabiran (2009), administration of 2-hydroxy 4-methoxy benzoic acid**,** is beneficial in normalizing the altered carbohydrate and lipid metabolism in diabetes, and also protects the liver by restoring the levels of liver specific enzymes.

There are no systemic pharmacological studies to establish the antidiabetic effect of *D.hamiltonii*. Hence, the present investigation was carried out to study the antidiabetic, hypolipidemic and antioxidant activities of the ethanolic extract of *D. hamiltonii* **(EDH)** in alloxan-induced diabetic rats.

Materials and methods

Plant material and extraction

D.hamiltonii roots are freshly collected during the month of August-September from the forest area Dhulepalli, Hyderabad. The Plant material was authenticated by Dr.V.S.Raju, Department of Botany, Kakatiya University, Warangal. Voucher specimen was deposited at Vaagdevi College of Pharmacy, Warangal. The roots were shade dried, powdered and material was extracted with ethanol (0.5kg powder and 2liter 95% ethanol) by reflexing over a boiling water bath for 4hours. The extract was dried under reduced pressure using a rotary vacuum evaporator. The % yield was 4.2% w/w and the extract was kept in refrigerator for further use.

Drugs and chemicals

Alloxan was purchased from Sigma Chemicals, USA. Standard drug glibenclamide was procured from Cipla ltd. Diagnostic kits used in this sourced from Span Diagnostics Ltd. India. All the other chemicals used were of analytical grade.

Animals

Wistar albino rats weighting 120-180g were procured from Mahaveer Enterprises, Hyderabad. (CPCSEA Regd.No:146/1999/CPCSEA). They were housed in individual polypropylene cages under standard laboratory conditions of light, temperature and relative humidity. Animal experiments were designed and conducted in accordance with the guidelines of Institutional animal ethical committee (IAEC-VCOP, Warangal). (1047/ac/07/CPCSEA, dated 13-06-2009).

Acute toxicity studies

The oral acute toxicity study of the plant extract was carried out in adult Swiss albino mice of both sexes. This method was carried out according to OECD guidelines by adopting fixed dose method. Four animals per treatment group and different dose range 5, 50, 300, 2000mg/kg respectively, the animals were observed continuously for any change in autonomic or behavioral response for first 2 hours, then intermittently and at the end of 24 hours, the mortality/survivor was recorded. (Veeraraghavan, OECD-420 guidelines).

Oral glucose tolerance test (OGTT)

Rats were fasted overnight and divided into five groups with 6 animals in each group. Group-I received distilled water, served as control. Group-II animals were treated with glibenclamide (0.5mg/kg p.o.) to serve as standard. Group-III to GroupV animals were treated with EDH in three different extract doses (100mg/kg, 200mg/kg and 400mg/kg b.w.). The groups control, standard and test were treated with drugs 30 minutes prior to the glucose load (2g/kg p.o.) (Leng *et al.,* 2004). Blood samples were collected at 30, 60, 90 and 120min after glucose loading, by retro orbital venous puncture and glucose levels were measured immediately after separation of serum.

Experimental induction of diabetes in rats

Alloxan monohydrate was used to induce diabetes. Animals were allowed to fast for 16hr and were injected intraperitoneally (i.p.) with freshly prepared alloxan monohydrate in normal saline in a dose of 150mg/kg. After 48 hours the rats which have blood glucose levels of 200mg/dl and above were considered to be diabetic.

Experimental design

After induction of diabetic the rats were divided into six groups of six animals each.

Group 1: Normal rats.

Group 2: Diabetic rats treated with the vehicle solution (2% gum acacia ml/kg, *os*)

Group 3: Diabetic rats treated with glibenclamide (5mg/kg, *os*).

Group 4: Diabetic rats treated with ethanolic extract of D.hamiltonii (100mg/kg, *os*).

Group 5: Diabetic rats treated with ethanolic extract of D.hamiltonii (200mg/kg, *os*).

Group 6: Diabetic rats treated with ethanolic extract of D.hamiltonii (400mg/kg, *os*).

