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ANTIDIABETIC EFFECT OF ETHANOLIC SEED EXTRACT OF *DECALEPIS HAMILTONII* WIGHT AND ARN ON OXIDATIVE STRESS AND ENZYMIC ACTIVITIES IN ALLOXAN INDUCED DIABETIC RATS

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Keywords:

Decalepis hamiltonii; Antidiabetic activity; Hyperglycemia; Glucose metabolism; Glutathione; Lipid peroxidation; Enzymic activities

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ABSTRACT: *Decalepis hamiltonii* Wight and Arn. is an indigenous medicinal plant, which has a folk reputation in Western and southern India as hypoglycemic agent. The present investigation was carried out to evaluate the antidiabetic effect of ethanolic seed extract of *Decalepis hamiltonii* in alloxan induced diabetic Albino rats. Blood glucose levels and body weights of control and diabetic rats were monitored. In the present study activities of liver enzymes such as glucokinase, glucose -6- phosphatase and fructose -1-6-diphosphatase were also determined. Glibenclamide an antidiabetic oral drug was used as reference in the present investigation. Oral administration of ethanolic seed extract (20 mg/kg body weight) for 21 days resulted in a significant ($P<0.05$) decline in blood glucose from 264.03±10.07 to 90.16±2.82 mg/dl and significant recovery in body weight of diabetic rats. There was also a significant ($P<0.05$) reduction in the activities of glucose-6-phosphatase and fructose-1-6-disphosphatase , glutathione levels, lipid peroxidation as oxidative stress γ-Glutamyl transpeptidase, Glutathione S-transferase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid and alkaline phosphatases in liver were also evaluated. Further there was significant ($P<0.05$) increase in the activity of glucokinase in liver of diabetic rats when compared with that of diabetic control. Results indicate increased metabolization of glucose in treated rats. Increased levels of lipid peroxidation measured as 2-thiobarbituric acid reactive substances (TBARS) indicative of oxidative stress in diabetic rats were also normalized by treatment. The study clearly shows that the ethanolic seed extract of *Decalepis hamiltonii* possesses potent antidiabetic activity.

INTRODUCTION: Nature has provided a complete store-house of remedies to cure all ailments of mankind. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. A number of plants have been documented for their medicinal potential, which is in use by the traditional healers, herbal folklorists and in Indian system of medicine namely Siddha, Ayurveda and Unani.

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The use of herbal medicine has become increasingly worldwide popular and medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Standardized extracts from them or pure phyto pharmaceuticals need to be studied extensively for their quality, purity, potency, safety and efficacy [1]. *Decalepis hamiltonii* Wight and Arn is an endangered climbing shrub belonging to the family Asclepiadaceae.

It is an endemic and endangered medicinal plant is commonly known as Magali Kizhangu in Tamil. This plant roots are seasonal and grow wild, which contains pure form of antifungal compound 2-hydroxy-4-methoxybenzaldehyde (2H4MB) was isolated from volatile oil of in *Decalepis hamiltonii* roots. Based on ethnopharmacological information,

Decalepis hamiltonii has been used to treat diabetes by the tribals in and around tropical and subtropical areas. The plant extracts have not produced any toxic symptoms within the treated animals. In Vivo and In Vitro conservation methods have been standardized to this endangered plant by developing rapid micro propagation techniques [2].

FIG.1: SEED OF *DECALEPIS HAMILTONII*

Diabetes mellitus is recognized as a syndrome, a collection of disorders that have hyperglycemia and glucose intolerance as their hallmark, due either to insulin deficiency or to the impaired effectiveness of insulin's action, or to a combination of these. In order to understand diabetes it is necessary to understand the normal physiological process occuring during and after a meal. Food passes through the digestive system, where nutrients, including proteins fat and carbohydrates are absorbed in to the blood stream. The presence of sugar, a carbohydrate, signals to the endocrine pancreas to secrete the harmone insulin. Insulin cause the uptake and storage of sugar by almost all tissue types in the body, especially the liver, musculature and fat tissues. The cause of diabetes continues to be anonymity, although both genetics and environmental factors such as obesity and lack of exercise appear to play roles [3].

