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Anti Diabetic Potential of Asparagus recemosus Stem Bark in Alloxan Induced Diabetic Rats

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ABSTRACT

The anti diabetic potential of the water extract of Asparagus recemosus (Liliaceae) ,a medicinal plant widely used in the traditional Ayurveda and siddha systems of medicine for the treatment of diabetes mellitus was evaluated in the alloxan monohydrate induced diabetic model. Graded doses of the water extract were administered to normal and experimental diabetic rats for 10 days .Significant ($p \leq 0.05$) reduction in fasting blood glucose levels were observed in the normal as well as in the treated diabetic animals. Increase in Serum insulin levels was observed due to pancreatic β cell regeneration. In addition, changes in body weight, serum lipid profiles and liver glycogen levels assessed in the extract treated diabetic rats were compared with diabetic control and normal animals. Significant results were observed in the estimated parameters, thereby justifying the use of the plant in the indigenous system of medicine.

Key words: Anti Diabetic Activity, Alloxan Monohydrate, Glibenclamide, Asparagus Recemosus.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerves. As the number of people with diabetes multiply world-wide, the disease takes an ever-increasing proportion of national and international health care budgets. It is projected to become one of the world's main disablers and killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two- to three-folds than the present rates. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations In the recent years traditional or complementary medicine has seen an upsurge and according to two studies, almost 48.5% Australian respondents, and 34% of American respondents had used at least one form of unconventional therapy including herbal medicine [1,2].

Asparagus recemosus (Liliaceae) is found through out the warmer parts of India and called as Satavari, Satawar, Satawari, Satmooli.It is a perennial shrub, with a tuberous root-stock, stems covered with recurved spines, linear leaves arranged in a tuft, white flowers and sweet-scented appears in October. The plant occurs through out India upto 1500 metres elevation. *Asparagus racemosus* is recommended in Ayurvedic texts for prevention and treatment of gastric ulcers as galatogogue and nervine tonic. The decoction of root has been used in blood diseases, diarrhoea, dysentery, cough, bronchitis and general debility [3-5]. 'shatavari', has been used as antidiarrheal, antiuclerogenic, refrigerant, tonic, anti bacterial,demulcent, diuretic,hypolipidemic, galactogogue, aphrodisiac and antispasmodic in Ayurvedia, Siddha and Unani systems of medicine.[6-13] Besides, *Asparagus racemosus* has also been found to have antioxidant, galactagogue, immunostimulant and hepatoprotective activities[14-16]. In folk medicine, stem bark and roots of *Asparagus racemosus* are used for the treatment of diabetes and gonorrhoea [17, 18].

In view of alleged antidiabetic potential of Asparagus recemosus, we have investigated effect of extracts of its stem bark on fasting blood

sugar levels and serum biochemical analysis in alloxan mono hydrate (AMH)-induced diabetic rats.

2. MATERIALS AND METHODS

The stem bark of the plant were collected from the Alagarkovil region, Madurai District in the month of july and cleaned to remove the debris. The collected plant was identified and authenticated by a botanist Dr. D. Stephen Department of Botany, American college of Arts and Science, Madurai. A voucher specimen (CS/AUG/BOT/02) has been kept in our museum for future reference. The bark was cut in to pieces and shade dry at room temperature ,the dried bark was subject to size reduction to a coarse powder by using a dry grinder (Philips India) was passed through sieve No. 60.

2.1. Animals

Healthy, adult male Wistar rats weighing 180-200 g were used for study. The animals were housed in large and spaces polypropylene cages, maintained under standard condition (12 h Light/12 h dark cycle, 25ºC and 30-35% humidity) and fed with standard pellet diet (M/S. Hindustan lever Ltd., Bangalore, India) and water *ad libitum*. The study was approved by institutional animal ethics committee of Ultra College of Pharmacy, Madurai. All the animals experimental procedure were carried out as per CPCSEA guideline. (CPCSEA No.890/ac/05/ CPCSEA).

2.2. Preparation of plant extracts

2.2.1. Preparation of hot water extract (HE)

Asparagus recemosus stem bark were air dried for 3–5 days in the shade and cut into small pieces. Five hundred grams were boil with 2.5 L of distilled water for 4 h. The hot water extract was concentrated under vacuum at 60^{0}C, freeze-dried at −20 ◦C (yield 34.2%, w/w, dry weight basis) and stored at 4^{0}C until use.