The drugs and vehicle were administered orally by an intragastric tube daily for 28 days.

The body weight was measured on days 7, 14, 21 and 28 and blood samples were drawn from the retro orbital venous plexus of rats under ether anesthesia using a glass capillary tube after they had been fasted for 12 hours. The blood samples were used for the biochemical analysis of triglycerides (TG), total cholesterol (TC), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL).

Bio-chemical analysis

The blood samples were centrifuged at 5000rpm for 20 min and serum was separated and stored at -20^{0}C

until analysis was done. Samples were analyzed spectrophotometrically for blood glucose by GOD-POD method (Kaplan *et al.,* 1984), using commercial kit (Span diagnostics, India). TG (Kaplan and Lavernal, 1983) was estimated by GPO-POD method, TC (Herbert, 1984) was estimated by CHOD-PAP method, HDL- was analyzed by kits (Roche diagnostics, Germany) LDL and VLDL-cholesterol using Friedevald's equation (Roche diagnostics, Germany). Glycosolated hemoglobin by Gould *et al.,* 1982 and serum insulin was estimated by ACS: 180 automated chemiluminescence system. On 28th day serum glycosylated hemoglobin and insulin estimations were carried out at Vijaya diagnostic center, Hanamkonda, A.P.

***In-vitro* antioxidant activity**

Estimation of DPPH scavenging activity

The hydrogen atom of electron donating abilities of the resultant compounds was measured from the bleaching of the purple-colored ethanol-solution of DPPH. This Spectrophotometric assay uses the stable radical DPPH as a reagent (Cuedet *et al*., 1976). 0.1mM solution of DPPH in ethanol was prepared and 0.1ml of this solution was added to 3ml of different concentration of the extract (5, 10, 20,40,60,80 and 100µg/ml). After a 30 min incubation period at room temperature, the absorbance was read against a blank at 517nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (µg/ml) of extract that inhibits the formation of DPPH radicals by 50%.

Estimation by reducing power method

The reducing power of the compound was evaluated according to Oyaizu *et al.,* 1986. Different concentration of the extract (5-100µg/ml) were dissolved in distilled water and added with 2.5ml of 0.2M phosphate buffer (pH6.6), and 2.5ml of 1% of $K_3Fe(CN)_6$. The mixture was incubated at 50^{0}c for 20 min. 2.5ml of 10% TCA (Trichloro acetic acid) was added to the blend and centrifuged at 3000rpm for 10min. The upper layer of the solution (2.5ml) was assorted with distilled water (2.5ml) and $FeCl_3$ (0.5ml, 0.1%) and the absorbance was measured at 700nm. Increase in absorbance of the reaction mixture indicated reducing power.

Estimation by hydroxyl radical scavenging activity

The scavenging ability for hydroxyl radical was measured according to the modified method of Halliwell *et al.,* 1987. Stock solution of EDTA (1mM), $FeCl_3$ (10mM), ascorbic acid (1mM), H_2O_2 (10mM) and deoxyribose (10mM) were prepared in distilled de-ionized water. The assay was preformed by adding 0.1ml EDTA, 0.01ml of $FeCl_3$, 0.1ml of H_2O_2, 0.36ml of deoxyribose, 1ml of different concentrations of extract (5-100µg/ml) dissolved in distilled water, 0.33ml of phosphate buffer (50mM,pH 7.4) and 0.1ml of ascorbic acid added in sequence. The mixture was then incubated at 37^{0}C for 1hr. A 1.0 ml of incubated mixture was mixed with 1ml of 10% TCA and 1ml of 0.5% Thio-barbituric Acid (TBA) in 0.025M NaOH containing 0.025% BHA (Butylated hydroxyl anisole) to develop the pink chromogen measured at 532 nm. The hydroxyl radical scavenging activity of the extract is reported as % incubation of deoxy ribose degradation.

$$I\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

***In- vivo* antioxidant studies**

Preparation of liver post mitochondrial supernatant (Liver-PMS)