Diabetes mellitus is a serious health problem with continuously increasing rates of incidence and mortality. Unfortunately, there is no cure for diabetes yet, but by controlling blood sugar levels through a healthy diet, exercise and meditation the risk of long–term diabetes complications can be decreased. (cataracts, retinopathy, kidney failure, neuropathy, ulcers, feet infections, hardening of arteries , heart disease and stroke). As a very common chronic disease, diabetes is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases, because of its high prevalence, morbidity and mortality. Therefore once diagnosed, it is well regulated by means of various therapeutically effective drugs. Besides, the therapy based on chemotherapeutic agents, the present century has progressed towards naturopathy. Thus, medical plants have an ever emerging role to play in treatment or management of lifelong prolonging diseases like diabetes mellitus, especially in developing countries where resources are meagre [4].

In recent years, researchers turned attention specifically to oxidative stress and the key role it plays, as a common element in the pathogenesis of diabetes complications. Hyperglycemia generates reactive oxygen species which, in turn, causes membrane lipid peroxidation and degradation. Many of the complications of diabetes, including vascular atherosclerosis, major cause of mortality in DM, are closely related to oxidative stress and, thus, antioxidants play an important role in the treatment of diabetes. Herbal remedies contain large amounts of antioxidants such as flavonoids, polyphenolic acids, carotenoids, Vitamins C and E; experimental research has shown that the antioxidant activity may be an important property of medicinal plants used for their hypoglycemic effect in the treatment of D.M. The most common symptoms of Diabetes Mellitus are those of hypoglycemia, loss of weight, ketosis, and arterio sclerosis, pathological changes in eyes, neuropathy, renal disease and coma [5]. In the previous study, biochemical parameters like oxidative stress and enzymic activities were not evaluated [6].

In the present study we have evaluated antidiabetic activity of a Ethanolic seed extract of *Decalepis hamiltonii* and its effect on enzymes of carbohydrate metabolism to find out possible mechanism of hypoglycemic action. In addition to this the effect of extract was evaluated on glutathione levels, related enzymes and lipid peroxidation as oxidative stress is known to occur in diabetes. Effect of extract on other enzymes of pharmacological importance i.e. γ Glutamyl transpeptidase, Glutathione *S*-transferase, aspartate aminotransferase (AST), alanine aminotransferase (ALT), acid and alkaline phosphatases were also evaluated.

MATERIALS AND METHODS:

The *Decalepis hamiltonii* Wight and Arn seed **Fig.1** were collected from the market of Kumbakonam, Tamil Nadu state, India. They were identified and authenticated by Prof. N. Ramakrishnan, Head and Associate Professor and voucher specimens (Department of Botany) and voucher specimens (GACBOT-168) were deposited at the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

Processing and preparation of plant extract:

The seeds were shaded and powdered. The material was extracted with ethanol (0.5 kg powder and 2 litre 95% ethanol) by refluxing over a boiling water bath for 3 hours. The extract was dried under reduced pressure using a rotary vacuum evaporator. The percentage of yield was 10.2% w/w and the extract was kept in refrigerator for further use.

Experimental animals:

Male albino rats were maintained under standard experimental conditions (Temperature 27±2°C, relative humidity 60±5 and 12 hours light/dark cycle) and they were fed with standard rate feed. Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No.BDU/IAEC/2011/ 31/29.03.2011).

Induction of Diabetes:

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Loba Chemie, Bombay: 150 mg/kg). Alloxan was first weighed individually for each animals according to the weight and solubilized with 0.2 ml saline (154 m M NaCl) just prior to injection.

Experimental Design:

Male albino rats [weighing >200 (g)] were divided randomly in to four groups. Each groups has eight rates.

Group- I: Served as normal control.

Group- II: The Second group of rats with diabetes was induced by intraperitonial injection of alloxan.

Group- III: Alloxan treated rats were administrated the Glibenclamide (10 mg/kg) and served as standard.

Group- IV: Alloxan treated rats were administrated the Ethanolic extract (20 mg/kg).

Determination of antidiabetic actiity and change in body weight:

The blood glucose level (BGL) was monitored after alloxanisation in blood samples collected by amputation of the tail tip under mild anesthesia. Using a blood glucose test strip (Glucocard 01 sensor) and a glucometer ARKRAY Glucocard 01-mini Blood glucose testing system. Treatment with extracts was started 48 hours after alloxan injection, Male albino rates having blood glucose level above 90 mg/dl of blood were selected for the study. Blood samples were drawn at weekly intervals till end of study (i.e. 3weeks). Fasting blood glucose estimation and body weight measurement were done on 1,7,14 and 21 days respectively. (**Tables 1** and **2**) 32 rats were used for this study. Fasting blood glucose levels were monitered weekly along with untreated controls. Any reduction in blood sugar level in comparison to that of untreated controls was taken as antidiabetic activity.