2.2.2. Preparation of ethanolic extract (EE)

Asparagus recemosus stem bark were air dried for 3–5 days in the shade and 500 grams were extracted with absolute 80% ethanol using Soxlet apparatus for 6 hrs. The extract was evaporated to dryness under reduced pressure at 60 ◦C (yield 25.6%, w/w, dry weight basis) and stored at 4^{0}C until use.

2.3. Phytochemical screening

The alcoholic extracts obtained were subjected to preliminary phytochemical screening (Kokate CK.,et al.,) to identify the chemical constituents. The methods of analysis employed were those described by Harbone & Baxter,1993[19-20].

2.4. Acute toxicity studies

Healthy *Wistar* rats of either sex were used, starved overnight were orally fed with the aqueous and ethanolic extract in increasing dose levels of 500, 1000, 2000 and 4000,5000 mg/kg body weight. The animals were observed continuously for 2 h under the following profiles:

(i) Behavioural profile: Alertness, restlessness, irritability and fearfulness.

(ii) Neurological profile: Spontaneous activity, reactivity, touch response, pain response and gait.

(iii) Autonomic profile: Defecation and urination. After a period of 24 and 72 h animals were observed for any lethality or death.

2.5. Induction of diabetes in rats

Diabetes was induced by single intraperitoneal injection of freshly prepared alloxan mono hydrate (150 mg/Kg, i.v, Loba Chemie, Bombay) dissolved in normal saline after over- night fasting of 12 hr. Two days after alloxan injection, rats with plasma glucose levels of >140 mg/dl were included in the study. Treatment with plant extracts was started 48 h after alloxan injection. Blood samples were drawn at weekly intervals till the end of study (i.e. 3 weeks).To prevent the hypoglycemia which occurred during the first 24 h following the AMH administration, 5% glucose solution was orally given to the diabetic rats. In all experiments, rats were fasted for 16 h prior to AMH injection. Only rats found with permanent diabetic were used for the antidiabetic study.

2.6. Experimental design

In the experiment [21-23] a total number of 30 rats (24 diabetic rats, 6 normal rats) were used. The rats were divided into 4 groups of six each.

Group I : Control group (Vehicle treated)

Group I : Diabetic control (Alloxan mono hydrate 150mg/kg b.w i.p)

Group III : Diabetic rats receiving "HE of. *Asparagus racemosus*" (500 mg/kg b.w orally*)*

Group IV : Diabetic rats receiving "EE of. *Asparagus racemosus*" (500 mg/kg b.w orally*)*

Group V : Diabetic rats receiving Glibenclamide (0.25mg /kg b.w orally)

Standard drug and extract were prepared in 0.5% Carboxy methyl cellulose Suspension as a vehicle and administered orally, Treatment of experimental animals with bark extracts and reference drug were initiated 2 days post

Alloxon monohydrate injection and was carried out once daily, by gavage, for 14 days. Food and water were made freely available.On day 21, blood was collected by cardiac puncture under mild ether anesthesia from overnight fasted rats and fasting blood sugar was estimated.(18)and the blood was centrifuged (2500 rpm/10 min) to get serum. The serum was used for biochemical estimation of fasting blood glucose level, [24-27] Hemoglobin, and Glycosylated hemoglobin, Liver glycogen and serum insulin. The Blood glucose level was determined by using glucometer and blood glucose test strips (CONTOURTMTS).Hemoglobin leads to formation of hemoglobin throughout the circulatory life of RBC by addition of glucose to N- terminal of hemoglobin beta chain. This process is non enzymatic, reflects the average exposure of hemoglobin to glucose over an extended period. The insulin concentration was calculated by Enzyme linked immuno sorbent assay (ELISA).Initial and final changes in body weight was observed.

2.7. Glucose Tolerance Test

The blood glucose concentrations of the animals were measured at the beginning of the study. In brief, twenty-four rats were fasted for 16 h and assigned randomly into 4 equal groups (n = 6/group). These rats were orally treated in the following manner: Group 1 (1 ml of DW), Group 2 (400 mg/kg of HE), Group 3 (400 mg/kg of EE) and Group 4 (0.25 mg/kg of Glibenclamide). Blood glucose level was measured by using glucometer at 0 hr (pre- study). One hour later, all these rats were orally loaded with 5 ml/kg of 50% (w/v) glucose solution. The blood samples were collected at 1hr, 2hr, 3hr intervals after the administration of the glucose .The blood samples were collected with potassium oxalate and sodium fluoride solution and glucose levels were estimated using a glucose oxidase–peroxidase reactive strips and a glucometer.