At the end of the study, animals were decapitated and cut open to excise the liver. The excised livers immediately and thoroughly washed with ice-cold physiological saline. The tissue of 100mg was homogenized in 1ml of 0.1M cold tris-HCl buffer (pH7.4) in a potter-Elvehjam homogenizer fitted with a Teflon plunger at 600rpm for 30 min. (Folch *et al*., 1957). The homogenate was centrifuged at 10,000g for 20 min at 4^{0}c and the supernatant with firmly packed pellets were resuspeneded by homogenization in 100mM Tri-Hcl buffer containing 20%w/v glycerol and 0.1ml of 10mM EDTA, pH 7.4. (Naveen Tirkey *et al*, 2005). The post mitochondrial supernatant was used to assay TBARS, reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) activity.

Estimation of lipid-peroxidation (LPO) from liver PMS

LPO was induced and assayed in rat hepatic-PMS (Wright *et al.,* 1981). In 1ml of the reaction muddle, 0.58ml phosphate buffer (0.1 M, pH 7.4), 0.2ml of hepatic PMS (10%w/v), 0.2 ml ascorbic acid (100mM) and 0.02 ml ferric chloride (100mM) and was incubated at 37^{0}c in a shaking water bath for 1h. The reaction was clogged by the addition of 1ml TCA (10%, w/v), subsequently 1ml TBA (0.67%w/v) was added and all the tubes were kept in a boiling water bath for 20 min. The tubes were shifted to ice-bath and centrifuged at 2500×g for 10 min. The amount of malonldialdehyde (MDA) formed in each of samples was assessed by measuring the optical density of the supernatant at 535nm allied with reagent blank without tissue homogenate. The molar extinction coefficient for MDA was taken to be $1.56\times105M^{-1}cm^{-1.}$

Calculation

3×absorbance of sample/50.156× (mg of tissue taken) = µM /mg tissue

Estimation of Reduced glutathione (GSH) from liver PMS

Glutathione was assayed by the method of Jollow *et al.,* 1974. An aliquot of 1ml of hepatic PMS

(10%w/v) was mixed with 1ml of sulphosalicylic acid (4%w/v) and centrifuged at 1200g for 5 min and filtered. From the above, 0.1ml filtered aliquot, 2.7ml phosphate buffer (0.1M,pH 7.4) and 0.2ml DTNB (40mg/10ml of phosphate buffer 0.1M, pH7.4) in a total volume of 3.0ml. The yellow color developed was comprehended at 412 nm on a spectrophotometer.

Estimation of super oxide dismutase (SOD) from liver PMS

Super oxide dismutase activity was estimated by Fridovich *et al.,* 1973 method. The reaction mixture consisted of 0.5ml of hepatic PMS, 1ml 50mM sodium carbonate, 0.4ml of 25µM NBT (Nitro blue tetrazolium) and 0.2ml, 0.1mM EDTA. The reaction was initiated by addition of 0.4ml of 1mM hydroxylamine-hydrochloride. The change in absorbance was recorded at 560nm. The control was simultaneously run without liver homogenate. Units of SOD activity were expressed as the amount of enzyme required inhibiting the reduction of NBT by 50%.

Estimation of catalase (CAT) from liver PMS

CAT activity was assayed by the Claiborne *et al.,* 1985. The assay mixture consisted of 1.95ml phosphate buffer (0.05M, pH 7), 1ml H_2O_2 (0.019M), 0.05ml of hepatic PMS (10%w/v). Changes in absorbance were recorded at 240nm for 2min with 60 seconds interval using a spectrophotometer (Model 106).

Statistical analysis

Data for various parameters were analyzed using analysis of variance (ANOVA) and the group means were compared by Tukey-Kramer test (Graph Pad Version 3.06, La Jolla, CA, USA). Values were considered statistically significant when at $P < 0.05$.