Evaluation of effect on biochemical variables:

Blood glucose levels were tested before the treatments and on 21st day of treatment on fasting mice. The Albino rate of all four groups were fasted and sacrificed by cervical decapitation. Blood was drawn from the heart. The liver was removed, washed with chilled saline, small weighed portion of the liver were processed for determination of glycogen and glutathione immediately after their removal and the homogenate was used for estimation of, glucokinase [7], glucose 6-phosphatase [8] and fructose 1, 6- diphosphatase [9] after fasting. Ten percent homogenate (w/v) of liver was prepared in 150 mM KCl using Potter– Elvehjem homogenizer at 4 °C. Two milliliter aliquots of crude liver homogenates were used for assay of lipid peroxidation and rest of the homogenates were centrifuged at 3000×*g* for 15 min at 4 °C and supernatants were divided into aliquots and frozen at −20 °C until assayed for different enzymes. Blood plasma was recovered by

centrifugation at 1000 *g* for 10 min at 4 °C. Enzymic activities of γ-glutamyl transpeptidase [10], glutathione S-transferase (GST) [11], Lipid peroxidation(MDA) [12] and enzymes of toxicological importance, i.e. Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) [13], acid phosphatise and alkaline phosphatise [14] activities are given in **Table 5**.

Effect on glucose metabolism:

In vivo effect of treatment with *Decalepis hamiltonii* ethanolic extract at dose 20 mg/kg for 21 days on glycogen levels and glucose metabolizing enzymes are given in **Tables 3** and **4**.

Statistical analysis:

Values were recorded as Mean ± standard error of the mean. Statistical difference between the means was determined by ANOVA followed by Dunnett's Multiple Comparisons Test P< 0.05 was accepted as significance level.

RESULT AND DISCUSSION: Seeds of *Decalepis hamiltonii* is used traditionally by diabetic patients in India and are taken as water decoction. Changes in body weight and blood glucose level in normal, diabetic and on treatment of diabetic rat with *Decalepis hamiltonii* ethanolic extract, glibenclamide are presented in **Table1** and **2.** Oral administration of *Decalepis hamiltonii* ethanolic seed extract (20 mg/kg body weight) for 21 days showed significant (P<0.05) reduction in blood glucose (264.03±10.07 to 90.16±2.82 mg/dl) and an improvement in body weight in diabetic mice compared with untreated diabetic mice. This was almost similar to results obtained with reference drug glibenclamide (264.02±11.2-96.22±5.16 mg/dl). Effect on the hepatic glucokinase glucose 6-phosphatase and fructose 1-6, diphosphatase, due to administration of *Decalepis hamiltonii* ethanolic leaf extract and glibenclamide on diabetic mice is given in **Table 2**.

TABLE 1: EFFECT OF ETHANOLIC EXTRACT *D. HAMILTONII* ON BODY WEIGHT OF ALLOXAN INDUCED DIABETIC RATES.

S. No.	Treatment	Body weight (g)			
		1st day	7th day	14th day	21st day
1	Normal Control	202.01±1.6	204.42±1.34	205.80±1.45	207.00±1.63
2	Diabetic Control (Alloxan)	209.00±2.3	166.10±4.64	152.04±2.43	132.08±2.88
3	Standard (Alloxan+ glibenclamide 10 mg/kg)	204.00±1.0*	205.20±1.22	206.10±1.46	207.40±1.68
4	Alloxan + EtOH Ext. (20 g/kg)	205.28±1.89*	196.62±2.24	190.82±2.12	185.24±4.21

Values are expressed in Mean ± S.E.M values. P<0.05 Dunnett's - test); diabetic control was compared with the extracts and standard treated groups .

TABLE 2: EFFECT OF ETHANOLIC EXTRACT *D. HAMILTONII* ON BLOOD GLUCOSE LEVEL AGAINST ALLOXAN INDUCED DIABETIC RATES.