2.8. Histopathological study

On Twenty first day the animals were sacrificed by mild chloroform anaesthesia, the pancreas was excised and stored in 10% formalin after washing with normal saline. The tissues were washed, dehydrated with alcohol, cleared with xylene and paraffin blocks were made. Serial section of 5µm thickness were cut using a rotary microtome. The sections were deparaffinised with xylene and hydrated in descending grades of alcohol. The slides were then transferred to haemotoxylin for 10min, followed by rinsing with water, dehydrated with ascending grades of alcohol, cleared with xylene and mounted.

2.9. Statistical analysis

Results were expressed as mean ± standard error. Statistical analysis was done by using repeated measure one way anova followed by Dennett's multiple comparison test .$P<0.05$ was considered as significant.

3. RESULTS AND DISCUSSION

Literature survey indicates that there is no scientific evidence to support the antidiabetic effect of. *Asparagus racemosus*. Therefore the present study is undertaken to investigate the action of aqueous and ethanolic extract of *Asparagus racemosus* stem bark in different models of rats to ascertain the scientific basis for the use of these plants in the treatment of diabetes.

3.1. Phytochemical screening

The preliminary Phytochemical screening of both aqueous and ethanolic extracts revealed that the presence of flavonoids, alkaloids, glycoside, steroids, terpenes,saponins, phytosterols and tannins.

3.2Acute Toxicity Study

The preliminary acute toxicity revealed the nontoxic nature of *Asparagus racemosus* Experiments were carried out on normal healthy rats. The behavior of the treated rats appeared normal. No toxic effect was seen even with the dose of 5 g/kg b.w. and there were no lethality in any of the group. Body weight was normal. Therefore, the cut off dose for effective dose (ED50) was taken as500 mg/kg b. w.which is the 1/10th of LD50.

3.3. Effect on Glucose Tolerance Test

Both HE and EE significantly ($P < 0.05$) improved the glucose tolerance test up to 3 h (Table 1). HE and EE showing approximately 14, 11, 10% and 15, 12, 11% reduction in glycaemia from control values at the 1, 2 and 3 h, respectively. Glybenclamide also improved the glucose tolerance test up to 3 h. It showed that both extracts of Asparagus *racemosus* gave significant effect lower blood glucose level at the end of 60 min after glucose loaded and even lower level at the end of 120min and 180 min. The HE and EE of *Asparagus racemosus* enhanced glucose utilization, so the blood glucose level was significantly decreased in glucose loaded rats

3.4. Effect of HE and EE on blood glucose level in normal fasted rats

Overnight fasted rats were divided into five groups of six rats each. Group I ,II and V were administered distilled water(control),diabetic(untreated control) and standard drug (Glybenclamide 0.25 mg/kg) by oral route. A dose 500 mg/kg and of HE and EE

Table .1. Effect of Hot water Extract (HE) and cold Ethanolic Extract (EE) of *Asparagus racemosus* stem bark on oral glucose tolerance test (mean ± S.E.M., *n* = 6)

Group	Treatment	Glucose Concentration(mg/dl)			
		Pretreatment	Time following 50% oral Glucose load(h)		
		0hr	1hr	2hr	3hr
I	Control (1ml DW)	94.1±1.1	93.1±3.1	91.2±3.2	92.2±4.2
II	HE(400mg/Kg)	94.2±2.2	136.5±1.2*	126.2±4.2*	116.2±3.4*
III	EE(400mg/Kg)	91.6±.2.2	139.4±1.3*	128.9±2.3*	109.2±3.5*
IV	Glybenclamide(0.25mg/Kg)	88.4±2.7	132.1±1.4*	123.3±3.2*	104.2±3.4*

DW,Distilled water.*P<0.05, as compared with controls.