Results

Effect of *D. hamiltonii* ethanolic extract on blood glucose levels in Normoglycemic rats (OGTT)

In oral glucose tolerance test, D. *hamiltonii* significantly reduced the blood glucose levels in glucose loaded rats at 30min and 60 min (Table.1). A significant ($P<0.05$) decrease in the blood glucose level of treated glucose loaded rats as compared with control rats was observed. The ethanolic extract of *D. hamiltonii* was possessed significant effect in 30 min at the doses of 200mg/kg and 400mg/kg. The effect was similar to glibenclamide.

Effect of *D. hamiltonii* ethanolic extract on body weight in alloxan induced diabetic rats

Animals treated with alloxan in diabetic control group showed increase in body weight, which was persistently observed till the end of the study period i.e.28 days. Where as in ethanolic extract of *D.hamiltonii* treated groups the body weight was restored almost it's near initial values after 28 days of treatment (Table 5).

Effect of *D. hamiltonii* ethanolic extract on blood glucose levels in alloxan induced diabetic rats

A statistically significant ($p<0.001$) decrease was observed in the blood glucose levels of diabetic rats treated with ethanolic extract of *D. hamiltonii at* the dose of 200mg/kg when compared with diabetic control rats from the 7th day experimental period. Glibenclamide causes maximum reduction in blood glucose levels from 1st week on wards when compared to controlled diabetic rats (Table. 2).

Effect of *D.hamiltonii* ethanolic extract on insulin and glycosylated haemoglobin levels in alloxan induced diabetic rats

Significant change($P<0.05$) was noted in the serum insulin levels and glycosylated hemoglobin levels of the diabetic animals treated with ethanolic extract of *D.hamiltonii,* there by suggesting that *D.hamiltonii* probably exerts antihyperglycemic activity by a pancreatic mechanism dependent of insulin secretion (Table.3).

Effect of *D.hamiltonii* ethanolic extract on various lipid parameters in alloxan induced diabetic rats

The marked hyperlipidemic that characterize the diabetic state may therefore be regarded as a consequences of the uninhibited actions of lipolytic hormones on the fat depots. Administration of EDH at both 200mg/kg and 400mg/kg doses caused reduction of TC, TG, LDL, VLDL and improved HDL level significantly ($p<0.001$) (Tables. 4&5).

Effect of *D.hamiltonii* ethanolic extract on DPPH, reducing power, hydroxyl radical scavenging activity in alloxan induced diabetic rats

The effect of alcoholic extract of *D. hamiltonii* on inhibition of hydroxyl radical production was assessed by iron (II)-dependent deoxyribose damage assay. The capability of alcoholic extract of *D. hamiltonii* of reducing hydroxyl radical production at all concentration was shown in Table 6. Ascorbic acid, used as standard was highly effective in inhibiting the oxidative DNA damage, showing an IC_{50}=18.46µg/ml. Where as the plant product have IC_{50}=35.56µg/ml, which can be comparable to standard.

The dose dependent inhibition of DPPH radical indicated that ethanolic extract of *D.hamiltonii* causes reduction of DPPH radical in stoichiometric manner. The IC_{50} values of ethanolic extract of *D.hamiltonii* are found to be 29.86µg/ml, which can be comparable to ascorbic acid (IC_{50} = 25.20µg/ml) as a standard compound. In reducing power method the increase in concentration causes increase in absorbance. This reduction could have resulted from the antioxidant effect of the different concentration of

fraction of plant *D.hamiltonii*, whose phytochemical components include flavonoid, which is known for antioxidant effect (Table.6).

Effect of *D.hamiltonii* ethanolic extract on liver TBAR, SOD, GSH and catalase enzyme activity in alloxan induced diabetic rats

Diabetic subjects have been shown to have increased oxidative stress and decreased antioxidant level (Jain *et al.,* 1998). The alcoholic extract *D.hamiltonii* was found to have strong lipid peroxide scavenging activity and lower the TBARS levels in liver. Exposure of liver to elevated glucose levels result in the decreased activities of SOD, CAT and GSH, which contribute to the increased lipid peroxide in the liver (Mata *et al.,* 1996). Administration of alcoholic extract of *D.hamiltonii* and glibenclamide increased activity of CAT and GSH significantly and decreased activity of SOD significantly (Table.7).