S. No.	Treatment	Blood Glucose(mg)			
		1st day	7th day	14th day	21st day
1	Normal Control	92.73±2.85	92.22±3.42	92.64±6.16	92.56±2.14
2	Diabetic Control (Alloxan)	266.05±15.65	264.41±12.5	265.93±15.43	269.05±16.12
3	Standard (Alloxan + glibenclamide 10 mg/kg)	264.02±11.2	161.82±7.96	104.26±6.18	96.22±5.16*
4	Alloxan + EtOH Ext.20 mg/kg)	264.03±10.07	172.02±18.16	142.42±15.16	90.16±2.82*

Values are expressed in Mean ± S.E.M values. P<0.05 (Dunnett's - test); diabetic control was compared with the extracts and standard treated groups

The activity of hepatic glucokinase is significantly (P<0.05) decreased while activities of glucose-6-phosphatase and fructose 1-6-diphosphatase were significantly (P<0.05) elevated in alloxan diabetic control rat. The administration of *Decalepis hamiltonii* ethanolic seed extract for 21 days showed significantly (P<0.05) increased activity of hexokinase in diabetic mice when compared to that of diabetic control group. There was also significant (P<0.05) decrease in activities of glucose -6-phosphatase and fructose 1, 6-diphosphatase in diabetic rat (P<0.05) reduction in the blood glucose and improvement in body weight compared to untreated diabetic rat. There was also significant (P<0.05) decrease in activities of glucose -6-phosphatase and fructose 1, 6-

diphosphatase in diabetic mice when compared to those of diabetic control group. The administration of glibenclamide to diabetic rat also showed similar results. The results showed that *Decalepis hamiltonii* seed extract caused a significant (P<0.05) reduction in the blood glucose levels in diabetic rat by stimulating the activity of hepatic enzymes involved in glucose metabolism.

The higher blood glucose levels are expected in alloxan induced diabetic rat, since alloxan causes a massive reduction in insulin release, by the destruction of the β-cells of the islets of Langerhans and inducing hyperglycemia. In the present study oral administration of *Decalepis hamiltonii* ethanolic seed extract (20 mg/kg body weight) caused a significant (P<0.05) reduction in the blood glucose and improvement in body weight compared to untreated diabetic mice. The antihyperglycemia action of *Decalepis hamiltonii* ethanolic seed extract may be due to potentiation of pancreatic secretion of insulin. *Decalepis hamiltonii* ethanolic seed extract is also known for its liver protective action [15]. Liver is an insulin dependent tissue, which plays a vital role in glucose and lipid homeostasis and is severely affected during diabetes. Decreased glycolysis impeded glycogenesis and increased gluconeogenesis are some of the changes of glucose metabolism in the diabetic liver [16]. Hepatocytes also contain a form of hexokinase called hexokinase D or glucokinase that is more specific for glucose and differ from other forms of hexokinase in kinetic and regulatory properties [17]. Glucokinase catalyzes the conversion of glucose to glucose -6- phosphate and play a central role in the maintenance of glucose homeostasis. In the liver this enzyme is an important regulator of glucose storage and disposes [18].

In the present study, the glucokinase activity was decreased in alloxan induced diabetic mice which may be due to insulin deficiency. Insulin stimulates and activates glucokinase in the liver. *Decalepis hamiltonii* ethanolic seed extract or glibenclamide elevates the activity of glucokinase in liver. *Decalepis hamiltonii* ethanolic seed extract like glibenclamide, may stimulate insulin secretion which may activate glucokinase thereby increasing utilization of glucose and this increased utilization leads to decreased blood sugar level [19].

TABLE 3: EFFECT OF ETHANOLIC EXTRACT D. HAMILTONII ON LIVER GLYCOGEN LEVELS AND GLYCOGEN SYNTHASE ACTIVITY OF ALLOXAN INDUCED DIABETIC RATES.

Group	Liver glycogen (mg/g wet tissue)	Glycogen synthase (mol UDP formed/min/mg protein)
Normal	4.74±0.74	3.12 ±0.18
Diabetic	4.29±0.91	0.76±0.18
Diabetic treated (20 mg/kg)	20.22±4.69	1.24±0.28 *

Values are expressed in Mean ± S.E.M values. P<0.05 (Dunnett's - test); diabetic control was compared with the extracts ..