Table .2. Effect of Hot water Extract (HE) and Ethanolic Extract (EE) of *Asparagus racemosus* stem bark on fasting blood glucose levels in alloxan induced diabetic rats(mean ± S.E.M., *n* = 6)

Group	Treatment	plasma glucose Concentration(mg/dl)			
		0th day	7th day	14th day	21st day
I	Vehicle	77.14±1.23	79.16 ± 1.37	79.8 ± 1.69	80.4 ± 2.12
II	Diabetic control	215.1±1.29	249.2±2.31	279.2 ± 1.83	283.6 ± 1.38
III	HE (500mg/Kg)	211.21±2.77	172.2±1.05	130.33±1.78	112.2±2.26***
IV	EE (500mg/Kg)	217.83±3.20	162.9±2.11	137.73±2.54	111.44±2.46***
V	Glybenclamide(0.25mg/Kg)	213.23±3.12	162.03±1.43	114.13±2.92	93.93±2.43***

****p < 0.001* significant from diabetic control animals

Table.3. Effect of Hot water Extract (HE) and Ethanolic Extract (EE) of *Asparagus racemosus* stem bark on body weight of rats(mean ± S.E.M., *n* = 6)

Group	Treatment	Body weight changes		
		1st Day	7th Day	21st Day
I	Control(1ml DW)	194.21±1.4	193.55±1.6	192.23±2.3
II	Diabetic control	199.53±1.1	181.21±1.2	165.14±2.6
III	Diabetic+HE(500mg/Kg)	191.15±2.7	174.23±1.2	185.23±1.4***
IV	Diabetic+EE(500mg/Kg)	190.35±1.4	171.53±1.7	192.23±1.2***
V	Glybenclamide(0.25mg /kg)	188.96±1.8	170.23±1.1	186.27±1.8***

DW-Distilled water, **p < 0.001* highly significant from diabetic control animals

Table 4: Effect of Hot water Extract (HE) and cold Ethanolic Extract (EE) of Asparagus *racemosus* stem bark on serum insulin,liver glycogen and glycosylated hemoglobin levels in diabetic rats.

Group	Treatment	Glucose Concentration(mg/dl)		
		Serum Insulin (ng/ml)	Glycosylated Hemoglobin(%)	Liver glycogen(mg/g)
I	Control (1ml DW)	0.24 ± 0.21	3.73 ± 0.09	12.76±0.21
II	Diabetic control	0.19±0.01	6.88±0.25	5.12±0.12
III	Diabetic + HE(500mg/Kg)	0.30 ± 0.00**	3.25 ± 0.44***	12.84 ± 0.71*
IV	Diabetic + EE(500mg/Kg)	0.31 ± 0.00**	3.44 ± 0.04***	12.741± 0.71*
V	Glybenclamide(0.25mg/Kg)	0.32 ± 0.02**	3.71 ± 0.17***	13.71 ± 0.11*

Values are given as mean ± S.E.M from six rats in each group**p < 0.05* significant, ***p< 0.01* most significant, ****p< 0.001* highly significant from diabetic control animal

were suspended in drug vehicle and administered to group III and group IV orally. The drug vehicle, standard drug and test substance were administered once daily, per orally for the period of 21 days. All the drug administration procedure was carried out between 8-9:30 am of the day. The blood glucose levels were estimated on 0 (pre- study) and 7, 14, 21 day of the study. The rats were restrained in rat restrainer and blood samples were collected from the tail vein by making a small incision on the tail tip and 0.5-1.0 ml of the blood was collected for estimation of blood glucose, The results were tabulated in Table.2. After one and two weeks of the treatment,the blood glucose level return to normal at 110-112mg/kg indicating that the hypoglycaemic effect of plant extract is achieved through repeated and not single administration

3.5. Effect of HE and EE on body weight in AMH induced diabetic rats:

There was a significant body weight loss in the diabetic rats (Diabetic control)during 21 days, Vehicle control animals were found to be stable in their body weight whereas animals treated with HE and EE at the doses of 500 mg/kg p.o. showed the significant increase in weight 14th day onwards, indicating that the HE and EE had beneficial effects in preventing loss of body weight of diabetic rats due to increases glucose metabolism.(Table.3)

3.6. Changes of Serum Insulin, Liver Glycogen and Glycated Hemoglobin:

To assess the effect of long-term treatment of the extract on serum insulin, liver glycogen and glycosylated hemoglobin in AMH-induced chronic diabetic rat model. Rats were treated with 500 mg/kg b.w. both extract once a day in the morning for 21 day.Administration of both extracts of *Asparagus racemosus* stem bark showed significant ($p < 0.01$) increase in the levels of serum insulin. The possible mechanism by which aqueous extract brings about its hypoglycemic action in diabetic rats may be potentiating the insulin effect of plasma by increasing either the pancreatic secretion of insulin from the existing beta cells or by its release from the bound form. The significant increase in the glycogen level of the aqueous and ethanolic extract-treated diabetic animals may be because of the reactivation of glycogen synthase system. This focuses the one possible way of antidiabetic action of this both extracts by improvement of glycogenesis process.