Discussion

The present study was undertaken with the objective of exploring the antidiabetic potential of *D. hamiltonii*, in alloxan induced diabetic rats, through blood glucose levels, lipid metabolism and antioxidant status. No toxic reactions were observed thereby suggesting the non toxic nature of *D. hamiltonii* at the selected doses of 100mg/kg, 200mg/kg and 400 mg/kg till the end of the experimental period. Alloxan causes diabetes by the rapid depletion of β-cell and there by brings about a reduction in insulin release. As well as alloxan produces oxygen radicals in the body, this causes pancreatic injury and role in the alleviation of diabetes. Hyperglycemia causes oxidative damage by the generation of reactive oxygen species (Jorns *et al*., 1997, Sakurai *et al*., 2001) and results in the development of diabetic complications (Sabu *et al*., 2002). Decreased antioxidant enzyme levels and enhanced lipid peroxidation have been well documented in alloxan induced diabetes (Sepici-Dincel *et al*., 2007).

In our study administration of alloxan increased serum glucose levels when similar effect of alloxan was also observed in previous studies on the evaluation of Persea Americana (Anita *et al*., 2005) for antidiabetic activity.

In oral glucose tolerance test, at 60, 90 and 120 min, a significant decrease in the blood glucose levels was observed in treated rats as compared with control rats. From the OGTT data, it is clear that administration of EDH at the doses 200mg/kg and 400mg/kg effectively prevented the increase in serum glucose level without causing a hypoglycemic state. Antidiabetic activity of *D.hamiltonii* may be through the stimulation of surviving β-cells of islets of langerhans to release more insulin. A number of other plants have been observed to exert antidiabetic activity through insulin-release stimulatory effects, such as *Musa sapientum* (Pari *et al*., 2000).

HbA_{1C} was found to increase in patients with diabetes mellitus to about 16 %(Koenig *et al*., 1976) and the amount of increase is directly proportional to the fasting blood glucose levels (Jacksone *et al*., 1979, Al-yassin and Ibrahim, 1981). There is evidence that glycation itself may induce the formation of oxygen-derived free radicals in diabetic condition (Gupta *et al.,* 1997). In the present study, the diabetic rats had shown higher levels of HbA_{1C} compared to those in normal rats. Treatment with EDH and glibenclamide showed a significant decrease in HbA_{1C} levels in diabetic rats that could be due to an improvement in glycemic status. Under normal circumstances, insulin activates enzyme lipoprotein lipase causes hydrolysis triglycerides (Sharma *et al.,* 1997). In diabetic rats, an increase in TC, TG, LDL and VLDL-cholesterol, decrease in HDL cholesterol. The abnormal high concentration of serum lipids in diabetic subject is mainly due to increase in the mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase (Pari and Latha, 2002). Improved insulin concentration significantly, when treated with EDH indicates the insulin secretogague activity as well as antihyperlipidemic activity.

The effect of alcoholic extract of *D. hamiltonii* on inhibition of hydroxyl radical production was assessed by iron (II)-dependent deoxyribose damage assay. Ferrous salts can react with hydrogen peroxide thus forming hydroxyl-radical via Fenton's reaction (Halliwell *et al*., 1981). The iron required for this reaction is obtained either from the pool of iron or the heme containing proteins. The hydroxyl radical thus produced may attack the sugar of DNA bases, which leads to sugar fragmentation; base loss and DNA strand breakage. Addition of transition metal ions such as iron at low concentration to deoxyribose causes degradation of the sugar into malondialdehyde and other related compounds which form a chromogen with thio-barbituric acid (TBA). The DPPH radical is considered to be a model of lipophilic radical. A chain reaction in lipophilic radicals was initiated by lipid auto oxidation (Blois *et al.,* 1958). The radical scavenging activity of ethanolic extract of *D.hamiltonii* was determined from the reduction in absorbance at 517 nm due to scavenging of stable DPPH radical.