Insulin decreases gluconeogenesis by decreasing the activity of key enzymes such as glucose-6-phosphatase, fructose 1, 6- diphosphatase, phosphoenolpyruvate, carboxykinase, and pyruvate carboxylase. In the present study, increased activities of glucose-6-phosphatase and fructose-1, 6- diphosphatase were observed in the liver of alloxan-diabetic rat. Glucose 6-phosphatase is one of the key enzymes in the homeostatic regulation of blood glucose levels, it catalyzes the terminal step in both gluconeogenesis and glycogenolysis [20] and fructose 1, 6-diphosphatase, catalyzes one of the irreversible step in gluconeogenesis, and serves as a site for the regulation of these process [21]. Increased activities of these two gluconeogenic enzymes in alloxan induced diabetic mice may be due to insulin insufficiency. In alloxan induced diabetic mice treated with Decalepis hamiltonii ethanolic seed extract, activities of these two enzymes were reduced. This may be due to increased insulin secretion which is responsible for the repression of the gluconeogenic key enzymes.

The present observation provide evidence that ethanolic extract of *Decalepis hamiltonii* ethanolic seed extract exhibited antidiabetic or hypoglycemic activity on alloxan induced diabetic. Glycogen levels in liver which were low in diabetic animals, increased several folds in *Decalepis hamiltonii* ethanolic seed extract treated diabetic animals (**Table 3**). Although glycogen synthetase activity decreased in diabetic animals significantly, the

treatment however could not normalize activity. Glycogen content of normal animals in fasting stage was only slightly higher than diabetic animals and this may be due to degradation of glycogen to maintain normal blood glucose levels, whereas glycogen levels in diabetics were found to be very low despite high blood glucose levels possibly due to lower levels of glycogen synthetase activity. Accumulation of glycogen in liver of treated animals is somewhat similar to that reported during insulin therapy. When insulin therapy is instituted, hepatic glycogen accumulation begins rapidly and glycogen content rises to 300% of normal levels within 24h and this inordinate accumulation of glycogen may account for up to 60% of dry liver weight in diabetic animals [22].

TABLE 4: EFFECT OF ETHANOLIC EXTRACT *D. HAMILTONII* ON THE ACTIVITIES OF HEPATIC ENZYMES IN CONTROL AND EXPERIMENTAL ANIMALS AFTER 21 DAYS OF TREATMENT.

Groups	Glucokinase [x]	Glucose-6-phosphatise [y]	Fructose 1-6-diphosphatase [z]
Normal Control	130.62±2.28	0.162±0.26	0.256±0.028
Diabetic Control (Alloxan)	97.94±1.82	0.248±0.026 *	0.421±0.06*
Standard (Alloxan + glibenclamide 10 mg/kg)	141.28±2.26	0.168±0.02*	0.278±0.023*
Alloxan + EtOH Ext.20 mg/kg)	125.48±2.78	0.192±0.028 *	0.282±0.024*

Values are expressed in Mean ± S.E.M values. P<0.05 (Dunnett's - test); diabetic control was compared with the extracts and standard treated groups .

x -moles of glucose phosphorylated/g/h., y- moles of pi liberated/min/mg., z - moles of pi liberated/min/mg.

Oxidative stress appears to be a key element of the production of secondary complications in diabetes. Glutathione, a tripeptide present in millimolar concentrations in all the cells is an important antioxidant Decreased glutathione levels in diabetes have been considered to be an indicator of increased oxidative stress [23]. In the present study not much change was observed in GSH levels either in blood or liver of diabetic animals, however in treated animals GSH levels were marginally high in both blood as well as liver. Lipid peroxidation was found to be increased in liver of diabetic animals which became normal in *Decalepis hamiltonii* ethanolic seed extract treated animals. This indicates that the extract may be helpful in the prevention of damage caused by oxygen free radicals.

We have not studied oxidized glutathione levels and this is a limitation of this study. There was not much change in glutathione *S*-transferase activity in diabetic and diabetic treated animals. γ-Glutamyl transpeptidase activity was decreased significantly in liver of diabetic animals and with Decalepis hamiltonii ethanolic seed extract treatment activity increased (**Table 4**). γ-Glutamyl transpeptidase has a key role in amino acid transport across membranes and catalyzes the initial step in breakdown of glutathione, i.e. transfer of γ-glutamyl moiety of glutathione to a variety of amino acids and peptides [24]. Increase in γ-glutamyl transpeptidase activity in plasma is an indicator of impairment in liver function. In the present study there was an increase in γ-glutamyl transpeptidase activity in plasma of diabetic animals.