The excess of glucose is present in the blood during diabetes, which react with hemoglobin and form glycosylated hemoglobin.Glycated hemoglobin levels were found to be increased in the untreated diabetic control group. Treatment with both extracts of *Asparagus racemosus* stem bark showed a significant decrease in the glycated hemoglobin levels, which could be due to an improvement in insulin secretion, which confirms the antidiabetogenic action of both extracts. The both extracts did not produce any significant effects on normal animals.. At the end of the treatment, the animals when compared with diabetic control, showed significant ($p < 0.01$) difference in serum insulin level and glycosylated hemoglobin level and significant ($p < 0.05$) effect on liver glycogen level (Table 3).

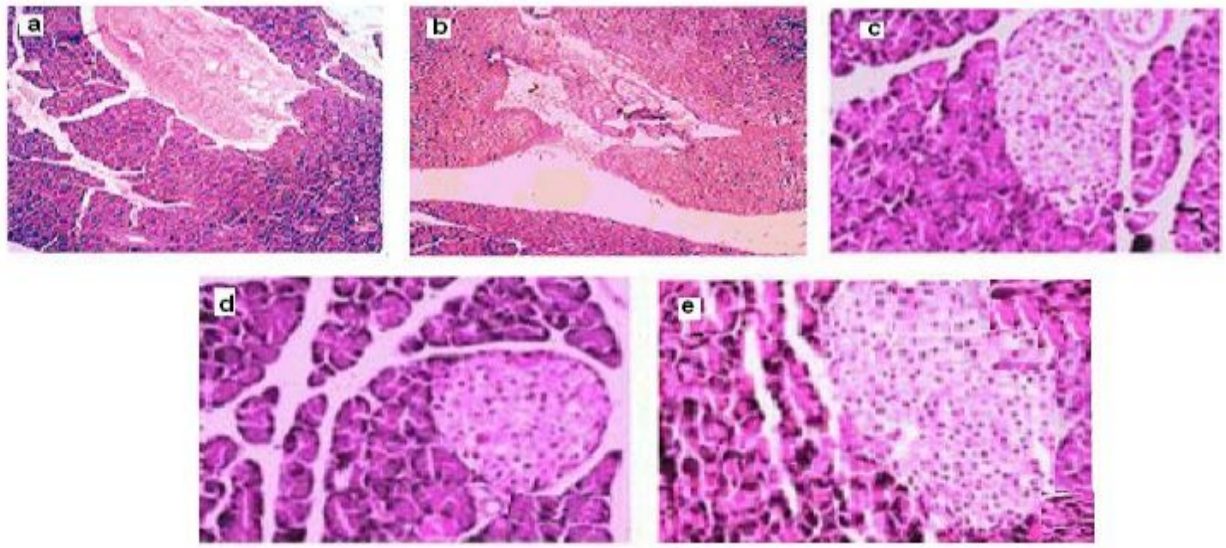

Fig 1. Histology of rat pancreas stained by haematoxylin and eosin of (A) untreated and (B) AMH induced diabetic rats and effects of(C) glibenclamide, (D)Hot water extract, (E) ethanolic extract of *Asparagus racemosus* stem bark Microscope magnification: 400×.

3.7. Histopathological studies

Histopathology studies also support our findings. AMH was suspected to destroy pancreatic β cells. The histopathological examination revealed extensive alterations in pancreas of AMH-induced diabetic rats (Fig. 1 (a-e)). The pancreas of control rat (Fig. 1a) showing normal islets. Extensive damage to the islets of langerhans and reduced dimensions of islets(Fig. 1b) , restoration of normal cellular population size of islets with hyperplasia by glibenclamide (c) was also shown. The partial restoration of normal cellular population and enlarged size of β-cells with hyperplasia was shown by Hot water and ethanolic extracts (Fig 1d-e).It suggests the possibility of the islets regeneration and recovery of normal carbohydrate metabolism in both hot water and ethanolic extracts of *Asparagus racemosus* stem bark treated group

4. CONCLUSIONS

It is concluded from the data that both Ethanolic and aqueous extracts of *Asparagus racemosus* stem bark exhibited significant antihyperglycemic activities in alloxan-induced diabetic rats. These extracts showed improvement in parameters like body weight and lipid profile as well as regeneration of β -cells of pancreas and so might be of value in diabetes treatment.However ,longer duration studies on chronic models are necessary to elucidate the exact mechanism of action so as to develop it is a potent anti diabetic drug.

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