Lipid peroxide was found to be significantly high in diabetic group as compared to normal group, which increases TBARS activity. This may be due to the presence of phenols and flavonoids, which may have a major role in reducing oxidative stress associated with diabetes (Morel *et al*., 1989). Apart from lipid peroxides, catalase, super oxide dismutase, glutathione synthetase are examples of enzymatic antioxidants. Superoxide dismutase and catalase

are considered as primary enzymes, since they are involved in the direct elimination of reactive oxygen species. SOD is an important defense enzyme which catalyzes the dismutase of super oxide radicals and CAT is a hemoprotein, which catalyze the reduction of hydrogen peroxides and protects tissues from highly reactive OH radicals. Glutathione synthetase, the most important biomolecule protecting against chemical induced toxicity, participates in the elimination of reactive intermediates by reduction of hydro peroxide in the presence of glutathione peroxides (Meister *et al.,* 1984). The decreased level of glutathione synthetase observed in diabetic animals represents an increased utilization resulting from oxidative stress. (Anuradha *et al.*, 1993). Restoring the all the enzymatic and nonenzymatic antioxidant parameters in liver is similar to previously reported plants by Pradeep reddy *et al*., 2010.

Conclusion

The results of the present investigation clearly indicate that the ethanolic extract of *D.hamiltonii* have glucose lowering effect on alloxan-induced diabetic rats. It was also found to be highly effective in managing the complications associated with diabetes mellitus, such as body weight maintenance, hyperlipidemia, and as an antioxidant prevents the defects in lipid metabolism. Therefore *D. hamiltonii* roots show therapeutic promise as a protective agent against the development of the major complication because of free radicals. This could be useful for prevention or early treatment of diabetic disorder. Further studies are in progress to isolate, identify and characterize the active principles.

Table.1 Effect of alcoholic extract of *D.hamiltonii* roots on oral glucose tolerance test (OGTT)

Groups	Dose mg/kg b.w.	Mean blood glucose concentration (mg/dl)				
		0min	30min	60min	90min	120min
Normal control(I)	-----	87.5±3.5	154.6±28.4	159.6±7.8	125.6±9.7	118.4±11.8
Glibenclamide(II)	5	86.6±3.6	126.5±19.5	107.6±10.5b	95.6±11.4b	75.4±3.6b
EDH(III)	100	89.4±2.3	156.4±3.4	117.6±10.5b	96.5±4.5b	73.4±7.8b
EDH(IV)	200	87.5±5.8	167.5±34.5a	128.9±12.4b	103.4±7.8b	83.4±8.7b
EDH(V)	400	89.4±2.1	134.2±5.6	100.5±12.3b	94.4±3.4b	79.3±3.4b

Values are mean ± S.D; N=4,
a = p<0.05 values when compared to diabetic rats, b= p<0.001 when compared to normal rats.

Table.2 Effect of ethanol extract of *D.hamiltonii* root extract on blood glucose levels

Groups	Dose	Blood glucose levels (mg/dl)				
		0days	7days	14days	21days	28days
Control(I)	----	86.7±3.12	88.3±4.5	87.4±3	87.3±4.3	88.4±5.6
Diabetic (II)	------	210.34±4.2	217.8±8.4	234.8±9.4	248.9±12.3	269.9±19.3
Glibenclamide(III)	5mg/kg	210.9±15.5	165.03±14.5	109.83±4.47a	94.9±15.4a	84.9±16.8a
D.hamiltonii(IV)	100mg/kg	252.3±21.9	223.4±11.3	164.8±10.4a	123.4±24.3a	84.5±8.9a
D.hamiltonii(V)	200mg/kg	248.4±10.2	202.8b±10.8	142.4±11.84a	101.4±18.3a	79.4±9.9a
D.hamiltonii(VI)	400mg/kg	264.9±9.4	221.4±11.4	184.4±16.8a	112.4±11.2a	89.4±9.8a

Values are mean ±S.D. (N=6).
A=P<0.001 when compared to diabetic control rats, b= p<0.05 when compared to normal rats.