In *Decalepis hamiltonii* ethanolic seed extract treated to animals and activity of this enzyme shows a decrease in plasma and was close to normal activity. Measurement of enzymic activities of aminotransferases (AST and ALT) and phosphatases (acid and alkaline) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants or in disease conditions. AST and ALT activities in liver of diabetic animals remain unchanged in liver though AST activity was little less than normal. Plasma levels of AST were increased around twice that of normal in diabetic animals and diabetic animals treated with extract show improvement.

Recovery of plasma AST levels of diabetic rats towards normal shows that the *Decalepis hamiltonii* ethanolic seed extract extract has no adverse effect on liver functions. Liver alkaline phosphatise activity was found to be significantly increased in diabetic animals. Treatment with extract further caused increase in activity (**Table 4**). Increase in alkaline phosphatase activity in testes and prostate at 20 mg/kg for 21 days by ethanolic seed extract of *Decalepis hamiltonii* is

reported in normal animals. Acid phosphatase activity of liver of diabetic rats was also found to be increased [25]. Detection of hypoglycemic activity in *Decalepis hamiltonii* ethanolic seed extract along with protective effect against alloxan challenge and preventive action on lipid peroxidation provides scientific rationale of use of *Decalepis hamiltonii* as antidiabetic plant. Antidiabetic activity seems to be a result of increase in glucose utilization. Further chromatographic fractionation of the extract may be useful in improving activity and reduction of dose [26].

In preliminary toxicity study it is safe, however chronic toxicity evaluation will be required for human use and there is a need for further studies on this aspect.

TABLE 5: EFFECT OF ETHANOLIC EXTRACT *D. HAMILTONII* ON GLUTATHIONE AND ACTIVITES OF ϒ-GT, GST, AST, ALT, ACP AND ALP ACID AND ALKALINE PHOSPHATASE.

Variable	Organs	Normal	Diabetic Control	Diabetic treated (20 mg/kg)
Glutathione (GSH)	Blood (mg/dl)	112.8±16.5	106.6±9.2	122.5±26.4
	Liver (_g/g wet tissue)	2831.4±182.0	3268.4±291.0	3384.5±546.0
Lipid peroxidation	Liver (_mol MDA/g wet tissue)	13.81±0.44	16.92±0.40	34.98±1.60
γ-Glutamyl transpeptidase	Liver(nmol *p*-nitroaniline released/min/mg protein)	4.62±0.27	2.76±0.78 *	5.76±0.75*
	Plasma(nmol *p*-nitroaniline released/min/ml)	14.20±1.22	22.44±2.02*	18.48±2.00*
Glutathione *S*-transferase	Liver(nmol pyruvate /min/mg protein)	0.61±0.04	0.46±0.08 *	0.46±0.02*
Aspartate aminotransferase	Liver (nmol pyruvate /min/mg protein)	88.98±7.80	73.46±8.32*	78.14±6.02*
Alanine aminotransferase	Liver(nmol pyruvate /min/mg protein)	84.55±2.62	80.26±8.22*	80.22±8.64*
	Plasma(nmol pyruvate formed/min/ml)	80.18±6.02	166.72±24.28	108.08±26.22
Alkaline phosphatase	Liver(nmol *p*-nitrophenol released/min/mg protein)	0.64±0.06	1.08±0.20*	1.18±0.24*
Acid phosphatase	Liver(nmol *p*-nitrophenol released/min/mg protein)	8.24±0.16	8.46±0.83 *	8.88±0.68*

Values are expressed in Mean ± S.E.M values. P<0.05 (Dunnett's - test); diabetic control was compared with the extracts.

CONSLUSION: The present observation provide evidence that ethanolic extract of *Decalepis hamiltonii* seed exhibited antidiabetic or hypoglycemic activity on alloxan induced diabetic rat. It may be due to enhancing the peripheral utilization of glucose, by correcting the impaired liver or kidney glycolysis and by suppression of its gluconeogenic activity similar to insulin. The result suggests that it is worth undertaking further studies on possible usefulness of the *Decalepis hamiltonii* ethanolic seed extract in diabetes mellitus. Detection of hypoglycemic activity in Decalepis hamiltonii ethanolic seed extract along with protective effect against alloxan challenge and preventive action on lipid peroxidation provides scientific rationale of use of *Decalepis hamiltonii* as antidiabetic plant. Antidiabetic activity seems to be a result of increase in glucose utilization. In preliminary toxicity study it is safe, however chronic toxicity evaluation will be required for human use.

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