Table.3 Effect of ethanolic extract of *D.hamiltonii* roots on different parameters

Groups	On 28th day	
	Glycosolated hemoglobin (%)	Insulin (µIU/ml)
Normal(I)	4.05±0.403	11.5±2.6
Diabetic(II)	12.29±1.210	10.7±1.7
Glibenclamide(III)	3.95±0.68	6.9±2.43a
EDH(100mg/kg)(IV)	5.6±0.76b	9.3±1.9a
EDH(200mg/kg)(V)	4.89±0.548b	8.6±1.03a
EDH(400mg/kg)(VI)	4.92±0.678b	10.3±2.4a

a= p<0.001 values when compared to normal, b= p<0.05 values when compared to diabetic rats. (N=4).values are Mean ± S.E.M.

Table.4 Effect of alcoholic extract of *D.hamiltonii* roots on various lipid parameters

Groups	Treatment in days											
	HDL(mg/dl)				LDL(mg/dl)				VLDL(mg/dl)			
	7	14	21	28	7	14	21	28	7	14	21	28
Normal (I)	36.52 ±2.3	33.07 ±6.4	34.84 ±4.41	35.4± 5.5	11.76 ±1.3	10.12 ±2.4	11.42 ±3.1	12.43 ±4.32	8.13 ±2.1	7.93 ±2.3	7.98 ±2.1	7.35 ±9.1
Diabetic (II)	29.19 ±1.34	16.24 ±1.02	13.91 ±2.2	10.46 ±2.33	156 ±16.8	214.36 ±32.4	226.59 ±20.9	232.92 ±22.1	24.09 ±3.2	25.12 ±4.5	27.9 ±2.8	29.8 ±3.5
Glibenclamide(5mg/kg) (III)	24.85 ±1.24a	35.42 ±2.43a	38.55 ±2.64a	41.38 ±3.4a	105.38 ±2.9a	45.96 ±3.1a	17.61 ±3.5a	3.04 ±2.01a	13.2 ±5.43a	11.1 ±0.98a	12.3 ±1.34a	9.8 ±2.34a
D.hamiltonii (100mg/kg) (IV)	26.78 ±1.964	34.5 ±2.43a	38.4 ±3.398a	39.9 ±4.489a	111.1 ±2.4a	47.78 ±2.09a	23.10 ±2.2a	11.92 ±1.67a	17.54 ±1.3c	14.08 ±1.3ab	11.68 1.3a	8.68 ±2.1a
D.hamiltonii (200mg/kg) (V)	28.45 ±1.084	35.4 ±2.66a	39.5 ±3.503a	40.9 ±2.94a	105.56 ±1.6a	20.93± 16.23a	12.71 ±3.4a	7.75 ±2.3a	15.98 ±2.1a	14.686 ±1.56ab	10.08 ±2.1a	7.88 ±1.34a
D.hamiltonii (400mg/kg) (VI)	29.86 ±1.612	39.5 ±3.44a	40.4 ±3.432a	43.4 ±3.744a	106.46 ±2.13a	25.646 ±3.4a	29.15 ±2.05a	3.35 ±1.2a	17.08 ±2.45c	14.284 ±2.34a	9.88 ±3.4a	7.7 ±4.3a

Values are mean ± SEM. (n=6).

a=p<0.001 when compared to diabetic rats, b=p<0.05 when compared to normal control rats. c= p<0.05 when compared to diabetic rats.

Table.5 Effect of alcoholic extract of *D.hamiltonii* roots on different lipid parameters

Groups	Treatment in days											
	Serum triglyceride (mg/dl)				Serum total Cholesterol(mg/dl)				Body weight (g)			
	7	14	21	28	7	14	21	28	7	14	21	28
Normal (I)	35.88 ±3.8	37.42 ±3.4	37.08 ±5.4	38.4 ±4.9	56.41 ±4.32	56.12 ±5.9	54.21 ±6.6	55.21 ±4.4	180.23 ±2.3	184.5 ±1.3	193.3 ±2.1	185.5 ±2.3
Diabetic control(II)	121.4 ±10.7	124.99 ±12.4d	138.09 ±14.2	142.37 ±14.2	207.96 ±12.5a	255.72 ±14.3	268.4 ±15.6	272.46 ±13.9	142 ±2.5d	140.1 ±3.7d	138.3 ±2.2d	135 ±5.2d
Glibenclamide(III)	60.9 ±4.4a	52.3 ±4.3ad	50.4 ±3.2**a**	45.8 ±4.2**a**	143.43 ±11.2a	92.46 ±3.34a	68.46 ±4.3a	54.22 ±4.5a	140.4 ±4.1d	145 ±3.2d	151.3 ±3.5d	155.8 ±4.1d
D.hamiltonii (100mg/kg)(IV)	87.9 ±2.3a	70.4 ±4.3ad	58.4 ±5.6a	43.4 ±3.2a	55.43 ±3.47a	96.36 ±4.22a	73.18 ±5.09a	60.50 ±4.43a	156.7 ±3.2d	132.5 ±4.5d	154.3 ±4.5d	153.4 ±4.3d
D.hamiltonii (200mg/kg)(V)	89.9 ±4.1a	73.43 ±3.2ad	50.4 ±4.5a	39.4 ±5.6a	149.99 ±12.15a	71.01 ±2.22a	62.29 ±4.43a	56.53 ±9.77a	156.46 ±2.5d	125.4 ±2.4d	148.3 ±5.6d	167.3 ±4.5d
D.hamiltonii (400mg/kg)(VI)	85.4 ±3.2a	71.42 ±4.3ad	49.4 ±5.9a	38.5 ±4.3a	153.4 ±15.43a	79.43 ±5.4a	60.43 ±5.9a	54.45 ±5.6a	165.4 ±3.4d	147.8 ±5.6d	168.5 ±4.7d	176.5 ±3.56d

a= $p<0.001$ when compared to diabetic control
d=$p<0.001$ when compared to normal rats.
Values are Mean ± SD, n=6

Table.6 Effect of alcoholic extract of *D.hamiltonii* roots *in-vitro* DPPH scavenging activity

Concentration(µg /ml)	% inhibition of DPPH peak area	
	Ascorbic acid	Ethanolic extract DH
5	18.34±0.340	12.45±0.04
10	26.87±0.560	21.40±0.08
20	34.56±0.230	35.60±0.09
40	56.78±0.060	48.90±0.12
60	78.90±0.080	67.80±0.11
80	87.60±0.090	78.90±0.21
100	100.00±0.088	123.40±0.22
IC_{50}	25.20 µg/ml	29.86 µg/ml

Absorbance was read in triplicate at all concentrations. Values are Mean ± SEM.

Table.7 Effect of alcoholic extraction of *D.hamiltonii* roots on various *in-vivo* antioxidant activities

Group	Dose b.w. (mg/kg)	CAT (µM/mg tissue)	GSH (µM/mg tissue)	LPO (µM/mg tissue)	SOD (U/mg tissue)
Normal	-----	0.0576±0.00168	0.185±0.0033	0.0492±0.00062	0.03174±0.0077
Diabetic control	-----	0.0043±0.00014	0.0405±0.00144	0.476±0.00522	0.00432±0.0037
Glibenclamide	5	0.0666±0.00186a	0.179±0.00393a	0.0582±0.00034a	0.0258±0.0043a
D.hamiltonii	100	0.0456±0.00123a	0.145±0.0056a	0.0345±0.0025a	0.01987±0.0035a
D.hamiltonii	200	0.0563±0.00145a	0.201±0.0067a	0.0510±0.0034a	0.02015±0.0045a
D.hamiltonii	400	0.0743±0.0047a	0.245±0.0021a	0.0621±0.0045a	0.3450±0.0035a

Values are Mean±SEM; n=4
A = $p<0.001$ compared to diabetic control.

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